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Soil seed bank pattern of *Adesmia tristis* Vogel from Campos de Cima da Serra ecosystem in southern Brazil*

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ABSTRACT – This study evaluated the soil seed bank (SSB) of *Adesmis Tristis* Vogel, plant deaths in the ecosystem, the interference of the soil potential acidity on the SSB and a seedling survey *in situ.* After simulating SSB in levels of acidity and surveying seedlings of *A. tristis* in plots, we confirmed that *A. tristis* naturally reseeds; with an aggregated or random distribution depending on the cutting management; the soil potential acidity reduces the activation of *A. tristis* SSB; the establishment of new individuals requires openings in the environment due to the death of mother plants.

Keywords: acidity, management, mother plants, reseeding, seedlings

RESUMO – **Padrão do banco de sementes do solo de *Adesmia tristis* Vogel no ecossistema dos Campos de Cima da Serra, no sul do Brasil**. Objetivou-se avaliar o banco de sementes do solo (BSS) de *A. tristis*, a morte de plantas no ecossistema, a interferência da acidez potencial do solo no BSS e o levantamento de plântulas *in situ*. Após simulação do BSS em níveis de acidez e o levantamento de plântulas de *A. tristis* nas parcelas, nós confirmamos que *A. tristis* promove ressemeadura natural; possui distribuição agregada ou aleatória em função do manejo de corte; a acidez potencial dos solos atenua a ativação do BSS de *A. tristis*; novos indivíduos necessitam de aberturas no ecossistema ocasionadas com a morte de plantas-mãe.

Palavras-chave: acidez, manejo, plantas-mãe, plântulas, ressemeadura

INTRODUCTION

Adesmia tristis is a decumbent, ascending legume from Campos de Cima da Serra (Miotto & Leitão Filho 1993) and is a native species with properties that can be used by the community in the future (Coradin *et al.* 2011). It has many branches, reaching 1.5 m in length (Miotto & Leitão Filho 1993). Besides being an endemic plant in the fields of Campos de Cima da Serra (Overbeck *et al*. 2007), it is cited as one of 18 species of the genus *Adesmia* found in Brazil (Iganci & Miotto 2011). It fixes nitrogen as a result of bacterial symbiosis and being endemic, it has adapted to soils with little phosphorus, low pH, and high aluminum levels, characteristic of the region. It is a micro thermal plant, used as a winter cover crop for grazing during the most critical time.

Adesmia tristis uses seeds to propagate which reproduce from October to March, peaking between December and January (Miotto & Leitão Filho 1993). During this period, the soil seed bank (SSB) is enriched by natural reseeding. Seed supply is increased by the dispersion, density, and spacing of the plant source diaspores (Lopes *et al*. 2010), improving species reproduction success. It is a complex process involving interactions among plants, seeds, and other biotic and abiotic factors from the environment. In this context, the SSB has a key role in the maintenance of individuals in the ecosystem (Garcia 2009), and shows the history of the local flora. Its viability is a function not only of inputs and outputs from system seeds, but also of anthropogenic disturbance, predation, decay, and transport (Maia & Maia 2008).

Microorganisms associated with abiotic soils influence SSB seed germination (Singh *et al*. 2008, Yamashita & Guimarães 2011), interfering on their size and dynamics. It is vital to understand dispersion processes in the environment, as well as the dynamics, size, and SSB pattern to be able to use this species (Favreto & Medeiros 2006), and there are virtually no studies on *A. tristis*.

This study aimed to evaluate the size and distribution pattern of *A. tristis* SSB on the environment, the consequences of plant death on species dispersion, and the soil pH interference on SSB stimulation for its conservation and application in tall grassland ecosystem.

MATERIALS AND METHODS

The field activities were carried out at the Pró-Mata Center for Research and Nature Conservation, Pontifical Catholic University of Rio Grande do Sul, Brazil, (Pró-Mata, PUCRS), (29°27'-29°35'S and 50°08'-50°15'W) (Bertoletti

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& Teixeira 1995). It is located in the geomorphological region of Planalto das Araucárias, in the municipality of São Francisco de Paula, RS, 900 m mean altitude, Cfb climate, temperate maritime humid and mean annual temperature of 14.5 °C with frequent fog, frost, and occasional snow. The mean annual rainfall is 2,252 mm well distributed over the year (Bertoletti & Teixeira 1995, Bond-Buckup 2008). The soil of the selected area was classified as Bruno Cambisoll, being Humic and Alic properties the main features with high acidity and low phosphorus (Streck *et al*. 2008). The predominant vegetation in the region is composed by Mixed Ombrophilous Forest (Araucaria Forest), Dense Ombrophilous (Atlantic Forest), and Altitude Fields (Overbeck *et al*. 2007).

This work was installed in July 2008 and involved three studies. The experimental area was 15.0 m x 30.0 m, and subdivided into six randomly distributed 3.0 m x 15.0 m plots. All the experimental area was planted with 1,800 seedlings of *A. tristis* with a plant density of 4.0 per m^2. This condition is found naturally in environment parches with *A. tristis*. Three of these plots received cuts (disorders) simulating grazing, and the remaining portions were taken as control (uncut).

The first study was divided into two parts. The first part was held from May to July 2009, assessing the size of *A. tristis* SSB and its distribution pattern in the environment. Fifteen soil subsamples per plot were randomly collected with an auger of 3.7 cm diameter at 5.0 cm depth from the ground surface. The soil was transported to the Department of Forage Plants and Agrometeorology (DFPA) of the Federal University of Rio Grande do Sul, (UFRGS) (greenhouse), where it was fragmented and homogenized manually after drying in the shade (Ferreira *et al*. 2008), and placed in plastic trays (one per plot) with dimensions of 20 cm x 15 cm x 5 cm. SSB was irrigated according to the need to keep the soil near field capacity. Two germination cycles of 20 days were performed, trying to exhaust the *A. tristis* SSB. At the end of the first cycle, the irrigation was suspended for seven days and the soil was turned over, starting the new cycle. All SSB species that germinated after the two cycles were quantified and identified to family level, except *A. tristis*.

Descriptive statistics were used to analyze the data involving *A. tristis* SSB size (seeds estimate/m^2), percentage of total participation in total SSB, and the Morisita index (MI) to identify the species distribution pattern in the system (Medeiros & Steiner 2002, Ferreira *et al*. 2008) with or without disorder (cut). MI was calculated according to the equation $MI = n\,[\Sigma(X^2)-N]/N(N-1)$, where n = the number of parts, X = the number of individuals per plot, and N = the species total number in all plots. For MI ≈ 1, individuals are randomly dispersed, MI<1, individuals are uniformly distributed, and, if MI>1, individuals are aggregated. Statistical significance was tested by X^2 value (chi-square). $X^2 = n\,(\Sigma x^2/N)-N$ to (n-1) degrees of freedom at a significance level of 1%. Results were compared with tabulated values of the chi-square defining significance.

The second part took place from February 2009 to November 2011. In this period, there was a pattern of plant death (drying), resulting in root diseases after the reproductive period. The individuals death interfered seed dispersion and SSB formation. In the last year of the study, a survey of surviving plants was carried out, estimating the percentage loss in the period.

In April 2010, 60 seedlings were transplanted in black polyethylene pots with a volume of 10.0 L (one plant per pot) in an area outside the DFPA. Originally, these seedlings were grown in a germination chamber BOD type (Biochemical Oxygen Demand). Over one year, the death pattern after the reproductive period was observed and quantified. In May 2011, the percentage of plant loss in pots was estimated. Samples were collected from roots of diseased plants and seeds stored in the experimental field.

The samples were taken to the Department of Phytosanity of UFRGS in order to isolate and cultivate the possible etiologic agents. At the same time, new seedlings were transplanted to a plastic container (volume of 0.5 L) for further isolate inoculation. The inoculant was prepared in 10 mL of distilled water in a petri dish with isolated pathogen. Then, the material was homogenized to form a mixture rich in spores and sclerotia. The inoculation was undertaken using individuals with a length of 6.5 ± 0.8 cm and 5.0 ± 0.7 mature leaves. Root galling was made with further addition of a 1.0 mL mixture for five seedlings, and pieces of sclerotia for five other seedlings. After inoculation, two observations were performed at intervals of 10 days, using a stereo microscope to observe the root system and to collect material with tweezers and stylets to form laminas stained with a drop of blue Aman. Such laminas were examined using a microscope, looking for the formation of pathogenic structures in root samples.

The second study was carried out from April to December 2010, which consisted of SSB natural simulation. Soils were collected from five distinct areas. Three areas near the study: burned area (BA), experimental area (EA), and preserved area (PA), and two areas in the physiographic region called the Central Depression in the state of Rio Grande do Sul, the UFRGS area (UA) and red soil area (RA). Samples of these soils were sent to the soil analysis laboratory of UFRGS, where the potential acidity was determined by the SMP Index method based on the buffer power of the soil, dictated by the Sociedade Brasileira de Ciência do Solo (2004). Later, liming needs were estimated (limestone with 100% Total Neutralizing Relative Power-TNRP) for the soil correction to reach pH 6.0, according the Sociedade Brasileira de Ciência do Solo-SBCS (2004).

Each soil type was placed in an open plastic container (11.0 cm x11.0 cm x 3.5 cm) with 2.0 cm layer of soil and four replicates. One hundred *A. tristis* seeds were used without prior treatment to break dormancy. SSB models were monitored in the Laboratory of Seed Analysis of the DFPA and were exposed to natural light and temperature variations. The replacement of the soil moisture content was performed with distilled water as needed. After eight months of daily germination monitoring, irrigation was suspended for the soils (substrates) to dry. The soils were then fragmented by hand and sieved through Granutest sieves with an opening of 0.71 mm, Tyler 24. The remaining seeds were separated by soil type. A germination test of the remaining seeds was undertaken over 20 days. The seeds were subjected to mechanical scarification (sandpaper number 180) to break dormancy. Plastic containers to germination (gerboxes) were used with two sheets of germitest paper and BOD germination chamber. Irrigations with distilled water were added when necessary. The temperature was 20 °C without photoperiod control.

A linear regression analysis was performed to compare the limestone need with germination rates (BioEstat 5.0 application, with 5% of probability), in addition to descriptive statistics with mean, standard deviation, and percentage. The means of the remaining seeds were compared by Student's t-test at 5%, using a completely randomized design through the statistical application Assistat 7.6 beta, version 2011.

The last study was carried out in December 2010, in the experimental area. It consisted of surveying *A. tristis* seedlings, resultant of natural reseeding in six plots, three with cut disorders and three uncut. A metal rectangle of 0.5 m^2 was used randomly with 150 replicates (50 per sample unit) for each treatment (disorder with and without cutting) for a total sampled area of 75 m^2. Means were compared by Student's t-test at 1%, and graphs were prepared with their standard errors.

RESULTS

The evaluation of SSB showed predominance of *Poaceae* and *Asteraceae* seeds, following the general pattern found on the fields in southern Brazil.

Part one: SSB size and distribuition patern of *Adesmia tristis*

Thirty species were observed distributed among nine families (*Asteraceae*, *Caryophillaceae*, *Fabaceae*, *Iridaceae*, *Oxalidaceae*, *Poaceae*, *Solanaceae*, *Plantaginaceae* and *Cyperaceae*) (Tab.1).

On average, the SSB revealed 4,166.7 (± 905.1) species per m^2. From this total, *A. tristis* contributed with 41.3 seeds (±13.1) per m^2, corresponding to 1% of the total SSB. The Morisita index (MI) for the uncut plots was MI = 3.0 ($p<0.01$) with an aggregated pattern. Plots that had suffered cutting disturbances had MI = 1.0 ($p<0.01$), indicating random distribution.

Table 1. List of botanical families and their species present in the studied SSB from Pró-Mata, PUCRS, municipality of São Francisco de Paula, in 2009.

Family	Species	Common name
Asteraceae	*Achyrocline satureioides* (Lam.) DC.	Marcela
Asteraceae	*Baccharis trimera* (Less.) DC.	Carqueja
Asteraceae	*B. dracunculifolia* DC.	Vassoura
Asteraceae	*Chaptalia nutans* (L.) Polak	Lingua-de-vaca
Asteraceae	*Calea phyllolepis* Baker	Cálea
Asteraceae	*Senecio brasiliensis* (Spreng.) Less	Maria-mole
Asteraceae	*Solidago chilensis* Meyen	Erva-lanceta
Asteraceae	*Trichocline catharinensis* Cabrera	Cravo-do-campo
Asteraceae	*Vernonia catharinensis* Cabrera	Assa-peixe
Caryophyllaceae	*Cerastium humifusum* Cambess. ex A. St.-Hil.	Cerástio
Cyperaceae	*Kyllinga brevifolia* Roottb.	Quilinga
Cyperaceae	*Rhynchospora globosa* (Kunth) Roem. & Schult.	Rincospora
Cyperaceae	*R. setigera* (Kunth) Boeck.	Rincospora
Fabaceae	*Desmodium affine* Schltdl.	Pega-pega
Fabaceae	*Adesmia tristis* Volgel	Babosa
Iridaceae	*Calydorea campestres* (Klatt) Baker	Íris-do-campo
Iridaceae	*Sisyrinchium sellowianum* Klatt	Canchalágua
Oxalidaceae	*Oxalis conorrhiza* Jacq.	Azedinho amarelo
Poaceae	*Axonopus affinis* Chase	Axônopos
Poaceae	*Agrostis montevidensis* Spr. ex Nees	Capim-mimoso
Poaceae	*Andropogon lateralis* Nees	Capim-caninha
Poaceae	*A. leucostachyus* Kunth.	Capim-colchão
Poaceae	*Aristida megapotamica* Spreng.	Arístida
Poaceae	*Briza calotheca* (Trin.) Hackel	Capim-brisa
Poaceae	*Calamagrostis viridiflavescens* (Poir.) Steud.	Calamagrostis
Poaceae	*Eragrostis airoides* Nees	Eragrostis
Poaceae	*E. neesii Trin.*	Eragrostis
Poaceae	*Paspalum pumilum* Nees	Grama-baixa
Plantaginaceae	*Polygonum punctatum* Elliott	Erva-de-bicho
Solanaceae	*Solanum aculeatissimum* Jacq.	Joá

Part two: Plant death pattern

Adesmia tristis plant mortality in the experimental area was 58% over the three years of the experiment. Similar patterns were observed with plants in pots, with 55% loss.

Two fungi, *Fusarium* sp. and *Rizoctonia* sp., were identified in both samples of adult plant roots (desiccation symptoms of shoot and root necrosis) as in seeds. In inoculated seedlings, there were no deaths or disease signs. Subsequently, the inoculated seedlings were transplanted to pots (10.0 L) for observation over five months. There were no disease symptoms in this period.

2. Second study: SSB natural simulation

In *A. tristis* SSB simulation, the seed germination process occurred over the eight months of observations. In relation of germination to the need for the soil correction to pH 6.0 level (Tab. 2), there was a negative linear regression, according to the Y=58.98-0.65 X equation (Fig. 1). The adjusted coefficient of regression (R^2) is responsible for 74% ($p<0.05$) of the variations presented on seed germination.

After eight months, some seeds were still hard (Tab.3) and a small fraction was lost by random causes, common event in the SSB, mainly after extended periods.

Means followed by same letter do not differ statistically from one another. The Student's t-test at probability 5% level was applied.

The viability of the remaining seeds was found with breaking dormancy by mechanical scarification with subsequent germinability ranging from 92.9% to 100% (Tab. 2).

The germination of the remaining seeds was statistically similar in most studied soils ($p<0.05$). There was a difference only between the treatment of burned area (BA) in relation to treatments with soil of UFRGS area and red soil area (Tab. 3). By the way, these last two soil samples are from a region far away (about 150 km) from the study area. There is a tendency to form a single block with treatments of similar characteristics from the study area (BA, EA, and PA) (Tab. 3), demonstrating the specificity of those soils, possibly one reason for *A. tristis* endemism.

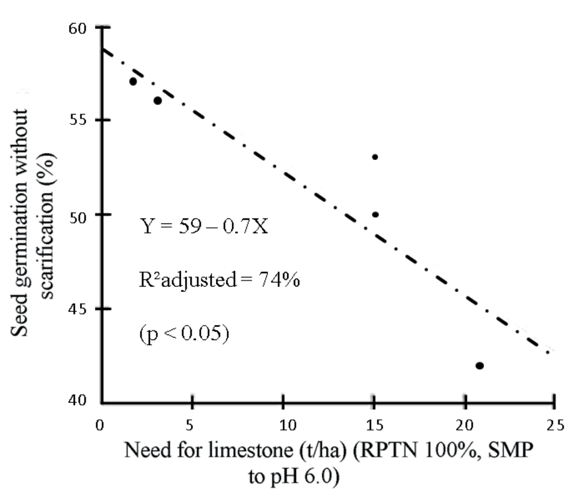

Fig. 1. Negative linear regression relating the germination of non-scarified *Adesmia tristis* seeds after eight months with addition of limestone to five different types of soils to corrected to pH = 6.

3. The last study: surverying *Adesmia tristis*

In the last study, surveying seedlings *in loco*, undisturbed plots (cuts) had an average of 0.1 seedling/m² (Fig. 2). The opposite occurred in plots with simulated grazing (cut) with 1.9 seedlings/m². The mean of the two treatments were highly different ($p<0.01$).

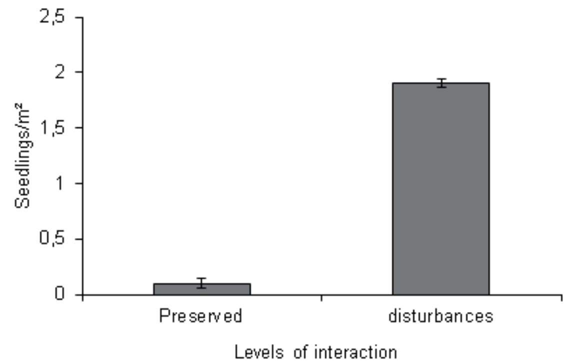

Fig. 2. Compilation of *Adesmia tristis* seedlings/m² in plots with disorders (cuts) and preserved plots (undisturbed) from Pró-Mata, PUCRS, municipality of São Francisco de Paula, in 2009.

Table. 2. Results of soil analyses from Pró-Mata, PUCRS, municipality of São Francisco de Paula, in 2009, carried out by the Soil Analysis Laboratory at UFRGS to determine the potential acidity by SMP index. UA = soil of UFRGS area, RA = soil with red horizon area, PA = preserved soil area (attached to the study), EA = soil of the experimental area, and BA = soil from the burned area (attached to the experiment).

Soils	SMP Index	*Liming (t/ha)
Substratum		Recommendation
RA	6.3	1.8
UA	6.0	3.2
EA	4.6	15.1
BA	4.6	15.1
PA	4.3	21.0

*Committee for Chemistry and Soil Fertility - RS/SC, 10th ed. (2004)

Table 3. Comparison of means from remaining *Adesmia tristis* seeds from Pró-Mata, PUCRS, municipality of São Francisco de Paula, in 2009, after eight months of monitoring the germination percentage and after dormancy breaking by mechanical chiseling. UA. Soil of UFRGS area; RA. Soil area with red horizon; PA. Soil of preseved area (attached to the study); EA. Soil of the experimental area and BA. Soil from the burned area (attached to the experiment).

Germination Substratum	Remaining Seeds	Germinated after Scarification (%)
UA	29.8 (±2.4) a	100.0
RA	26.0 (±5.8) a	93.2
PA	21.8 (±3.9) ab	92.9
EA	21.0 (±5.5) ab	95.3
BA	12.8 (±2.9) b	99.2

DISCUSSION

When establishing the experiment level of 4.0 plants/m² *A. tristis,* we were simulating the natural conditions.

The contribuition of 1% from the soil seeds of *A. tristis* of the total SSB expresses the maintenance potential of the species in the system. In natural reseeding, seed losses are common, mainly due to deterioration, parasitism, or predation (Ludwig *et al*. 2011), where some of these factors may occur in association.

In diaspore samples, both in the experimental area and in five different points on the edges of RS 020 and RS 235 highways, we found that some seeds were damaged by Coleoptera. These insects belonged to the genus *Apion* (Brentidae). In the seed set of 24.9 g, 444 adult insects and 187 larvae were found. However, *A. tristis* produced enough seeds to compensate for losses. The residual seeds are sufficient to ensure the species in the native grasslands.

Based on these data, we concluded that *A. tristis* is an opportunistic species, benefitting from stochastic and timely disturbances to establish itself in the system. In natural grasslands, the aggregation is confirmed by the formation of small and sparse clusters of individuals. Disorders with low impact on system resilience inhibit the dominance of few species, allowing resources for less competitive plants (Connell 1980). A more random distribution of the plant in the environment is favored, when vegetation cover is reduced by cutting the plots. Introducing animals into the system possibly promoted the same phenomenon for *A. tristis,* improving forage supply.

During the reproductive phase, *A. tristis* has a very reduced leaf area (Miotto & Leitão Filho 1993), compromising plant photosynthetic potential. Energy reserves are channeled for intense flowering and seed production. It is possible that the physiological resilience is broken (individual depletion). This makes the plant more susceptible to infection and the soil biological agents can change from saprophytic to pathogenic states. This process allows diseases to emerge as recorded, with consequent death of the mother plant.

For the establishment of new individuals in the system, the occurrence of some disturbance is necessary with open microspaces in the environment (aggregated distribution). These impacts are characterized by the death of the mother plant, allowing viability for new individuals of the same species. This phenomenon is characterized as a kind of "altruistic" strategy of dispersion for species preservation in natural grasslands.

Natural soil acidity of the studied region (pH 4.0 to 4.8) is responsible for decreasing *A. tristis* seed germination contained in SSB. However, other mechanisms that promote dormancy breaking often occur in nature, but more slowly (Zaidan & Barbedo 2004). The humic cambisol soils are found in the region of *A. tristis* endemism. These soils are constituted by highly acidic substrates, with the "A" horizon rich in organic matter (Streck *et al.* 2008) that promote complex interactions among soil properties and seeds. Exogenous signals to the plant from the humic acids of organic soil matter may cause similar effects to those orginating from the phythormones that promote plant growth by stimulating germination (Canellas *et al*. 2008). These intercrosses of factors distribute germination in time and space benefiting the maintenance of the legume in the environment. *Adesmia tristis* SSB may be classified between transitory and permanent (transitory are viable seeds for less than one year after dispersion, and permanent are viable seeds after a year or more after dispersion) (Christoffoleti & Caetano 1998).

Tegument hardness may be influenced by interactions amongst the characteristics and environmental variations, reflected in the germination distribution in space and time (Bryant 1989). *Adesmia tristis* has tegumentary dormancy (arrangements of macrosclereids and osteoesclereids in palissades) associated with a tanniferous parenchyma that regulates water entry into the seed (Ferreira *et al*. 2011). In addition, traqueoids and astroesclereids form intercellular spaces that enhance the seed adaptability to moisture variation in the environment (Ferreira *et al*. 2011).

Vegetation cover density possibly served as a physical barrier for seeds to reach the ground, leaving them more exposed to weather and predation (Maia & Maia 2008, Ludwig *et al*. 2011). Simulated cut management controls grazing that improves the flowering synchrony and reduces plant mass, allowing natural reseeding. Spaces emerge for seed contact with soil, with subsequent establishment of new seedlings. Positive results in seed production and reseeding are linked not only to species improvement but also to a rational management of the system (Lopes & Franke 2009), targeting the reproductive process in detriment of biomass production. This fact was noted in *Lotus subbiflorus* Lag. "El Rincón", which benefited in natural seeding, by reducing the amount of aerial phytomass (Gomes *et al*. 2011). This reduction can be spontaneous or caused by management. In a spontaneous condition *Lotus subbiflorus* reseeding was favored in the summer, the season with more defined and dry period, according to Gomes *et al*. (2011).

The promotion of spaces in the soil facilitates better seed dispersion and seedling establishment in the environment. The dispersive efficiency is considerable in *Adesmia* (Miotto & Waechter 1996) and *A. tristis* appears to be a diplocory species (dispersion by two agents). The glandular trichomes lining the hemicraspedium suggest a epizoochory primary dispersion (Miotto & Leitão Filho 1993). A second dispersion by myrmecochory is likely to occur after the cladodios dehiscence. *A. tristis* has seeds with attractive arils to ants (Miotto & Waechter 1996), as occurs in other taxa that have arils or elaiossomes in seeds (Howe & Westley 1988, Peternelli *et al.* 2004).

The natural soil acidity decreases the germination of *A. tristis* from the SSB and consequently the establishment of new individuals into the environment. Furthermore, the loss of the mother plants are necessary to open spaces in the system after the breeding season. The cut management provides a better *A. tristis* dispersion and establishment of new individuals in grassland.

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