Project's Report

INFLUENCE OF EMERGENCE TIMING OF WEEDS ON THE ESTABLISHMENT OF WOODY NATIVES AND GROUND COVER SPECIES

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Key words: invasion, survival index, interspecific competition, intraspecific competition, weeds, native grass, growth rates, seedlings, seed size, seasonal effects.

Abstract

Catalan
Les invasions biològiques són produïdes per espècies transportades per l'home fora de la regió d'origen a altres regions on s'estableixen i expandeixen. Són actualment de les majors causes de perduda de biodiversitat, amb el canvi d'usos del sòl, tret rellevant en zones insulars. Comprendre mecanismes de competència amb les espècies autòctones és clau per gestionar el problema.

L'experiment evidencia diferències de creixement de 7 plantes natives australianes (3 espècies d'eucaliptus, 3 espècies d'acàcia, 1 pasturatge natiu), competint intraespecífica (entre mateixa espècie) i interespecíficament (acàcies o eucaliptus convivint amb pasturatge natiu) plantejant tres tractaments (sense males herbes, males herbes i males herbes a posteriori) per definir la naturalesa de la interacció dels diferents tipus funcionals d'espècies.

S'analitzen tendències temporals de creixement de plàntules, així com la supervivència. S'ha detectat una moderada correlació entre taxes de creixement d'espècies i mida de la llavor, ($\rho \approx 0.6$), així com una correlació entre la supervivència i la humitat del sòl ($\rho \approx 0.5$); efectes estacionals.

A curt termini i en escenari de primavera la convivència amb males herbes reporta creixement nul. Tractaments sense males herbes, presenten major supervivència en escenaris en competència interespecífica. A llarg termini les espècies amb major supervivència són les que conviuen amb pasturatge natiu i sense males herbes, indicant un efecte beneficiós en espècies millor adaptades a la sequera (*E. loxophleba*).

Spanish
Las invasiones biológicas son producidas por especies transportadas por el hombre fuera de la región de origen a otras regiones donde se establecen y expanden. Son actualmente de las mayores causas de pérdida de biodiversidad, con el cambio de usos del suelo, factor relevante en zonas insulares. Comprender mecanismos de competencia con especies autóctonas es clave para gestionar el problema.

El experimento evidencia diferencias de crecimiento de 7 plantas nativas australianas (3 especies de eucaliptos, 3 especies de acacia, 1 pastoreo nativo), compitiendo intraespecífica (entre misma especie) y interespecíficament (acacias o eucalipto conviviendo con pastoreo nativo) planteando tres tratamientos (sin malezas, con malezas y malezas a posteriori) para definir la naturaleza de interacción de los diferentes tipos funcionales de especies.

Se analizan tendencias temporales de crecimiento de plántulas, así como la supervivencia. Se ha detectado una moderada correlación entre tasas de crecimiento de especies y tamaño de la semilla, ($\rho \approx 0.6$), así como una correlación entre la supervivencia y la humedad del suelo ($\rho \approx 0.5$); efectos estacionales.

A corto plazo y en escenario de primavera la convivencia con malezas reporta crecimiento nulo. Tratamientos sin malezas, presentan mayor supervivencia en escenarios en competencia interespecífica. A largo plazo las especies con mayor supervivencia son las que conviven con pastoreo nativo y sin malezas, indicando un efecto beneficioso en especies mejor adaptadas a la sequía (*E. loxophleba*).

English
Biological invasions are caused by species transported by man outside the source region to other regions where are established and expanded. Thus are currently the greatest causes of biodiversity loss, the change in land use, relevant actor in insular areas. Understanding the mechanisms of competition with native species is the key to managing the problem.

The experiment demonstrates differences in growth of 7 Australian native plants (three species of eucalyptus, three acacia species and one native pasture), competing intraspecific (between same species) and interspecific (acacia or eucalyptus living with the native grass) proposing three treatments (no weed, weed and weed after) aiming to define the nature of the interaction of the different functional types of species.

It's analysed the temporal trends of seedling growth and survival. Note a moderate correlation between growth rates of species and seed size ($\rho \approx 0.6$), as well as a correlation between survival and soil moisture ($\rho \approx 0.5$); seasonal effects.

In the short-term spring scenario the coexistence with weeds reported zero growth. Treatments without weeds present higher survival scenarios in interspecific competition. Long-term species survival are greater when living with the native grass and without weeds, indicating a beneficial effect on species better adapted to drought (*E. loxophleba*).

Introduction

Biological invasions are caused by species transported by humans outside their region of origin to other regions where established and expanded. They are currently one of the major causes of biodiversity loss, along with the change in land use; therefore, to understand the mechanisms by which compete with native species is the key to manage the problem.

Australia is one of the world's centres of 'mega-diversity' of plants and animals. The south west of Western Australia is known as one of the world's biodiversity "hotspots" with some of the richest and most threatened reservoirs of plant and animal life on earth. In some areas of the state the agricultural development has involved the clearing of forest, woodland and shrubland since settlement. Clearing and consequential salinity are having a devastating effect on land and water, on biodiversity through the direct loss of plant species, and the associated loss of mammals, birds and other animals which depend upon areas of bush for food and shelter. As a result, many of the original ecosystems are very poorly represented in agricultural and urban areas.

The 1588 ha farming property at West Pingelly UWA Ridgefield is located 25 km north-west of Pingelly, being the target study area.

Today, land use continues to be based on dryland agricultural production using annual, winter growing, pastures and crops.

Deep, sandy soils characteristic of Perth, on which the woody species typically occur, are low in nutrients. While the native grass it's well adapted to this circumstance (Lambers et al. 2010), it may not be conducive to the achievement of target growth rates for revegetation projects. In summer, its long tap root seeks access to deep ground water, while in winter, near-surface cluster roots take up nutrients when soil is moist (Marschner et al. 2005).

Acacia and Eucalyptus woodlands are widespread in the Perth region of south-west Western Australia, with Mediterranean climate. They have declined substantially because of various factors, including land clearing, altered fire regimes, declining rainfall and ground water drawdown.

The species used in this project are 3 species of Eucalyptus (*E. accedens*, *E. astringens* and *E. loxopheba*), 3 species of Acacias (*A. acuminata*, *A. pulchela*, *A. sessilis*) and the native grassland (*Austrodanthonia caespitosa*), some of the most characteristic species of Australian flora. That is why this experiment has used this species as an object of study. In addition to the abundance of these species, these were selected because its characteristics of nutrient acquisition (arbuscular mycorrhizal, ectomycorrhizal and nitrogen fixers), to have broad distribution in the study area, high germination rate and seed availability through local suppliers.

Probably the best research work to compare this project with is Stevens, J.M. and Fehmi, J.S. (2011) where the early establishment of native grass in Arizona that reduces the competitive effect of a non-native grass (Buffelgrass) is studied.

In this report it's concluded that the competitive effect of the Buffelgrass plants it's noticed when the age of the Arizona cottontop it's the same or younger than the invasive grass. It's find, as well, that the interspecific competition between the native and the invasive plant is several than the intraspecific competition between the same aged species.

These results suggest that establishing native plants immediately following a disturbance event could be a practical technique for restoring or retaining diversity on sites with high potential for invasion by buffelgrass.

Also, at Jurado, E. and Westoby, M. (1999) the relationship between the size of the seed and the growth rate and the biomass was analyzed. Their results were that the bigger the seed is, the lower the growth rate and the higher the biomass will be.

Purpose

The investigation described in this report is a part of a larger investigation carried out by the School of Plant Biology of the University of Western Australia collaborating with the Universitat Autònoma de Barcelona, Bellaterra, aiming to find the influence timing of weeds on the establishment of woody natives and ground cover species.

In this project a field experiment would be set up to achieve the scope of the main research question. The target species are subject to more realistic conditions like longer time of competition against a whole community of invasive species and stressors like water scarcity and high radiation. With this field experiment it's also intended to describe possible differences between target species when they grow as monocultures and their growth when they are mixed with a native grass Austrodanthonia caespitosa (Wallaby grass). This interaction also will be examined under presence of a weed community in order to identify the nature of interaction between these three components.

The purpose of this trial is to define the nature of interaction between three different functional types of species, trying to follow the next questions as the project shaft.

- *How does the late emergence of the weed community affect the establishment of five woody natives and one native grass?*
- *Does the late emergence of weeds affect their abundance under competition with woody natives and ground cover species?*
- *How do woody natives and native ground covers species affect the establishment of each other?*
- *How does the late emergence of weeds affect the interaction between woody natives and ground cover species?*

Using field data were obtained during the stay in Ridgefield, the study allows to conduct a trend analysis of growth towards the weed, wed after and no weed treatments.

Methods

The experimental plots were established in an isolated area for purposes of ecological restoration and include 21 ha. It's located in an area traditionally known as the "Wheatbelt" in the South West of Western Australia, to 174 km from Perth (31 ° 57'11 "E).

To evaluate the competitive response of target plants and the interspecific and intraspecific competitive effect, a complete block design with 10 replications at random it's established. Each block is made up of three parcels 5 X 2.5 m corresponding to three different moments in the emergence of the weed community: 1) natural emergence, 2) delayed emergence and 3) control, without weed.

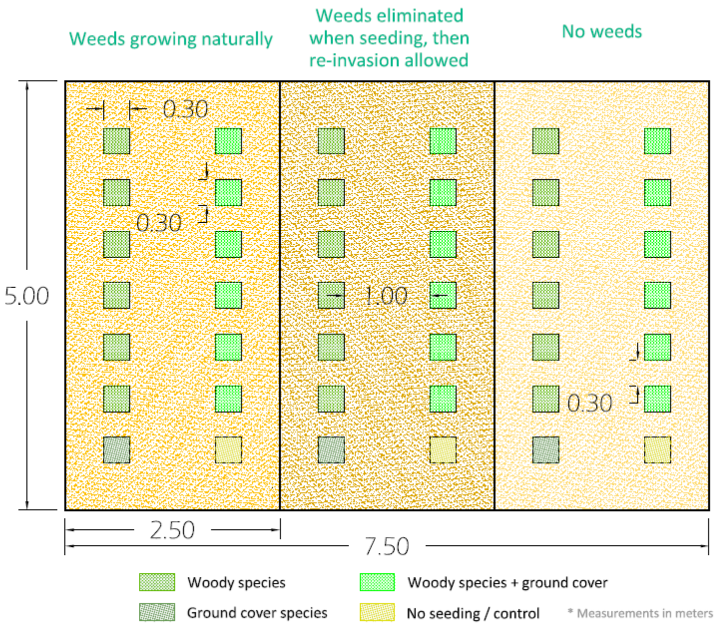


Figure 1. Plot crops organization

By the time that were sowing the seeds of the species studied, the resident community of weeds in treatment 1 had a height between 10 and 15 cm. The eradication of weeds in treatments 2 and 3 was made by a single spraying glyphosate (diluted commercial 360ml/l) with a fumigator a week before planting the species studied.

Within each plot were located, distributed in two parallel rows of similar size, 14 subplots of 0.3 X 0.3 m, which were sown in three different types according to the composition of seed mixtures 1) monospecific, with woody seeds of each species, 2) monospecific with seeds of *Austrodanthonia caespitosa*, 3) mixed with seeds of each species of woody and *Austrodanthonia caespitosa*. In one of the 14 subplots there was no seed to be used as a control. The location of the mixtures in each sub-parcel was random. The distance between each row of subplots was 0.3 m and the distance between rows was 1m. The amount of time used by each species was defined based on the expectation of having a sufficient number of seedlings emerging and survivors throughout the experiment and seed size for each species. This was set weighing 10 lots with a known number of seeds (20 to 100) to *E. loxophleba* and *E. astringens* and weighing a batch of 250 seeds for *E. accedens*, Acacia species and *Austrodanthonia*.

The planting was the last week of June 2012 (as shown in the *Gantt chart*, in the main project) raking the soil surface with a screwdriver in order to create favorable microsites for germination. The removed soil was placed back in the original place to protect the seeds.

Given the data collected in the field by the author of this project, it has ten independent samples for each stage of the project and for each count carried out, having a total of four counts over time.

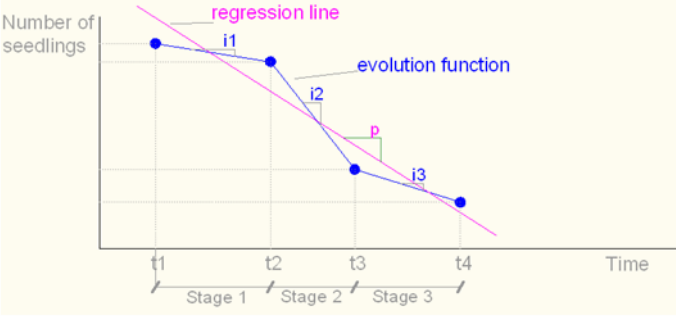
Each count, involve the number of plants have been recorded in each plot existing at the moment of the count in question, having made measurements of biomass, soil moisture measurements and size characteristics of the plant species in each plot.

A selection of the best estimators used to synthetize data it's performed. Normality tests have been done (Shapiro-Wilk tests, and Q-Q graphs), and after that, it's concluded that MEDIAN and intercuartílic deviation are the most accurate central tendency and deviation estimators to be employed in this project according the data collected.

For the tendency analysis of seedlings growth, a discussion about the goodness between proceeding according linear regression models or proceeding according to Perish rate procedure, it's done.

Perish rate it's a methodology proposed by the author of this project to analyze growth rate, understanding this along this project as the velocity of mortality of the seedlings along a specific period of time, being the absolute value of the formal definition of the first derivative's evolution function.

This procedure allows including different growth/decline rate in the different stages contemplated in the project, it's to say, contemplating thus the different growth rate due to seasonal effects. Noticing that a linear regression model cannot capture the seasonal effect.



$$i_j \rightarrow \textit{Prerish rate at stage } j\textit{, calculated as the first derivative of the evolution function at stage } j$$

$$p \cong \frac{i_1 \cdot (t_2 - t_1) + i_2 \cdot (t_3 - t_2) + i_3 \cdot (t_3 - t_2)}{t_4 - t_1} \rightarrow \textit{Average of the perish rate}$$

Figure 2. Linear regression model vs. evolution function

Graphing survival and perish rate in each stage will be performed in a discussion of the different growth trends for each plant variety depending on the type of treatment used, as in the coexistence between the species in question.

The tool used to establish relationships between the different indicators (indicators of growth/decline rate, survival indicators and soil moisture) is the analysis of the Pearson's correlation coefficient. Noticing thus the different types of correlation between variables (direct or inverse correlation), and also showing the powerful of its relation, it's to say this relation became harder as the absolute value of the Pearson coefficient tends to one (|ρ|→1).

RESSULTS

Temporal evolution of seedlings

If one analyse emergence, could observe that: The results of *A. sesslis* have not been present for the null emergency. One can observe different behaviours in both acacia trees such as eucalyptus and native grass. In reference to the acacia trees, there is a greater emergency in cases of Weed After treatment than in the Weed treatment, as may be apparent. In contrast to the cases of No Weed, the emergency has been null. In reference to eucalyptus trees, there is a major emergency in case of No weed that not in the rest of the cases, such emergency being higher in the case of emergency contemplated in pure plots than in mixed plot, happening the same to the crops of native grass. In the particular case of *E. loxophleba*, it can be seen that in the first stage the largest emergency is in the scenario Weed after, but then in a second stage the emergency is higher over the scene not weed.

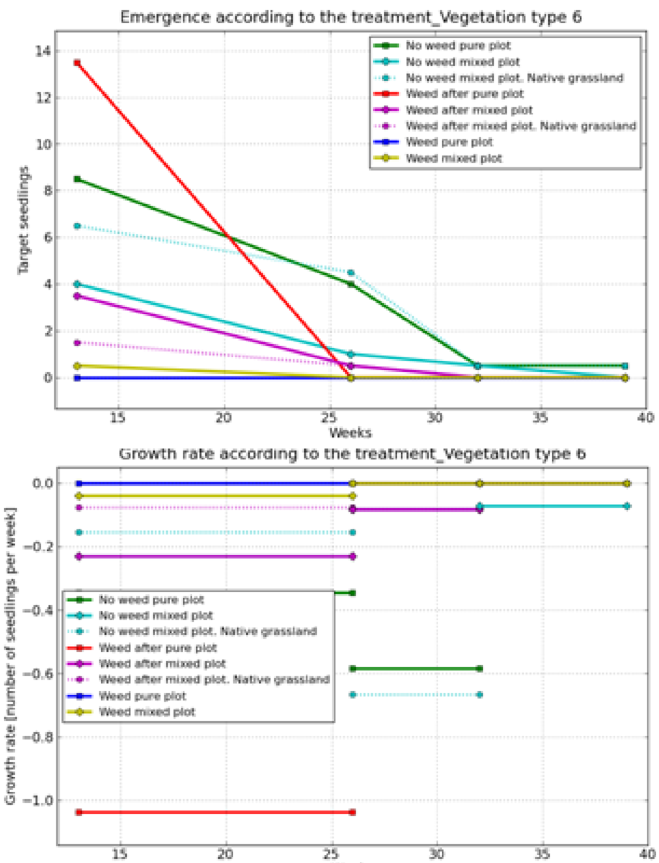


Figure 3. Emergence and growth/decline rate evolution for *E. loxophleba*

In the case of contemplate scenarios with coexistence of the native grass, at all times the emergence of the native grass is higher than the natives woody varieties in question.

Survival of seedlings in Spring stage

At week 26, the scenarios of no weed pure plot have higher survival of *E. astringens*, *E. loxophleba* and *A. caespitosa* that for other scenarios. In contrast, *A. acuminata* and *E. accedens* survival is greater in scenario weed after pure plot. As curious information and as it can be seen in the figure 4, *A. pulchela* only survives in no weed mixed scenario and the only specie that survives in the weed after mixed plot scenario is *E. loxophleba*.

In general there is more survival in pure scenarios than in mixed scenarios. It may be due to nutrient competition between the woody species and native grass, interspecific competition, this being worse than intraspecific competition, between the same species.

In weed scenarios scenarios, there are no survival plants; weeds prevent the growth of all varieties.

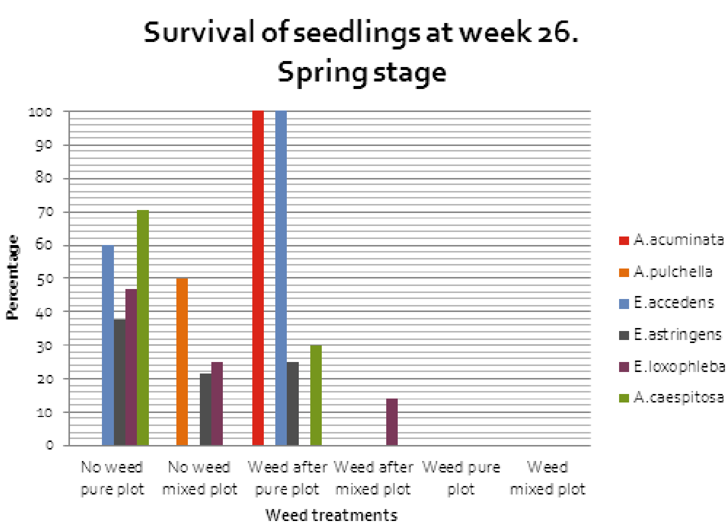


Figure 4. Survival of seedlings at spring stage

Survival of seedlings in Summer stage

At week 32, there is only *E. loxophleba*, the more resistant variety, which only survives in no weed treatment, being better when competing with native grass than when competition is intraspecific. One might think that the benefits of mycorrhizae in long term are greater or sufficient to eliminate the effect of competition for nutrients with native grass, in comparison to no weed pure plots where survival is lower due to intraspecific competition. Even so, the variety survives at 32 weeks, with twice the number of plants found in the no weed mixed plot treatment than in the no weed pure plot treatment.

At week 32, the native grass under weed after treatment has already died because of the appearance of the weed community and thus has hindered the development of the native grass. In contrast in the no weed pure plots treatment, the native grass is able to survive because it has any kind of competition, indicating that these specie is well acclimated.

All other species have died at 32 weeks in weed after treatments because of the interspecific competence between the weed community and the woody species and the native grass. The lack of water in the soil will also be an influence factor.

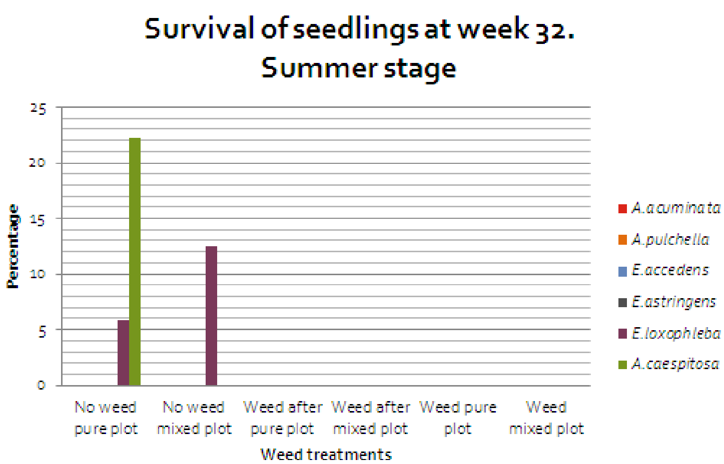


Figure 5. Survival of seedlings at summer stage

Discussion

Comments about the tendency evolution procedure

Using the methodology developed by the author of this project about of Perish rates, captures better the behaviour of seedling growth, being able to carry out the analysis of trends for each seasonal stage in a more accurate way than using the slope of the regression as a benchmark indicator. Indeed, information is lost about the behaviour proceeding with the regression models if the linearity assumption is not valid, although the slope of the linear regression is an indicator of global trends is more synthetic.

In the absence of further experimental data, it was concluded that the growth trend analysis using the methodology perish rates is sufficient to analyse the growth of seedlings over time for the scenario in question, as it includes information similar to that provided with the methodology of regression models, in addition to providing the possibility to see different growth behaviours during the stages studied. Although the coefficients of goodness of fit of the linear models are not too bad, because the few data available to perform the interpolation, it's concluded that the best method to use in the case in question is the relative to the perish rates procedure developed in this project.

The findings set out on trends in the growth of the different plant varieties are similar proceeding with the raised two methodologies, although it prefers the methodology of growth rates because it has the potential to indicate the local developments of each stage, indicating more accurately the behaviour of the seedlings along the time (seasonal effects).

Comments about the growth/decline tendency results at spring stage.

it can be concluded that in general rules, the mortality rate of coexistence scenarios with native grass is lower than for native crops by the same treatment. The scenarios No Weed have higher mortality rates than the rest.

There is also a strong rate of mortality of *E. loxophleba* in the crops of weed after pure plot, being notorious the mortality rate of *E. astringens* and *E. loxophleba* above the others species. Aspect that may be related with the seeds size, 0.55 and 0.61 respectively.

Acacias however remain low mortality rates, less than 0.1 seedlings per week.

Comments about the growth/decline tendency results at summer stage.

In summer, if it's compared the magnitude of the mortality rates respect to spring, there is a high mortality in the no weed pure plot crop varieties respect to the scenario no weed mixed plot which could be explained by the mycorrhized roots as beneficial reducing the mortality rate in the case of eucalyptus.

The scene weed after pure plot also presents moderate mortality rates (approx. 0.2 seedlings died per week) in comparison to weed after mixed plot scenario. weed scenarios have zero mortality rates since the emergency in both scenarios is negligible for all varieties, cause around 32 weeks are already dead all the seedlings.

Influence of the seed size in growth/decline rate

In general terms, it seems that there are different growth/decline trends over time, effect certainly due to seasonal effects (E. JURADO and M. WESTOBY, 1999), where is evidenced a decrease of the soil's moisture, affecting thus to the seedlings development. It's also observed that the magnitude of the growth rates/decline is different for acacia and eucalyptus, suggesting a correlation between growth/decline rates and the seed size.

The acacias have lower perish rate in comparison with eucalyptus, being the seeds of acacias more heavy or large than the eucalyptus.

To corroborate the suggestion about correlation between growth/decline rates and the seed size, a correlation analysis using Pearson's coefittient its performed. For this particular analysis, will be contemplate the average growth rate (expressed as the slope of the linear regression model), due to the simplicity to analyze growth evolution with only one parameter, knowing that seasonal effects isn't contemplate, but being easy to summarize the growth behavior along the full time of the experiment. These average growth rate it's relative to each plant variety depending on the type of treatment.

Pearson correlation coefficient for variables: Seed size and Growth rate depending on the treatment				
Project scenario	NWP	NWM	WAP	WAM
Correlation coefficient ρ	0,67232141	0,62134894	0,4671479	0,68488083

Table 1. Pearson correlation coefficients between seed size and growth rates depending on the treatment

As it can be seen from the table 1, the Pearson correlation coefficient is positive in all scenarios contemplated in this project (NWP-no weed pure plot, NWM-no weed mixed plot, WAP-weed after pure plot, WAM-weed after mixed plot), using the average growth rate of the linear regression model.

In this project it's assumed that perish rate it's the same that "minus growth rate". Therefore it will indicate that, effectively, exists an inverse relation between the perish rate and seed size variables, been a moderated relation ($\rho \approx 0.6$).

Could be demonstrated in the experiment developed in this project the same result reached by (SALISBURY, 1942; SILVERTOWN, 1981), thus confirming the sentence where the species with larger seeds tend to have relative slower growth/decline rates regardless of treatment performed, the vegetal type, the possible seasonal effects, and the amount of time considered in the experiment.

It's relationship could be explained due to the greater reserves in large seeds, and their greater ability to quickly establish larger root systems (SANCHEZ-GOMEZ et al., 2006). BAKER (1972) observed that species in drier habitats on average had larger seeds, which could be interpreted as a hedge against water stress.

Fungus symbiosis influence in survival index.

In the survival, one could say that the mycorrhized roots provide long-term benefits, as one may think what has happened with *E. loxophleba* (see Figure 5). In survival graph for summer stage, was reflected that *E. loxophleba* only survives in no weed treatment, being better when competing with native grass than when competition is intraspecific

One might think that the benefits of mycorrhizae in long term are greater or sufficient to eliminate the effect of competition for nutrients with native grass, in comparison to no weed pure plots where, although in short-term may be due to other factors as seed size, seasonal effects…

Influence of seasonal effects and weed competition in Survival index

In the survival of the species there is an aspect that noticeably impacts, the season. The season influences the degree of soil moisture because the weather of each season presents different temperatures and rainfall affecting therefore to the soil of the different plots. The rate of soil moisture is a key parameter for the survival because of reducing water in the soil, the chances of survival and proliferation of plant species decreases

The study began with the planting of the seeds at the end of June, belonging to the Australian winter, and ended with the harvest in March, which coincides with the autumn. It has been able to verify that in autumn and in summer, the moisture has been virtually non-existent.

Another parameter that directly influences the soil moisture is the presence of weeds as they help to reduce soil moisture, on the understanding that they also require water to survive.

Attending to the results about soil's moisture for spring stage presented in the project, ona can conclude that the presence of weeds acts decreasing soil moisture by up to a third compared to scenarios with no weed treatment, affecting this fact to the survival of the species in terms of competition for the acquisition of water and nutrients. (See *Figure* 4) The seasonal variation influences substantially in the reduction of soil moisture, dramatically influencing the survival of the species, being the species more resistant and in coexistence with native grass those that presented survival in summer (case of *E. loxplheba* in scenarios no weed) (see *Figure* 5).

It will also carry out a correlation analysis between soil moisture and survival index, aiming to demonstrate the strong correlation between these two factors. If the Pearson's correlation coefficient is positive for all vegetal species studied, it will indicate that exist a direct relationship between both variables, it's to say, as the soil moisture increases, the expected survival of each plant species does it too.

Correlation coefficient for variables: Soil moisture and survival index depending on the vegetal type. Week 26					
Project scenario	*A. acuminata*	*A. pulchella*	*E. astringens*	*E. accedens*	*E. loxophleba*
Correlation coefficient ρ	0,04579286	0,4948753 5	0,38023744	0,7674504 2	0,761583

Table 2. Pearson correlation coefficients for soil moisture and survival index. Vegetal type dependence for week 26

In the case of measurements of moisture for the week 30, corresponding to summer, these have been non-existent, being similar behaviour observed in survival for the same period of study. The varieties more resistant to the lack of moisture in the soil are the ones that have been able to survive in the summer season (as in the case of *E. loxophleba*).

Conclusions

Conclusions concerning the trend of perish of seedlings

- The acacias have lower death rates than observed in eucalyptus, due in part to the fact of the role of the seed size
- Observed mortality rates higher in summer than in spring for the varieties of acacias and *Eucalyptus accedens*. The lack of moisture in the soil triggers the decreased speed in seedlings.
- For the varieties and *Eucalyptus astringens* and *Eucalyptus loxophleba* notes that in the scenario no weed pure plot the perish rate is higher in the summer stage than spring. On the contrary, no weed and weed after mixed scenarios presented lower death rates in summer stage than in spring, probably due to the beneficial effect of long-term of the mycorrhized roots, being at the same time these varieties of eucalyptus more resistant to the lack of water that the other plant varieties analysed.

Conclusions relating to the survival of seedlings

- If the plant variety is resistant to the cohabitation with weeds and has not died in the short term, in a long-term the coexistence with native grass results beneficial, giving higher survival rates as it has been observed with *Eucalyptus loxophleba.*
- In scenarios with Weed, the competition for the acquisition of nutrients is very strong and does not survive anyone. Weed remaining soil moisture getting to the point where the survival is null.
- In scenarios with weed after, survival is lower than in no weed scenarios due to the non-competition with weeds, although the *Acacia acuminata* survives better in the short term in a weed after scenario than in no weed.
- In general, in short term, the varieties die in mixed scenarios with weeds because of nutrient competition, as has happened with *Eucalyptus astringens* and *Eucalyptus accendens.*

Conclusions regarding the presence of weeds

- The presence of weeds, especially in scenarios Weed notably affects the growth of different plant varieties, as competition for the acquisition of soil nutrients is very high, which is manifested by low soil moisture to the point of not surviving seedlings at the first evaluation in spring.
- In scenarios of weed after, the competition for the acquisition of nutrients has not been as strong, enabling higher survival in the woody species. It is strange that *Acacia acuminata* and *Eucalyptus accedens* preferred the scenario weed after than no weed to survive.
- Finally, the no weed are the scenarios that have been behaving greater proliferation of seedlings with difference, mainly due to the non-competition for the acquisition of nutrients, as expressed with more moisture in the soil in reference to other scenarios.

Conclusions relating to the coexistence with native grass

- The coexistence of the different plant species with the native grass has not meant a relevant fact in regard to competition for the acquisition of nutrients, because the humidity values observed in pure and mixed scenarios are similar for all the three treatments done.
- In the long term, it is evidence of the beneficial effect of coexistence with native grass for *Eucalyptus loxophleba*. At the same time if the plant variety is resistant to drought episodes, it is beneficial the coexistence as such has been evident in the present experiment.

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