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**Monodominance of *Erythrina fusca* Lour.: influence of
environmental factors, chemical ecology and dendroecology**

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General abstract

In some tropical regions, areas with more than half of the total number of arboreal individuals where only one species occurs, and these formations are popularly known as monodominant stands. Although there is no pattern of environmental characteristics that determine the occurrence of all monodominant species, some factors are more commonly associated with these formations, such as soil characteristics, fire, and flooding, among others. In the Brazilian Pantanal the occurrence of monodominant species is common. The species *Erythrina fusca*, for example, is widely dispersed throughout the world, characteristic of flooded areas, and in the Pantanal subregion of Cáceres occurs as monodominant formation. This species is an important resource for fauna and has a strong relationship with the local ecosystem. Considering the importance of this species and the lack of knowledge on how environmental factors influence the occurrence of monodominant species, our work aimed to investigate the factors that could influence the dominance of *E. fusca* in the Pantanal subregion of Cáceres. We hypothesized that environmental events, as well as intrinsic characteristics of *E. fusca*, facilitate its dominance to the detriment of other species. To investigate this, we compared the structure of dominance areas of *E. fusca* with adjacent mixed forests by analysing the relationship between soil and flood with the species occurrence. We investigated the influence of flooding and fire on germination and secondary metabolism in early stages of development. We also performed dendroecological analyses to observe the influence of climatic events on growth and establishment of *E. fusca* and investigated the presence of allelopathic potential that could confer advantages in competition with other plant species. We observed that the monodominance of *E. fusca* seems to be favored by environmental factors, such as higher levels of flooding and low soil fertility. We detected that flooding and fire confer changes in germination patterns and secondary metabolism of this species. Establishment of individuals

is favored by higher levels of flooding and presence of allelopathic potential, especially in leaves, may provide advantages to competition with other species. Thus, we conclude that the dominance of the species *E. fusca* in the Pantanal subregion of Cáceres has a strong relationship with flood levels, soil, and intrinsic characteristics, which influences its occurrence, establishment, germination, and release of allelopathic compounds. Therefore, changes in these factors can be harmful to the occurrence of this important plant formation, since this monodominant stands depends on particular soil and flood conditions to occur.

Keywords: allelopathy, dendrochronology, flood pulse, germination, soil-plant interaction, Pantanal, wetland.

Resumo geral

Em algumas regiões tropicais, ocorrem áreas onde mais de metade do número de indivíduos pertencem a apenas uma espécie, conhecidas popularmente como formações monodominantes. Apesar de não existir um padrão de características ambientais que determinam a ocorrência de todas as espécies monodominantes, alguns fatores influenciam mais comumente, como características do solo, ocorrência de fogo e inundação entre outros. No Pantanal brasileiro é comum a ocorrência de áreas de monodominância. A espécie *Erythrina fusca*, por exemplo, é amplamente dispersa pelo mundo, sendo característica de áreas inundáveis, e na sub-região de Cáceres no Pantanal ocorre como monodominante. Essa espécie é um importante recurso para a fauna e possui uma forte relação com o ecossistema local. Assim, considerando a importância dessa espécie e a lacuna de conhecimento sobre como os fatores ambientais influenciam a ocorrência de espécies monodominantes, nosso trabalho objetivou investigar os fatores que podem influenciar a dominância de *E. fusca* na sub-região de Cáceres no Pantanal. Nossas hipóteses são que os eventos ambientais, bem como características intrínsecas de *E. fusca* facilitam a sua dominância em detrimento de outras espécies. Para investigar isso nós comparamos a estruturação de áreas de dominância de *E. fusca* com florestas mistas adjacentes analisando a relação do solo e inundação com a ocorrência da espécie. Investigamos as influências da inundação e do fogo sobre a germinação e metabolismo secundário nos estágios iniciais de desenvolvimento. Realizamos análises dendroecológicas para observar a influência de eventos climáticos sobre o crescimento e estabelecimento da espécie e investigamos a presença de potencial alelopático que poderia conferir vantagens na competição com outras espécies vegetais. Nós observamos que a monodominância de *E. fusca* parece ser favorecida pelos fatores ambientais, como os maiores níveis de inundação e baixa fertilidade do solo, detectamos que a inundação e o fogo

conferem mudanças nos padrões de germinação e metabolismo secundário da espécie, observamos que o estabelecimento dos indivíduos é favorecido por níveis mais altos de inundação e que a presença de potencial alelopático, principalmente nas folhas, pode fornecer vantagens na competição com outras espécies. Assim, nós concluímos que a dominância da espécie *Erythrina fusca* na sub-região de Cáceres no Pantanal possui forte relação com os níveis de inundação, características do solo e intrínsecas, que influenciam sua ocorrência, estabelecimento, germinação e liberação de compostos alelopáticos e que mudanças nesses fatores podem ser prejudiciais à ocorrência dessa importante formação vegetal, uma vez que essa monodominância depende de condições particulares de solo e inundação para ocorrer.

Palavras-chave: alelopatia, dendrocronologia, pulso de inundação, germinação, interação solo-planta, Pantanal, áreas úmidas.

General introduction

The interest in the occurrence of dominance in tropical areas started in the early 1950s, when Richards (1952) described large tropical vegetation formations dominated by one or a few species. Thereafter, the word monodominance began to be used to define areas where more than half of the total number of arboreal individuals belong to only one species (Connell & Lowman 1989, Hart et al. 1989).

These monodominant formations are often recorded in different regions of the world, such as Africa (Hart et al. 1989, Hart 1995, Vierling & Wessman 2000, Torti et al. 2001, Maisels 2004, Peh et al. 2011), America (Richards 1952, Veblen et al. 1979, Boucher 1981, Isaacs et al. 1996, Van Groenendael et al. 1996, Torti et al. 1997, Martijena 1998, Henkel 2003, Henkel et al. 2005) and Oceania (Connell & Lowman 1989). In Brazil, monodominant stands of different species were found and studied in Amazonia and the Pantanal (Nascimento & Cunha 1989, Arieira & Nunes da Cunha 2006, Ribeiro & Brown 2006, Nascimento et al. 2007, Pott et al. 2011, Amador et al. 2012, Dalmagro et al. 2016).

Although no uniform pattern of factors influence all monodominant species in exactly the same way, some factors more commonly influence them than others, such as flood, fire, succession, association with fungi and conditions and characteristic of the soil (Hart 1990, Torti et al. 2001). Periodic flooding and soil conditions are the main events associated with the dominance of species in tropical regions, as both influence the oxygen levels and increase CO₂ and anaerobic decomposition (Joly & Crawford 1982, IRRI 1985, Lieberman et al. 1985, Campbell et al. 1986, Hart 1990, Richards 1996). Therefore, dominant species seem to be more tolerant than other species to unfavorable conditions, and such characteristics seem to select for species that have greater tolerance or fewer nutritional requirements (Joly & Crawford 1982, IRRI 1985, Lieberman et al. 1985, Campbell et al. 1986, Hart 1990, Richards

1996). In addition, the dominant species may possess attributes that are beneficial, such as ectomycorrhizal association, allelopathic potential, massive fruiting, or other intrinsic characteristics (Hart 1990, Torti et al. 2001).

An interesting and favorable environment to study the occurrence and factors that influence the dominance of species is the Pantanal, where several monodominant species are found.

The Pantanal is a vast Quaternary floodplain traversed by the Paraguay River and its tributaries, which extends through Paraguay, Bolivia, and Brazil, with an area of approximately 140000 km² (RADAMBRASIL 1982), and two distinct seasons: a dry season from May to September and a rainy season from October to April. The flood pulse in the Pantanal is monomodal, with long duration and low amplitude (Junk et al. 2014). These fluctuating flood levels heavily influence the composition and dynamics of the Pantanal (Junk et al. 1989, Nunes da Cunha & Junk 2014). In terms of vegetation, the Pantanal is considered an evolving region, dominated by a complex mixture of species and communities, where the flora presents Bolivian-Paraguayan xerophyte elements, central Brazilian savanna elements, wild elements of oriental Brazil and the Amazon, and widely dispersed hydrophilic elements (Adámoli 1986, Godoi Filho 1986, Rizzini 1997).

In the Brazilian Pantanal it is possible to find several monodominant species, which often have a local common name (followed by the suffix “al”), such as *Byrsonima cydoniifolia* A. Juss. (“canjiqueiral”), *Curatella americana* L. (“lixerial”), *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore (“paratudal”), *Copernicia alba* Morong ex Morong & Britton (“carandazal”), *Attalea phalerata* Mart. ex Spreng. (“acurizal”), *Vochysia divergens* Pohl (“cambarazal”), *Elyonurus muticus* (Spreng.) Kunth (“caronal”), *Bromelia balansae* Mez (“gravateiro”), *Licania parvifolia* Hub. (“pimenteiral”), *Handroanthus*

heptaphyllus Mattos (“piuval”), among others (Pott et al. 2011). A curious characteristic about these monodominant species is that they are not always dominant everywhere they occur and individuals of these species can occur sparsely in different regions, whereas in some places, where conditions are favorable, they can dominate the area. One example is the species *Erythrina fusca* Lour. (Fabaceae), which is the most widespread species of the genus *Erythrina*, occurring sparsely in Asia, Oceania, some African islands, and Central and South America (Russo & Baguinon 1997, Parrini & Raposo 2010). It is also well known that this species can have large dimensions in low-lying freshwater swamps, making it possible to find large areas of monodominant stands (called “*Abobral*”) in the plains of the northern upper Paraguay River in the Pantanal sub-region of Cáceres (Appendix 1, Appendix 2 and Appendix 3), and along the banks of the Aquidauana River (Russo & Baguinon 1997, Pott et al. 2011).

Erythrina fusca is an arboreal species that reaches 20-30 meters in height, with globose crown and short and branched stem, which is thorny when young. The leaves are alternate, composite, trifoliolate with leathery, glabrous leaflets. The inflorescences are terminal racemes, with noteworthy yellow-orange flowers (Appendix 4 and Appendix 5). This species occurs frequently in riparian forests, mainly in the Pantanal and Amazon regions and blooms in the months of May to September, with intense fruiting starting in November (Lorenzi 2002). The species *E. fusca* is a very important resource for fauna, which probably reflects in a strong relationship between the monodominant stands and the local ecosystem. The showy flowers are food for birds and insects and the seeds are an abundant resource eaten mainly by fish (Pott & Pott 1994, Parrini & Raposo 2010).

Thus, considering the wide occurrence of monodominant species in the Pantanal, the lack of knowledge about the factors that influence the occurrence of these stands and the importance of the species *E. fusca* in the study region, this thesis explores these topics in four

chapters. In these chapters, through the use of distinct tools, we seek to obtain answers about factors that may influence/facilitate the dominance of the species *Erythrina fusca* in the Pantanal subregion of Cáceres, Mato Grosso, Brazil, and thus generate information that helps to better understand and conserve, and that can be tested on other monodominant species.

Stands dominated by *E. fusca* occur side-by-side with richer riparian forests, which can be influenced by variation of flooding and soil conditions between these nearby areas. Thus, in the first chapter “Effects of periodic flooding and edaphic factors on monodominance of *Erythrina fusca* in Paraguay River riparian forests” we compared the plant species composition, soil data, and flooding of areas dominated by *E. fusca* with adjacent mixed riparian forests with the objective to investigate the relationship between flooding and some soil characteristics in the context of monodominance. We hypothesized that the dominance of *E. fusca* is mainly associated with longer flooding periods and, perhaps, some soil conditions (as lower fertility), as both factors are frequently associated with the occurrence of monodominant species (Hart 1990, Torti et al. 2001).

The establishment of a new vigorous plant depends on the success of germination and seedling development (Chacón et al. 2013). And these phases are strongly influenced by the environment, such as the occurrence of flooding, fire and climate changes, which can affect the percentage of germination, the physiology and the metabolism of the new plant. This way, in the second chapter “Effects of environmental factors in early development stages of *Erythrina fusca*”, we aimed to understand about the variations in secondary metabolism between seeds and seedlings of *E. fusca*, examine if flooding and/or fire can affect germination and seedling development of *E. fusca*, and investigate if these events produce alterations in the arrangement of secondary metabolites in seeds and seedlings (very important phases of establishment). We hypothesized that flooding and fire stimulate seeds overcoming

of dormancy, increasing/accelerating germination and formation of seedlings, and that these events produce modifications in the composition of secondary metabolites in the early stages of development.

Analysis of tree ring growth in tropical areas is an interesting way to study the structure and dynamics of forests, since it is possible to understand how some factors influence the growth and establishment of arboreal species (Worbes et al. 2003), what also makes it easier to understand how climate could affect vegetation in the future (Brienen et al. 2010). Besides that, the spatial structures found in plant populations are the result of actions of abiotic and biotic factors that affect the individuals of these populations, and the spatial arrangement of the parent plants and the interactions between the environment and the antecessor plants can affect the spatial structure of populations (Hutchings 1997). Considering that, in the third chapter: “Dendroecology and population structure of the monodominant stands of *Erythrina fusca* Lour. (Fabaceae)”, we analyzed the population structure using dendrochronological techniques to build a chronology of *E. fusca* in monodominant stands, estimate the age of the monodominant population, evaluate effects of climate on its growth, and investigate if these factors influence the establishment of this species. We hypothesized that the establishment and growth of *E. fusca* in the Pantanal subregion of Cáceres is negatively affected by higher flooding and higher precipitation levels, and positively affected by the occurrence of *El Niño*, since this event can reduce rainfall.

The presence of allelopathic potential is another factor that can affect the interactions between plant-plant, and plant-environment (Hart 1990, Macías et al. 2008), since the secondary metabolites produced by the dominant species may have an adverse effect on others species. Thus, in the last chapter: “Allelopathic potential of *Erythrina fusca* Lour. (Fabaceae), a monodominant species of Brazilian Pantanal” we verified if *E. fusca* presents

allelopathic potential, to infer about the relationships between species that co-occur. Therefore, we evaluated the effects of extracts from leaves, bark, roots, and seeds of *E. fusca* on germination and development of the bioindicator *Lactuca sativa* L. (lettuce). Our hypothesis was that this species has allelopathic potential, which may be inhibiting and/or retarding seed germination and development of other species.

Chapter 1

Effects of periodic flooding and edaphic factors on monodominance of *Erythrina fusca* in Paraguay River riparian forests

Abstract

In the Pantanal, stands dominated by *Erythrina fusca* occur side-by-side with other riparian forests with greater richness. Considering the flood pulse concept, a theory which holds that the annual flood pulse is the most important aspect and the most biologically productive feature of a riverine ecosystem, we hypothesized that low richness and dominance of *E. fusca* is associated with longer periods of flooding and, perhaps, some soil conditions. Therefore, this work aimed to investigate the relationship between monodominance of this species and both flood pulse and some soil characteristics. To accomplish this, we demarcated 48 plots, including 24 in monodominant stands of *E. fusca* and 24 in adjacent riparian forests. In each plot, compound soil samples were taken at a depth of 0-20cm. We calculated the average number of days each area was subjected to flooding each year as the duration of flooding. We used Redundancy Analysis and a variance partitioning to find relationships between tree species composition in the 48 plots and environmental variables. We found 730 individuals representing 46 species out of which 200 were from monodominant stands (11 species), and 530 were from riparian forests (43 species). Against our expectations, we found that the set of soil variables explained more about monodominance of the studied species than flood pulse. Therefore, the occurrence of monodominant stands of *E. fusca* in the Pantanal subregion of Cáceres seems to be strongly related to higher levels of sulfur and lower fertility of soil and less related to longer periods of flooding.

Keywords: monodominant stands, Pantanal, soil-plant interaction, tree community, wetlands

Introduction

Monodominant stands are vegetation types where more than half of the total number of trees belongs to only one species (Connell & Lowman 1989, Hart et al. 1989). It is well known that no uniform pattern of characteristics affects all monodominant formations in exactly the same way. Nonetheless, these formations do seem to share some features, such as flood, fire, and conditions and characteristics of the soil. Under such selective pressures, less tolerant species will be excluded, while the dominant species may be benefited by, for example, ectomycorrhiza, massive fruiting and other intrinsic characteristics (Hart 1990, Torti et al. 2001).

In tropical and subtropical regions, periodic flooding is often associated with low diversity and the dominance of a single species (Campbell et al. 1986, Hart 1990), essentially because flooding induces changes in plants and soil, such as changes in oxygen levels (anoxia or hypoxia) and increase in CO₂, as well as products of anaerobic decomposition. Thus, such factors would tend to select against species intolerant to frequent flooding (Joly & Crawford 1982, IRRI 1985, Lieberman et al. 1985). According to Dalmagro et al. (2016), periodic flooding affects the physiological performance of some monodominant tree species such that a decrease in the rates of soil oxidation-reduction potential and soil O₂ concentration, for example, may favor some species.

Despite the presumed influence of flooding, soil is another factor commonly associated with the monodominance of some species. Dominant species seem to be more tolerant than other species towards unfavorable soil conditions, therefore, soil characteristics seem to select for species that have greater tolerance or fewer nutritional requirements (Richards 1996). As an example, Nascimento et al. (1997) observed that the concentration of magnesium was

higher in areas dominated by *Peltogyne gracilipes* than in other nearby forests without *P. gracilipes*.

The Pantanal is a Quaternary floodplain, extending over 140,000 km², crossed by the Paraguay River and its tributaries, running through Brazil, Bolivia and Paraguay (RADAMBRASIL 1982). Flooding in the Pantanal is a periodic event, and in the north, high flooding levels almost coincide with the rainy seasons. In addition to these annual water level fluctuations, pluriannual fluctuations can occur, creating periods of prolonged droughts and floods (Nunes da Cunha & Junk 2001).

The Brazilian Pantanal is a favorable environment to study species dominance because several monodominant trees are found there, such as *Byrsonima cydoniifolia* A. Juss., *Vochysia divergens* Pohl, *Tabebuia aurea* (Silva Manso) Benth. ex S. Moore and *Erythrina fusca* Lour. (Pott et al. 2011).

The species *Erythrina fusca* Lour. (Fabaceae) is scattered throughout various regions of the world. It is the most widespread species of the genus *Erythrina*, and it can reach large dimensions in low-lying freshwater swamps, developing almost pure stands (Russo & Baguion 1997). In Brazil, it is present in different regions in riparian forests and areas subjected to periodic flooding, such as the Amazon and floodplains of the Paraguay River. It is recorded as monodominant, as an almost pure stand, in the Pantanal. These monodominant stands occur as floodable forests of the northern Paraguay River, in the Cáceres subregion, and along the banks of the Aquidauana River (Pott et al. 2011).

Stands dominated by *E. fusca* occur side-by-side with other riparian forests that have much greater species richness. This most likely results from the fact that such features as flooding and soil conditions may vary between nearby areas and that these different

conditions affect species richness. For example, a small change in altitude could change the level and duration of flooding, as well as edaphic conditions.

According to the flood pulse concept, species diversity is expected to be limited in sedentary terrestrial and aquatic taxa because they experience the full impact of physiological stress resulting from flooding (Junk et al. 1989). Therefore, it is reasonable to attribute the low diversity and monodominance of a species to flood pulse, especially if such species, in particular *E. fusca*, is tolerant to flooding.

One way to understand the different factors that may facilitate the dominance of a single species and, hence, test the monodominance of different communities, is by comparing monodominant stands to adjacent mixed forests. In the Pantanal subregion Cáceres, we observed both mixed riparian forests and areas dominated by *E. fusca*. This provided the perfect study site to investigate the relationship between dominance and both edaphic and climatic features affecting tree species, in particular the monodominance of *E. fusca* at Taiamã Ecological Station, Pantanal, Brazil.

As predicted by the flood pulse concept, we hypothesized that the low diversity and dominance of *E. fusca* is mainly associated with longer periods of flooding and, perhaps, some specific soil conditions, such as lower fertility, both factors frequently related to the occurrence of monodominant stands. Thus, the present work aimed to investigate the relationship between flooding and some soil characteristics in the context of *E. fusca* monodominance.

Methods

Study area

This study was conducted at Taiamã Ecological Station (TES) and the nearby areas of Sararé Island and the Jubran Private Reserve in the Pantanal subregion of Cáceres. In this subregion, it is possible to find a sequence of floodplains covered by grasses and other herbaceous plants, riparian forests and large monodominant stands of *Erythrina fusca* (MSEF) (Appendix 1, Appendix 2 and Appendix 3).

We analyzed areas of riparian forests (RF) and monodominant stands of *E. fusca* (MSEF). The riparian forests consist of Semideciduous Seasonal Alluvial Forests, a frequent vegetation in the Pantanal, which borders the Paraguay River (Veloso et al. 1991). This area is characterized by riparian forest formations with abundant species, including *Sapium obovatum* Klotzsch, *Laetia americana* L., *Psidium nutans* Berg, *Zygia inaequalis* (Kunth) Pitt. and *Albizia polyantha* Spreng.f.) Lewis (Ikeda-Castrillon et al. 2011).

The monodominant stands, locally known as *abobral*, consist of *Erythrina fusca*, which is a deciduous species that blooms from May to September with intense fruitification in November (Pott & Pott 1994, Lorenzi 2002). Its seeds fall into the river and are ingested by fish. Flowers are visited by birds and pollinators (Pott & Pott 1994, Parrini & Raposo 2010).

The climate in the region is seasonal Aw in the Köppen classification, with two distinct seasons, a dry season from May to September and a rainy season from October to April. In this subregion of the Pantanal, high flooding levels almost coincide with the rainy seasons (Fig. 1). The average precipitation is 1227mm, and the average annual temperatures are around 26°C, ranging from 20°C (minimum average) to 31°C (maximum average). These values were calculated from the data obtained from the Instituto Nacional de Meteorologia (INMET – National Institute of Meteorology).

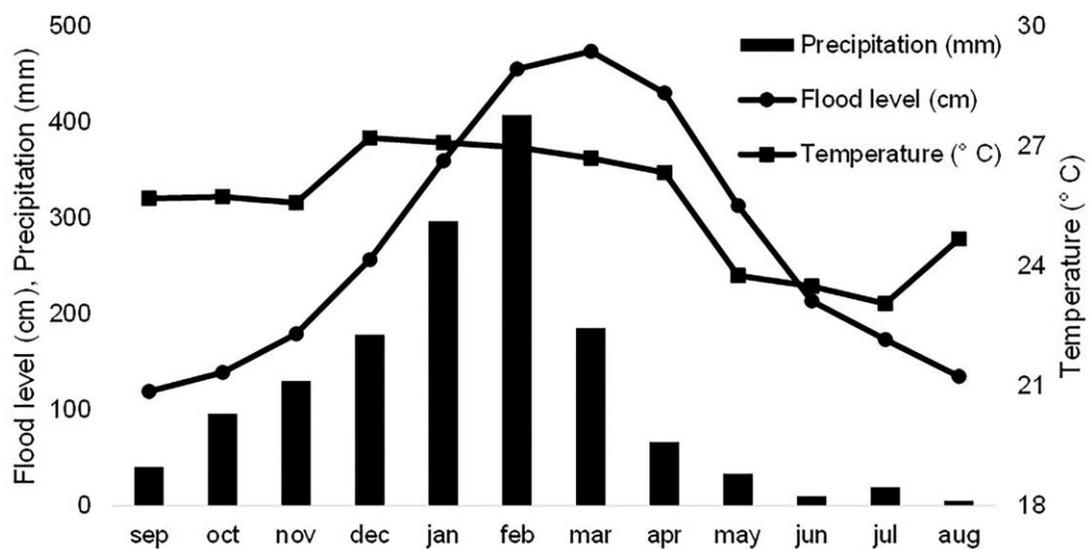


Fig. 1. Average ten-year flood levels of the Paraguay River (filled circle) with annual rainfall (filled bars) and temperature (filled square) in the northern Pantanal subregion of Cáceres, Mato Grosso, Brazil.

The predominant soil in the subregion is Eutrophic Gleysol (Fernandes et al. 2007). This kind of soil is periodically saturated. The water remains internally stagnant, or saturation occurs by lateral flow in the soil. These soils are characterized by strong gleization, as a result of the reducing environment, virtually free of dissolved oxygen in response to water saturation throughout the year, combined with oxygen demand by biological activity (Embrapa 2006).

Data collection

We carried out the sampling in October 2015 in TES and nearby areas. We selected 16 sampling areas, distributed throughout the TES, over 115 km², eight monodominant stands of *Erythrina fusca* (MSEF) and eight riparian forests (RF). In each area, we demarcated three plots 50x5 m each and a minimum distance of 20 m apart. Plots were allocated in order to better show different elevations and duration of flooding of the Paraguay River levees. MSEF were only located in the northwestern part of the study area, while RF were distributed throughout the study area. We distributed the plots between the coordinates 16°50'58.2"S 57°28'25.7"W and 16°52'57.4"S 57°30'22.2"W for MSEF and 16°50'35.9"S 57°35'07.9"W and 16°56'03.6"S 57°26'02.9"W for RF.

Trees with circumference at breast height (CBH) \geq 15 cm were included in the sampling. Bifurcated stems were included only if at least one had a circumference \geq 15 cm. Tree height was also measured with a digital distance measurer.

Samples were collected for identification. When found fertile, they were prepared with the usual herbarium techniques (Mori et al. 1989, Bridson & Forman 2004), and the vouchers were deposited in the CGMS Herbarium. For identification of plant material, we used regional checklists (Ikeda-Castrillon et al. 2011), as well as identification keys, specific

bibliography and comparison with samples in the CGMS Herbarium. The list of families was based on Angiosperm Phylogeny Group III guidelines (APG IV 2016).

Environmental parameters

For each plot, compound soil samples, with 15 subsamples, were taken at a depth of 0-20cm. The analyses were performed at the Soil Laboratory of the Universidade Federal de São Carlos - Araras (Brazil). The elements analyzed were phosphorous (resin P), aluminium (Al), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). Hydrogen potential (pH) was also measured, and organic matter (OM) was determined using the calorimetric method. Finally, based on chemical analysis, the percentage of base saturation (BS) was determined, and this value was used to define soil fertility.

To determine the duration of flooding for each plot, we calculated the level of flooding, measuring from the ground to the highest watermark left by the last inundation on each tree trunk inside the plots. We identified the darker watermark as last year's flooding. We used the values sampled for all trees to obtain an average per plot. The values of flooding level were compared with the highest level recorded at the hydrometric gauge of Cáceres. As the topography is very flat with no tributaries between the gauge and the sampled area, we considered variation in water level to be the same for both areas. We used ten years of data to calculate an average number of days each area was subjected to flooding each year, and we called it the duration of flooding (DF).

Data analysis

We analyzed the data using R software (R Development Core Team) and the R vegan package (Oksanen et al. 2016). We used a *t* test to compare density of trees. To obtain the contribution

of measured variables to tree species composition, we followed the methods proposed by Dray et al. (2012) and Legendre et al. (2012): (i) we used a Hellinger transformation (Legendre & Gallagher 2001) to reduce the effect of widespread species; (ii) we split the explanatory variables into two categories and matrices and prepared two redundancy analyses (RDA), including one for one properties of soil and species matrix and another for duration of inundation and species matrix; (iii) we applied a forward selection procedure to compile the significant soil variables (Blanchet et al. 2008); and (iv) we used a variance partitioning of the community composition matrix relative to the significant variables. This method uses the adjusted R^2 of the global model (RDA) as an additional stop criterion and reduces the risk of overparameterization (Borcard et al. 2011). To test the significance of the fractions of the variance partitioning, we applied a permutation test (999 permutations) for RDA.

Results

Tree communities

In the study area as a whole, we found 730 individuals (46 species) (Table 1). A total of 200 were in monodominant stands (11 species) and 530 in RF (43 species). The density was respectively 333 and 883 individuals/hectare. The density of trees in RF was higher than that in MSEF ($p > 0.05$).

From the 11 species found in MSEF, eight also occurred in RF, and three were exclusive to MSEF: *Sapium obovatum*, *Nectandra amazonum* and the monodominant species *Erythrina fusca*. From the 200 individuals, 153 belong to *E. fusca* (approximately 77%), followed by *Calophyllum brasiliense* with 20 individuals, and all other species had six or fewer individuals in MSEF.

Environmental parameters

In all MSEF, we observed a histosol layer, which is a low-density soil with a high proportion of organic matter (Brady & Weil 2008). The chemical analysis of soil samples indicated high fertility in RF, with BS values around 64%, while MSEF samples showed a mean value of 45% (Table 2). We found that MSEF was subjected to flooding (DF) for an average of 78 days per year compared with 44 days in RF (Table 2).

Association between community and environment

Forward selection retained four significant soil variables: percentage of base saturation (BS) which defines soil fertility, pH, sulfur and copper (Table 2). When we partitioned the variation explained by soil and duration of inundation, we found that soil explains 9.8 % ($P =$

0.001) of the variance in species composition, independent of DF, while DF explains 5.4 % ($P = 0.001$) independent of soil and 16.4 % variance was shared in common by

Table 1. Families, species and number of individuals in monodominant stands of *Erythrina fusca* (MSEF) and riparian forests (RF) at Taiamã Ecological Station (TES) and nearby areas, Pantanal wetland, Brazil.

Family	Scientific name	MSEF	RF
Anacardiaceae	<i>Mangifera indica</i> L.	0	4
	<i>Spondias mombin</i> L.	0	8
Arecaceae	<i>Attalea phalerata</i> Mart. ex Spreng.	0	1
Capparaceae	<i>Crateva tapia</i> L.	0	27
Clusiaceae	<i>Calophyllum brasiliense</i> Cambess.	20	17
Celastraceae	<i>Salacia elliptica</i> (Mart. ex Schult.) G.Don	0	1
Erythroxylaceae	<i>Erythroxylum anguifugum</i> Mart.	0	4
Euphorbiaceae	<i>Alchornea discolor</i> Poepp.	4	34
Fabaceae	<i>Albizia inundata</i> (Mart.) Barneby & J.W.Grimes	0	1
	<i>Cassia grandis</i> L.f.	0	19
	<i>Erythrina fusca</i> Lour.	153	0
	<i>Inga vera</i> Willd.	0	64
	<i>Pterocarpus santalinoides</i> L'Hér. ex DC.	5	8
Lauraceae	<i>Zygia latifolia</i> (L.) Fawc. & Rendle	0	43
	<i>Nectandra amazonum</i> Ness	3	0
	<i>Nectandra pulverulenta</i> Ness	0	1
	<i>Ocotea diospyrifolia</i> (Meisn.) Mez	0	5
	<i>Ocotea puberula</i> (Rich.) Nees	2	4
Malpighiaceae	<i>Bunchosia paraguariensis</i> Nied.	0	1
Malvaceae	<i>Ceiba</i> cf. <i>samauma</i> (Mart.) K.Schum.	0	4
	<i>Guazuma ulmifolia</i> Lam.	0	1
	<i>Sterculia apetala</i> (Jacq.) H.Karst.	0	5
Meliaceae	<i>Trichilia catigua</i> A. Juss.	0	44
Moraceae	<i>Brosimum lactescens</i> (S.Moore) C.C.Berg	0	19
	<i>Ficus</i> cf. <i>carautana</i> L.J.Neves & Emygdio	0	3
	<i>Ficus pertusa</i> L.f.	1	10
	<i>Sorocea sprucei</i> (Baill.) J.F.Macbr.	0	12
Myrtaceae	<i>Eugenia</i> cf. <i>polystachya</i> Rich.	0	2

	<i>Eugenia</i> sp1	0	4
	<i>Eugenia</i> sp2	0	1
	<i>Eugenia</i> sp3	0	3
	<i>Myrcia splendens</i> (Sw.) DC.	0	5
	<i>Psidium guajava</i> L.	0	2
Nyctaginaceae	<i>Neea hermaphrodita</i> S.Moore	0	31
Polygonaceae	<i>Coccoloba cujabensis</i> Wedd.	0	20
	<i>Symmeria paniculata</i> Benth.	0	1
	<i>Triplaris americana</i> L.	0	39
Rubiaceae	<i>Genipa americana</i> L.	0	21
	<i>Palicourea crocea</i> (Sw.). Roem. & Schult.	0	1
Salicaceae	<i>Banara arguta</i> Briq.	6	20
	<i>Casearia aculeata</i> Jacq.	1	14
	<i>Laetia americana</i> L.	0	5
Sapindaceae	<i>Sapium obovatum</i> Klotzsch ex Müll.Arg.	1	0
Sapotaceae	<i>Pouteria glomerata</i> (Miq.) Radlk.	0	1
Urticaceae	<i>Cecropia pachystachya</i> Trécul	4	18
Vochysiaceae	<i>Vochysia divergens</i> Pohl	0	2
Total		200	530

Table 2. Mean, minimum and maximum values of environmental parameters, including duration of flooding (DF) and chemical attributes of soil (P, OM, pH, Al, BS, S, B, Cu, Fe, Mn and Zn) at a depth of 0-20cm in monodominant stands of *Erythrina fusca* (MSEF) and riparian forests (RF) within the Taiamã Ecological Station (TES) and nearby areas, Pantanal.

We present values of adjusted R^2 ($R^2_{adj^a}$) and significance (p) only for soil variables significant to explain community variation.

	MSEF		RF		$R^2_{adj^a}$	p
	Mean	Min/Max	Mean	Min/Max		
DF (days)	78.00	42.00/117.00	44.00	34.00/58.00	-	-
P (mg.dm ⁻³)	13.40	6.00/22.00	12.40	7.00/29.00	-	-
OM (g. dm ⁻³)	48.80	27.00/80.00	29.10	15.00/48.00	-	-
pH (CaCl ₂)	4.10	3.70/4.70	4.00	3.50/4.90	0.24	0.002
Al (mmolc. dm ⁻³)	24.50	3.00/52.00	17.30	1.50/39.50	-	-
BS (%)	45.30	22.00/69.00	63.80	50.00/80.00	0.20	0.004
S (mg. dm ⁻³)	63.10	31.00/113.00	36.70	21.00/65.00	0.16	0.002
B (mg. dm ⁻³)	0.52	0.17/1.05	0.56	0.20/0.76	-	-
Cu (mg. dm ⁻³)	3.00	0.10/5.90	2.80	0.50/4.70	0.27	0.026
Fe (mg. dm ⁻³)	352.50	203.00/486.00	289.60	106.00/511.00	-	-
Mn (mg. dm ⁻³)	13.80	3.20/28.50	7.60	1.30/18.40	-	-
Zn (mg. dm ⁻³)	1.50	0.80/2.70	1.40	0.40/2.80	-	-

DF and soil variables (Fig. 2). All factors together explain 31.6 % of the variance in species composition, while 68.4 % of variation remains unexplained. All RDAs were significant at $p < 0.001$.

We could see a clear separation between the plots of MSEF and RF in both RDA, soil variables (Fig. 3a) and DF (Fig. 3b). For areas of *E. fusca* monodominance, we noticed that MSEF was negatively correlated with soil fertility and positively correlated with S and DF. Other species in the study area appear to be correlated with higher BS and other unidentified factors. These species did not occur in soil with higher concentration of S nor were they representative of more days of flooding. Cu and pH were also significant and seem to better explain the distribution of plots within each of the two groups.

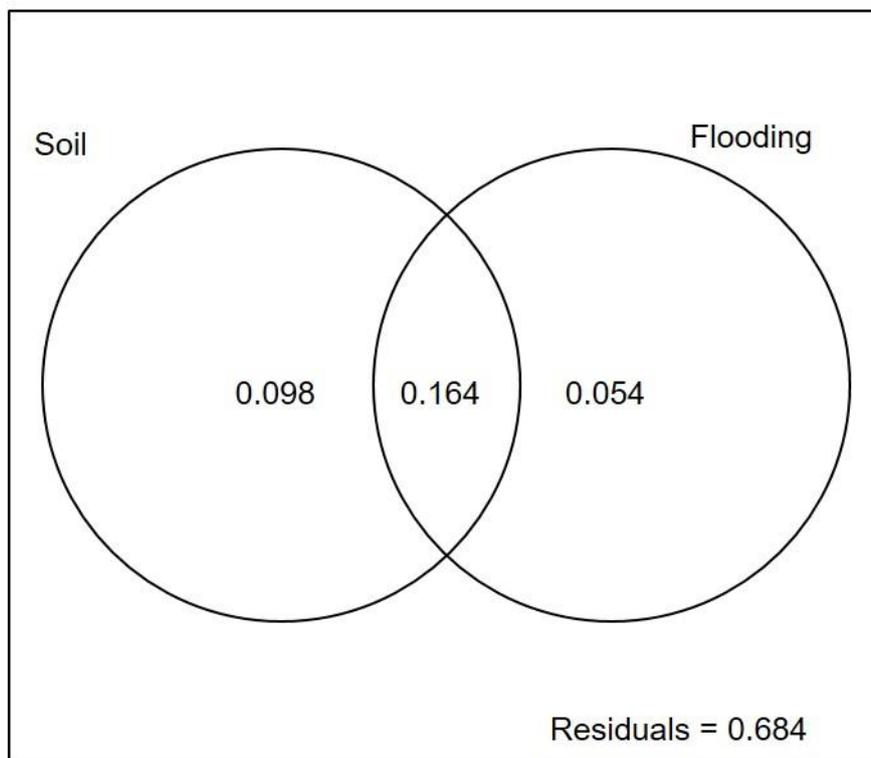


Fig. 2. Venn diagram of the variation partitioning of soil parameters (Soil) and flooding. Intersections between circles represent the variations shared (common contribution) by both groups of environmental parameters.

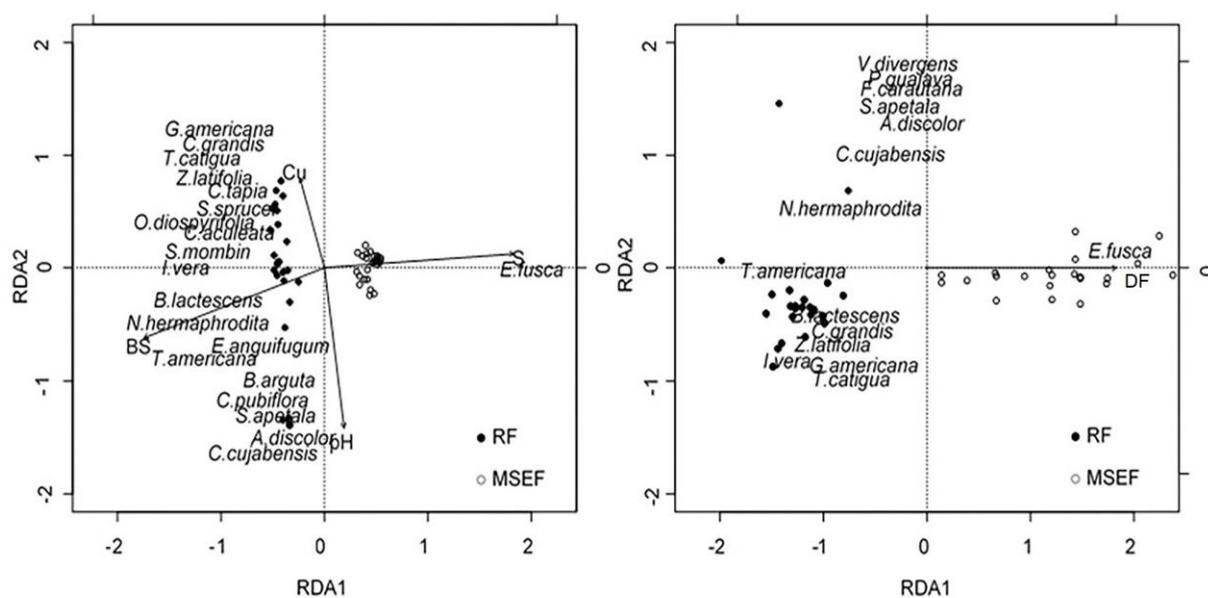


Fig. 3. Redundancy analysis showing the most significant species for each analysis with soil variables (a) and duration of flooding (b) in areas of monodominant stands of *Erythrina fusca* (MSEF: ○) and nearby riparian forests (RF: ●) at Taiamã Ecological Station, Pantanal subregion of Cáceres, Mato Grosso, Brazil. Copper (Cu), base saturation determining the fertility of soil (BS), pH, sulfur (S) and duration of flooding (DF).

Discussion

Compared with monodominant stands of *E. fusca* (MSEF), the total density of trees was higher in riparian forests (RF). RF was characterized by many small individuals, while MSEF had few large individuals, but enjoyed a very open canopy. Despite the higher density of trees in RF, we observed that the herbaceous strata were more open, with evidence of regenerating tree species, while MSEF showed closer herbaceous strata, but almost entirely dominated by the grasses *Hymenachne donacifolia* and *H. amplexicaulis*, with little regeneration of tree species (Appendix 2 and Appendix 3). The recruitment in MSEF was low, as we observed only a few young individuals of *E. fusca* usually associated with floating histosol (Appendix 6).

Low richness of tree species is a common pattern in floodable monodominant vegetation areas in different geographic regions (Hart et al. 1989, Marimon et al. 2001), a phenomenon also reported for the Brazilian Pantanal by several authors (Nascimento & Nunes da Cunha 1989, Arieira & Nunes da Cunha 2006, Soares & Oliveira 2009, Amador et al. 2012).

Considering the flood pulse concept, we expected that the differences between MSEF and RF would be mainly explained by seasonal flood patterns, however, we found that the set of soil variables explained more about monodominance of the studied species than DF. More specifically, nearby areas appear to have large differences in soil composition, and, as we have seen, MSEF are strongly associated with low soil fertility, an unfavorable condition which is, in turn, strongly associated with monodominant formations since only species tolerant to this stressor can grow and develop (Grime 1977). We did not find *E. fusca* in RF, supporting the study of Ikeda-Castrillon et al. (2011) who also found the notable absence of this species on others islands in Paraguay river near our study site. The absence of *E. fusca* in

these mixed riparian forests indicates that it is not a good competitor relative to other tree species in nearby RF. However, *E. fusca* is a pioneer species, and it seems to compete well with grasses abundant in MSEF. Compared with other tree species, it is more tolerant to low percentages of BS. As such, it has been successful in dominating areas of lower soil fertility.

When compared with RF, MSEF showed higher sulfur (S) values. In flooded soils with a normally high presence of organic matter, oxygen cannot supply the decomposition. Therefore, bacteria use sulfur to decompose organic matter (IRRI 1985), forming chemical compounds with a strong odor, which, in fact, we detected in these areas.

Increasing temperature also influences the reduction of sulfur at the surface (IRRI 1985). In the study area, the average air temperature during the period of data collection was close to 31°C, but temperatures between 35 and 40°C are quite common. Therefore, it is likely that the combination of flood conditions, high temperature and the presence of organic matter favors the process of bacterial decomposition of sulfur and the greater resultant release of this compound in these soils. In addition, species of the genus *Erythrina* are very rich in alkaloids, which are present in greater quantity in the seeds, and according to Folkers et al. (1944), some alkaloids like erysothiovine and erysothiopine contain sulfur in their composition, suggesting that this plant may accumulate and use sulfur for some still unidentified purpose.

Copper and pH were additional soil parameters that influence the distribution of species among plots. These two soil variables seem to be more related to axis 2, having less influence on the separation between MSEF and RF and more within-group influence, mainly involving RF distribution. Since flooding and high organic matter concentration decrease available copper concentrations, typically low in wetlands (Ponnamperuma 1985). The soil pH is strongly correlated with an availability of inorganic nutrients for plants. For example, in

alkaline soils, some cations, such as copper, are precipitated and become unavailable to plants (Raven et al. 2014). Thus, small variations in both variables seem to strongly influence plant community distribution, especially in RF.

Despite its lower explanation, DF did influence the plant community. In MSEF we observed a more superficial water table than that found in RF, suggesting that the soil would remain essentially waterlogged, even in the dry season, but certainly reaching high levels of flooding during the wet season. It is well known that the frequency, intensity and duration of flooding negatively affects species richness (Hart 1990, Junk 1996, Damasceno-Júnior et al. 2005, Parolin & Wittmann 2010). Flooding can induce changes in the environment and the community, such as anoxia or hypoxia and increase CO₂, which can exclude species intolerant to these unfavorable conditions, thereby reducing richness (Joly & Crawford 1982, IRRI 1985, Lieberman et al. 1985). Jirka et al. (2007) noted that differences in soil hydrology influence the distribution of plant communities. It is known that *E. fusca* is a species that occurs along the riverbank, often in areas subject to flooding (Pott & Pott 1994). Thus, it is likely that long flooding duration coupled with diminished development and persistence of other species has allowed *E. fusca* to flourish in these areas.

In addition, we found that individuals of *E. fusca* have a different pattern of prop roots such that trees in areas of prolonged flooding had more pronounced and higher prop roots (Appendix 7), whereas individuals in short-term flooding areas did not exhibit such pattern. This could be attributed to phenotypic plasticity of the species as an adaptation to long-term flooding, allowing a better fixation of plants and increase of gas exchange rates, along with avoidance of stem submergence for prolonged periods of flooding.

In the ESEC Taiamã, flooded grasslands also occur. Similarly to MSEF, they are dominated by herbaceous species such as *Hymenachne* spp., which are quite common in these

parts of the Pantanal. These flooded grasslands seem to have environmental characteristics similar to MSEF, especially those related to flooding, and since the altitudes are similar, the flood tables are superficial with frequent soil saturation. These flooded grasslands are dominated by herbaceous species, but a few trees are also present, all belonging to *E. fusca*, while in MSEF, apart from herbaceous species and *E. fusca* trees, we found ten other tree species (Table 1). Therefore, these areas of MSEF and flooded grasslands seem to differ only in relation to arboreal vegetation, and this fact suggests that *E. fusca* is a species more tolerant to unfavorable conditions, as long flooding periods. *Erythrina fusca* with its abundant organic matter and complex root system facilitates the retention of soil and organic matter which encourages the establishment of other trees in MSEF.

The expansion of *E. fusca* on these grassland areas maybe are occurring as in an encroachment process, where there is a gradual growth in the cover, biomass and density of individuals of a native woody species or shrubby plants in grasslands (Van Auken 2000, Moleele et al. 2002, Van Auken 2009). The process of expansion of woody species on flooded grasslands has been described for species of Fabaceae, for example, *Senna reticulata* (Willd.) H.S.Irwin & Barneby (Parolin et al. 2015), and for Pantanal woody species such as *Combretum laxum* Jacq. (Barbosa da Silva et al. 2016).

In conclusion, we discovered a strong relationship of the dominance of *Erythrina fusca* with soil conditions and flood pulse. However, while duration of flooding is an important factor contributing to monodominance, we found, contrary to our original hypothesis, that edaphic characteristics explain much more about the structure of this community. Therefore, the occurrence of monodominant stands of *E. fusca* at Taiamã Ecological Station in the Pantanal subregion of Cáceres seems to be negatively correlated with fertility and positively correlated with higher levels of sulfur in the soil and less affected by duration of flooding.

Chapter 2

Effects of environmental factors in early development stages of *Erythrina fusca* Lour.

Abstract

The species *Erythrina fusca* occurs naturally in the Pantanal, where it is subject to periodic flooding and sporadic fires. Understanding the germination patterns and changes in the secondary metabolism of seeds submitted to these events allows us to infer about effects on initial development and relationships between plants and their environment. Therefore, we hypothesized that flooding and fire stimulate germination and seedling formation of *E. fusca* and affect the composition/concentration of secondary metabolites. We aimed to identify differences in the secondary metabolism between seeds and seedlings of *E. fusca*, and investigate whether flooding and fire affect germination and seedling formation and generate changes in secondary metabolism in these early stages of development. We subjected seeds to simulated flooding (15, 30, 60, and 120 days) and fire (on soil surface and buried at 3 and 5 cm) and evaluated their effects on germination, seedling formation, and secondary metabolism in these stages of development. We observed that flooding leads to increased germination and seedling formation, while fire does not affect these parameters when seeds are buried, but was lethal to seeds on the soil surface. We observed that seeds and seedlings have quite distinct metabolite composition, in seeds we observed reserve metabolites, while seedlings presented more compounds that seem to be defense. Fire and flooding events caused very similar changes in the secondary metabolism of seeds and seedlings, leading to an increase in defense metabolites against pathogens and insects. Therefore, submission to these events causes changes in the early stages of development of *E. fusca*.

Keywords: fire, flooding, secondary metabolites, seed, seedling, wetland.

Introduction

Seed germination and initial phases of development are crucial for the establishment of new healthy plants (Chacón et al. 2013). Seed germination is measured by the imbibition and protrusion of radicle (embryonic axis) through the seed coat, followed by the establishment of a new seedling (Weitbrecht et al. 2011). The energy source for these processes and development are the reserves accumulated during embryogenesis and seed maturation, when a flow of nutrients and secondary metabolites occurs from the mother plant to the seed which is stored in the cotyledons and endosperm (Fait et al. 2006).

The composition/concentration of secondary metabolites may vary between different tissues or development phases within the same plant, depending on its needs (Suzuki et al. 2008). For example, these initial phases are the most vulnerable stages for a plant, and to complete their establishment, seedlings need an efficient defense against stressors and environmental hazards. The synthesis and accumulation of secondary metabolites could have been received from the mother plant or synthesized *de novo* during development, and represent a significant advantage in new seedlings survival, as they act in the competition with other plants species and defense against pathogens (Chacón et al. 2013, Raven et al. 2014).

During maturation, in addition to accumulating reserves and secondary metabolites, seeds lose water to the environment (90% or more), decreasing metabolic rates, which in some species can cause a state of seed dormancy (Raven et al. 2014). Dormancy means that some viable seeds are unable to complete germination even under favorable conditions, which can be due the water impermeable seed coat, an important kind of physical dormancy frequently found in leguminous species (Bewley 1997, Baskin & Baskin 2001, Finch-Savage & Leubner-Metzger 2006). Dormancy can be a beneficial process for species, as seeds do not

germinate as soon as they reach the substrate, allowing them to disperse farther from the mother plant or by causing a greater distribution of germination over time in the populations (Bewley 1997).

In species with integument dormancy, some environmental events such as floods or fire can lead to a scarification of the coat, allowing water to enter into the seed, imbibition to occur, metabolism to resume, which allow the germination process to begin (Baskin & Baskin 2001). Therefore, these events can initiate overcoming dormancy, or may be stress factors of seed germination and development for species subjected to these stressors.

The species *Erythrina fusca* Lour., for example, naturally occurs in riparian forests (Russo & Baguion 1997), where it is exposed to flood pulses and sporadically to fire events. In the northern Brazilian Pantanal, *E. fusca* can grow in monodominant stands, where this species represents approximately 77% of all the arboreal individuals (unpublished data –see Chapter 1).

Seed dormancy in the genus *Erythrina* is a known pattern, due to the impermeability of the tegument (Silva et al. 2006, Koszo et al. 2007, Matheus et al. 2010, Santos et al. 2013). Additionally, this genus is rich in secondary metabolites, such as erythrina alkaloids (tetracyclic spiro amine alkaloids), terpenoids, flavonoids (especially pterocarpan), coumarins, and saponins (Tanaka et al. 2002, Yenesew et al. 2003, Juma & Majinda 2004, Dao et al. 2009, Pérez et al. 2015, Guaratini et al. 2017).

Understanding the germination, development, and secondary metabolite patterns of plants subject to specific environmental conditions, such as flooding and fire, allows us to infer about the establishment and relationships between plants and their environments. Considering that *E. fusca* is subject to a flood pulse and sporadic fire events in the Pantanal, our hypotheses were: 1) these events stimulate seeds to overcome dormancy, increasing or

accelerating germination and formation of seedlings; and 2) these events generate changes in the composition/concentration of secondary metabolites in the early stages of development. Thus, our objectives were to identify differences of the secondary metabolism between seeds and seedlings of *E. fusca* to investigate whether fire and flooding events affected germination (including overcoming dormancy) and seedling formation of *E. fusca*, and if these events generate changes in the composition of secondary metabolites in these two important phases (seeds and seedlings).

Materials and Methods

Study area and seed processing

We collected the seeds in November 2014 from monodominant stands of *Erythrina fusca*, in the Taiamã Ecological Station, Pantanal subregion of Cáceres, Mato Grosso, Brazil. In this subregion of the Pantanal, the climate is seasonal Aw in the Köppen classification, with a dry season (May to September) and a rainy season (October to April). In this subregion of the Pantanal, high flooding levels almost coincide with the rainy seasons, and the species *E. fusca* has intense fruiting in this same period (Pott & Pott 1994, Lorenzi 2002). The average precipitation is 1227 mm, and the average annual temperatures are around 26°C, with temperatures between 35 and 40°C being quite common. These values were calculated from the data obtained from the Instituto Nacional de Meteorologia (INMET – National Institute of Meteorology).

A voucher was deposited in the CGMS Herbarium under registration number CGMS 40967. We manually cleaned the seed samples, discarded broken and predated seeds, until we obtained a uniform sample.

Flood and fire-simulated experiments

To simulate the effects of flooding on *E. fusca* seed germination, we maintained seeds in a plastic container filled with distilled water (which was changed every 7 days to avoid seed rot) and 4 aquarium air pumps (to simulate the water movement in a river). We maintained the water temperature at 25(±1)°C, because the average annual temperature observed for Pantanal subregion of Cáceres is around 26°C. To simulate seed responses in short and long flood durations, we used 4 different periods of flooding: 15, 30, 60 and 120 days. The

maximum period of 120 days was chosen because it corresponds to the maximum average time that the monodominant stands of *E. fusca* remain flooded (unpublished data - see Chapter 1).

To investigate effects of the fire on germination of *E. fusca* seeds we used a method adapted from Menezes & Rossi (2011). We used a metallic tray (aluminum) filled with soil and covered with litter. We determined the amount of litter used (100 g) from an estimate of an area of 40x20cm² (useful tray area). Both soil and litter were oven-sterilized (120°C for 1 hour). To allow for fast burning, as expected in the natural environment, we first heated the litter (60°C for 20 minutes) and used 50 ml of alcohol gel 70% deposited just above the litter. We performed three treatments (for each one we positioned seeds in the center of the tray, 0.5 cm from each other): seeds arranged on the surface of the soil (just below the litter), seeds arranged at 3cm deep, and seeds arranged at 5cm deep. We maintained the metallic tray at room temperature, and used a spit thermometer at each depth (surface, 3cm, and 5cm) to record the temperature every 10 seconds until stabilization and return to the initial temperature.

For both experiments (flooding and fire), we used the same control that consisted of undamaged seeds not submitted to any treatment. Using the oven method (105 ± 3°C for 24 hours), we calculated the moisture content of the control seeds and seeds from each of the treatments, using two subsamples of 12 seeds (ISTA 2017).

Immediately after performing the experiments, we left all treatments and control to germinate. We distributed the seeds on a paper towel moistened with 2.5 times its weight in water. We used 4 replicates for each treatment, with 20 seeds. The replicates were kept in biological oxygen demand (BOD), at 35 °C, which is a normal temperature for the period of fruiting of the species in the study area and was previously confirmed as the best temperature

for germination and seedling formation for *E. fusca*. We performed the evaluations every 24 hours for 60 days, which consisted of counting germinated seeds (seeds with 2 mm radicular protrusion) and normal seedlings (which must have had all the basic structure). We calculated the percentage of germination (G), germination speed index (GSI), the percentage of normal seedlings (NS), and the normal seedling speed index (NSSI) (Maguire 1962, Labouriau & Valadares 1976).

We used completely randomized experimental design and to submit the results to one-way analysis of variance followed by the Tukey 5% test, using the agricolae package in R software (Mendiburu 2016).

Preparation of samples for HPLC-DAD-MS/MS analyses

We used seven seeds and seven seedlings for each of the treatments. The seeds were collected immediately after experiments were executed (flooding and fire), while seedlings were obtained during the evaluation days. Both were kept in identified paper envelopes, immediately dried in an oven at 60 ° C for five days (pre-determined time for complete stabilization of water loss) and then maintained in a freezer at -20 ° C.

Seeds and seedlings were homogenized separately in an A11 basic Analytical mill (IKA). We prepared the extraction with 50 mg of each material with methanol:water (7:3 v/v and 1% of acetic acid) and placed them for 15 minutes in an ultrasonic bath. Then, we centrifuged the extracts at 3000 rcf for 10 minutes, and 1 ml of supernatant was filtered using millex PTFE 0.22 µ.

We analyzed the extracts promptly after we prepare them using a UFLC LC-20AD coupled to diode array detector (Shimadzu) and ESI-qTOF microTOF-Q III (Bruker Daltonics) mass spectrometer. We monitored the UV wavelength between 240-800nm and

operated the mass spectrometer in positive and negative ion modes. We used a Kinetex C-18 (2.6 μ , 150x2.2 mm, Phenomenex) chromatographic column protected by a pre-column packed with the same material. We used a ultrapure water mobile phase (solvent A) and acetonitrile (solvent B) both with 1% acetic acid, and the gradient elution profile used was: 0-2 min – 3% of B; 2-25 min- 3-25% of B; 25-40 min- 25-80% of B; 40-43 min - 80% of B followed by washing and reconditioning of the column (5 minutes). We injected a volume of 1 μ l, and the flow rate was 0.3 ml/min. We randomized the samples and injected each in duplicate. To use as a quality control we prepared a pool, which consisted of 50 μ l of each sample and injected this every eight samples.

We obtained the MS/MS data from the pool sample. We based the compound identification on the mass spectrometry (accurate mass and ion fragmentation) and UV data and compared the information with the literature. We determined each molecular formula based on mass errors within ± 5 ppm and mSigma below 30.

We selected the positive ion mode data for statistical analyses due to the highest number of peaks observed. For data processing, we used DataAnalysis 4.2 (Bruker Daltonics), and we aligned data using Metalign software (Lommen 2009) and reduced the entrance with MSclust (Tikunov et al. 2012), which provided 84 metabolites from the reassembled signals. We compared replicates and pools to evaluate the reproducibility and stability of compounds.

Statistical

We prepared matrices containing the interest data to better observe differences between groups. First, we used all samples only to observe grouping between seeds and seedlings. Second, considering our objective to observe the effects of stress factors on seeds and

seedlings, we prepared four analysis: 1) seeds subjected to flood treatments compared with controls; 2) seedlings subjected to flood treatments compared with controls; 3) seeds subjected to fire treatments compared with controls; 4) seedlings subjected to fire treatments compared with controls.

For statistical analysis, we used Metaboanalyst 3.0 platform (Xia et al. 2015) and ran a Principal Component Analysis (PCA) with data log transformation and normalization by median. To obtain the heatmap we performed Pearson distance, complete clustering algorithm, and random forest analyses ($p \leq 0.001$) to graphically represent the 30 most representative compounds. In addition, we used a t-test ($p \leq 0.05$) to statistically determine the most important compounds for separation of each group.

Results

Flooding and fire-simulated experiments

The germination of *Erythrina fusca* seeds was slow, and even in the control the percentage of germination was scarce (Table 1 and Table 2).

In flooding experiments, we observed an increase in the moisture content of treatments when compared with control. We also observed an increase in the percentage of seed germination with the increase of period in which seeds were subjected to submersion in water, with the highest percentage of germination at 120 days. Despite the increase in germination percentage, we did not observe an increase in germination speed index. Seedlings followed the same pattern; we found an increase in the percentage of normal seedlings and the normal seedling speed index in 120 days.

For the 3 treatments performed under fire, we observed differences in the temperatures reached: the seeds remaining on the soil surface were subjected to a maximum temperature of 86.8 °C, at 3 cm the maximum temperature reached was 45 °C, and at 5 cm was 30.7 °C. Also, the duration of heating until the return to the initial temperature was different, on the surface it was close to 60 minutes, while for the treatments of 3 and 5 cm it took 75 and 125 minutes, respectively. Therefore, we observed a decrease in the moisture content in seeds submitted to soil surface treatment, and no significant differences in the 3 and 5 cm depth treatments. We did not observe differences between the control and the treatments of 3 and 5 cm for any of the analyzed parameters, however, for seeds submitted to direct contact with fire (soil surface) there was no germination (Table 2).

Table 1. Results of moisture content in control and treatments (MC), and average results for percentage of germination (G), germination speed index (GSI), percentage of normal seedlings (NS) and normal seedling speed index (NSSI) for *Erythrina fusca* seeds submitted to different periods of flooding: 15, 30, 60, and 120 days.

	MC(%)	G(%)	GSI	NS(%)	NSSI
Control	7.96	30.00c	2.22ab	27.00b	1.26b
15 days	8.66	31.25c	1.13b	21.25b	0.72b
30 days	12.24	38.75bc	1.47b	28.75b	0.91b
60 days	9.66	58.75ab	1.76ab	42.50b	1.14b
120 days	9.38	76.25a	3.10a	68.75a	2.18a
CV(%)		44.53	48.56	52.81	49.78

Table 2. Results of moisture content in control and treatments (MC), average results of percentage of germination (G), germination speed index (GSI), percentage of normal seedlings (NS) and normal seedling speed index (NSSI) for *Erythrina fusca* seeds submitted to fire at the surface of the soil and at 3 and 5cm depth.

	MC(%)	G(%)	GSI	NS(%)	NSSI
Control	7.96	30.00a	2.22a	27,00a	1.26a
Surface	3.63	0.00b	0.00b	0.00b	0.00b
3 cm	7.76	31.25a	2.58a	30.00a	1.25a
5 cm	8.71	33.75a	1.99ab	30.00a	1.09a
CV(%)		65.04	79.16	67.87	65.63

Metabolomics analyses

Among the 84 metabolites from the reassembled signals of the positive ion mode data, we characterized by HPLC-DAD-MS/MS 55 (Table 3 and Fig. 1), which were essential to our analysis, as they were representative of all samples or they showed significant differences between the analyzed groups. We observed four main compound classes: erythrinian alkaloids; triterpene saponins; flavonoids (flavone, isoflavone, flavonol and pterocarpan); and aminoacids derivatives. We observed that in general chromatographic profiles presented similar distribution of peaks between the samples, differing only in the intensity of some compounds (Fig. 2).

We detected a notable difference between the general chromatographic profiles of *E. fusca* seeds (SD) and seedlings (SL) (Fig. 3 and 4), showing that the profile of metabolites in the species varies considerably before and after germination. Both stages presented compounds of similar classes, although differed in the degree of oxidation, polarity, glycosylation, among others.

In seeds, we observed the outstanding presence of erythrinian alkaloid with mid polarity such as the erythraline (**36**) and acetyl-*O*-hexosyl erysopine (**18**), and the reserve metabolite di-*O*-hexoside (**2**). The compounds found in seeds also occurred in seedlings, but with less intensity. For alkaloids we observed two different patterns between seeds and seedlings, some alkaloids as: **5**; **6**; **7**; **11**; **14**; **16**; **20**; **29**; **33**; **35**; **36** and **37** occurred in seeds and were transported to seedlings, while the alkaloids **3**; **9**; **13**; **15**; **17**; **19**; **24**; **25**; **26**; **27**; **28** and **38** were synthesized *de novo* from other metabolites that were present in seeds, which occurred exclusively in seedlings. For saponins we observed a similar pattern, where the compounds **47** and **49** were produced both in seeds and seedlings and showed a greater

Table 3. Compounds identified in seeds and seedlings of *Erythrina fusca*

Peak	RT (min)	Compound	Class	UV (nm)	[M+H] ⁺ (m/z)	Molecular Formula	MS/MS (m/z) (positive ion mode)
1	1.1	UNKNOWN	---	265	259.0947	C ₄₆ H ₄₂ N ₂ O ₁₁	325, 259, 163
2	1.1	di-O-hexoside	hexose	264	343.1245	C ₁₂ H ₂₂ O ₁₁	---
3	1.2	O-hexosyl-erysopitine	erythrinian alkaloid		466.2072	C ₂₃ H ₃₁ N ₉ O ₉	304, 286, 272
4	1.6	UNKNOWN	---	264	322.1065	C ₁₉ H ₁₅ N ₄ O ₄	187, 176, 175, 158
5	2.0	O-hexosyl-erysopitine	erythrinian alkaloid	269	466.2065	C ₂₃ H ₃₁ N ₉ O ₉	304, 286, 272, 254
6	2.3	erythrinian alkaloid	erythrinian alkaloid	275	224.128	C ₁₂ H ₁₇ N ₃ O ₃	---
7	3.9	O-hexosyl-erysopine	erythrinian alkaloid	282	448.1961	C ₂₃ H ₂₉ N ₈ O ₈	254, 237, 219, 208
8	4.8	UNKNOWN	---	277	188.0693	C ₁₁ H ₉ N ₂ O ₂	---
9	5.2	di-O-hexosyl-erysopine	erythrinian alkaloid	282	610.2495	C ₂₉ H ₃₉ N ₁₃ O ₁₃	254, 237
10	5.4	N-methyl-tryptophan	aminoacid derivative	279	219.1116	C ₁₂ H ₁₄ N ₂ O ₂	188, 170, 146, 143
11	6.8	O-hexosyl-erysodine	erythrinian alkaloid	287	462.2091	C ₂₄ H ₃₁ N ₈ O ₈	268, 253, 236, 219
12	7.4	hypaphorine	aminoacid derivative	283	247.1417	C ₁₄ H ₁₈ N ₂ O ₂	188, 170, 146
13	8.4	erythrinian alkaloid	erythrinian alkaloid	285	332.1477	C ₁₈ H ₂₁ N ₅ O ₅	314, 300, 282, 264, 254, 239, 213
14	8.8	O-hexosyl-erysopine derivative	erythrinian alkaloid	284	534.1927	C ₂₆ H ₃₁ N ₁₁ O ₁₁	254, 237, 219, 208, 191
15	9.8	erythrinian alkaloid	erythrinian alkaloid	285	332.1478	C ₁₈ H ₂₁ N ₅ O ₅	214, 257, 240, 228, 197, 179
16	10.1	Erythratine	erythrinian alkaloid	287	316.1521	C ₁₈ H ₂₁ N ₄ O ₄	298, 284, 266, 255, 237, 226, 214, 199, 185
17	10.5	erythrinian alkaloid	erythrinian alkaloid	285	332.1467	C ₁₈ H ₂₁ N ₅ O ₅	314, 282, 264, 254, 240, 213
18	10.6	acetyl-O-hexosyl-erysopine	erythrinian alkaloid	282	490.2065	C ₂₅ H ₃₁ N ₉ O ₉	254, 237, 219
19	10.8	erythrinian alkaloid	erythrinian alkaloid	285	348.1423	C ₁₈ H ₂₁ N ₆ O ₆	314, 300, 280, 256, 242, 226, 214, 211, 190, 174
20	10.9	erythratidine	erythrinian alkaloid	284	332.1834	C ₁₉ H ₂₅ N ₄ O ₄	314, 300, 282, 267, 253, 230, 214, 201, 175, 165
21	11.0	7,4'-di-O-hexosyl-daidzein	isoflavone	335sh	579.1695	C ₂₇ H ₃₀ O ₁₄	417, 297, 255
22	11.1	UNKNOWN	---	286	724.2386	C ₃₃ H ₄₁ N ₁₇ O ₁₇	---
23	11.2	UNKNOWN	---	284	326.1179	---	---
24	11.2	erysrovine/erysodine	erythrinian alkaloid	284	300.1575	C ₁₈ H ₂₁ N ₃ O ₃	268, 253, 238, 224, 219, 210, 191, 179
25	11.2	erythrinine/oxo-erythrinine	erythrinian alkaloid	285	314.1379	C ₁₈ H ₁₉ N ₄ O ₄	282, 264, 256, 226, 213, 198, 180, 149
26	11.3	erythrinian alkaloid	erythrinian alkaloid	287	330.1318	C ₁₈ H ₁₉ N ₅ O ₅	284, 255, 240, 226, 214
27	12.0	UNKNOWN	---	285	318.1228	---	264, 256, 238, 213, 284, 268, 256, 234, 207, 179
28	12.6	11-Hydroxyerythravine	erythrinian alkaloid	285	316.1523	C ₁₈ H ₂₁ N ₄ O ₄	
29	12.7	erythrinian alkaloid	erythrinian alkaloid	287	476.1892	C ₂₄ H ₂₉ N ₉ O ₉	264, 234, 207, 457, 439, 421, 391, 379, 355, 337, 325, 295
30	14.0	vicenin 2	flavone	280/330	595.1645	C ₂₇ H ₃₀ O ₁₅	

31	14.6	daidzin derivative	isoflavone		665.1724	C30H32O17	417, 255
32	15.1	Daidzin	isoflavone erythrinian	249/284	417.1163	C21H20O9	---
33	15.2	erythrinian alkaloid	alkaloid	270	562.1913	C27H31NO12	264
34	15.3	daidzin derivative	isoflavone erythrinian	250/285	665.1706	C30H32O17	417, 255 282, 251, 236, 208,
35	15.8	Erysotrine	alkaloid	279	314.1751	C19H23NO3	191
36	16.5	Erythraline	erythrinian alkaloid	286	298.1446	C18H19NO3	266, 251, 236, 208, 191
37	17.0	erythristemin	erythrinian alkaloid	285	344.1846	C20H25NO4	298, 282, 269, 267, 255
38	18.6	erythrinian alkaloid malonyl-daidzin	erythrinian alkaloid	280	350.1146	C18H20CINO4	264, 207
39	19.8	derivative	isoflavone	250/285	503.1168	C24H22O12	---
40	20.4	flavonol derivative malonyl-daidzin	flavonol	270/340	741.2025	C36H36O17	409, 391, 379, 337, 177
41	21.1	derivative	isoflavone	250/283	503.1173	C24H22O12	297, 255
42	22.3	acetyl-daidzin	isoflavone	250/286	459.1269	C23H22O10	---
43	22.7	malonyl-genistin	isoflavone	260/310	519.1118	C24H22O13	---
44	23.3	Daidzein	isoflavone	250/282	255.0619	C15H10O4	227, 199, 181
45	23.4	UNKNOWN	---	280/325	534.8842	C25H31N3O10	---
46	26.5	UNKNOWN	---	---	811.2014	C39H38O19	255, 177
47	34.2	triterpene saponin	saponin	---	797.4689	C42H68O14	441, 423
48	34.7	triterpene saponin	saponin	---	1069.5537	C54H84O21	---
49	35.3	triterpene saponin	saponin	---	923.4945	C48H74O17	441, 423
50	36.9	UNKNOWN	---	---	478.2941	---	---
51	37.0	UNKNOWN	---	---	520.3343	---	---
52	37.4	UNKNOWN	---	---	520.3359	---	---
53	37.9	sandwicensin	pterocarpan	286	339.1562	C21H22O4	283, 253, 240, 225
54	38.1	UNKNOWN	---	---	480.3075	---	---

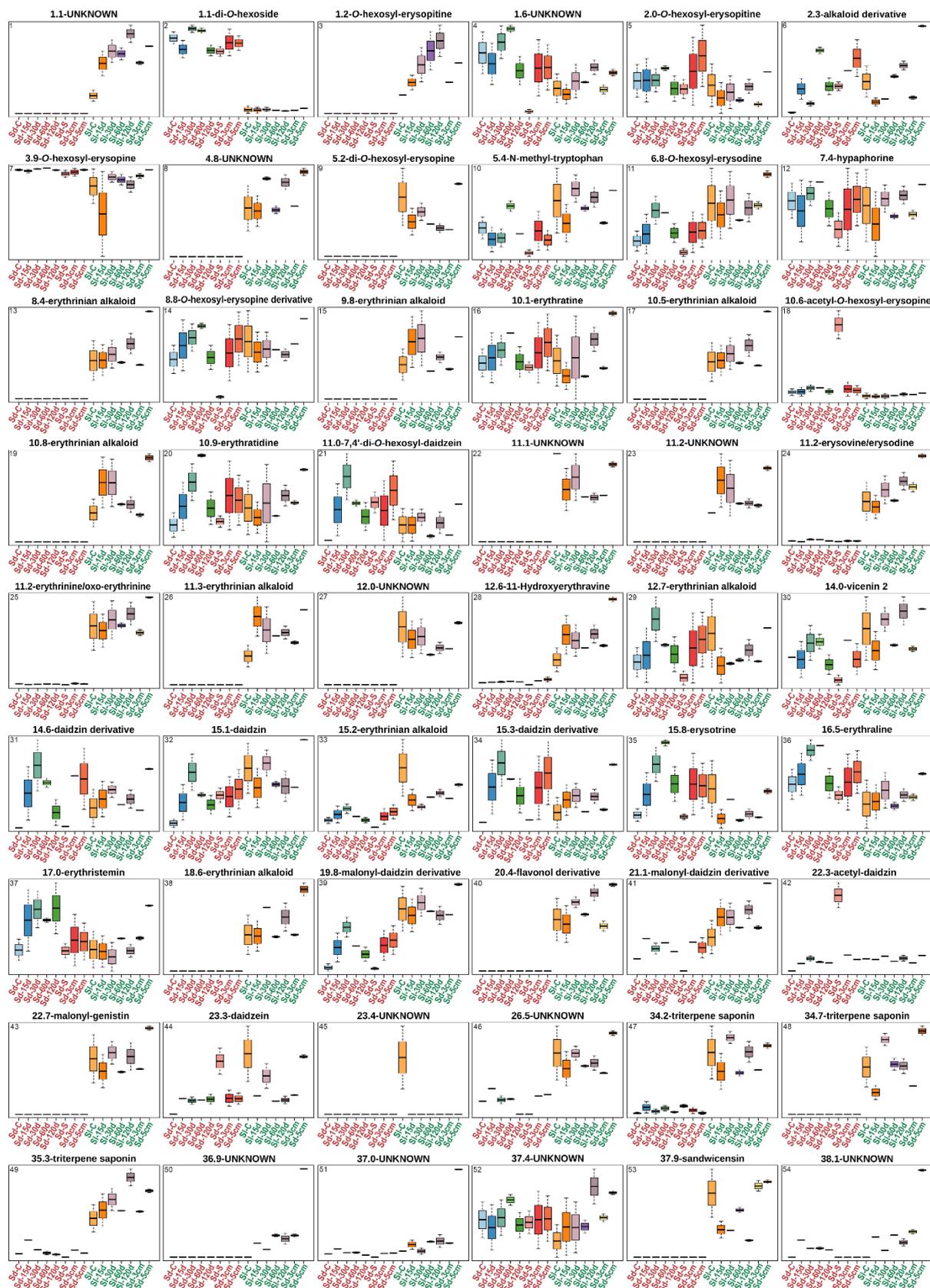


Fig. 1. Box plot presenting the averages distributions of the ions intensities for all treatments tested for seeds and seedlings of *Erythrina fusca*. Sd_C: seeds control; Sd_15d: seeds submitted to 15 days of

flood; Sd_30d: seeds submitted to 30 days of flood; Sd_60d: seeds submitted to 60 days of flood; Sd_120d: seeds submitted to 120 days of flood; Sd_S: seeds submitted to fire effect on the surface; Sd_3cm: seeds buried 3 cm from the surface and submitted to fire effect; Sd_5cm: seeds buried 5 cm from the surface and submitted to fire effect; SI_C: seedling control; SI_15d: seedlings obtained from seeds that were submitted to 15 days of flood; SI_30d: seedlings obtained from seeds that were submitted to 30 days of flood; SI_60d: seedlings obtained from seeds that were submitted to 60 days of flood; SI_120d: seedlings obtained from seeds that were submitted to 120 days of flood; SI_3cm: seedlings obtained from seeds buried 3 cm from the surface and submitted to fire effect; SI_5cm: seedlings obtained from seeds buried 5 cm from the surface and submitted to fire effect.

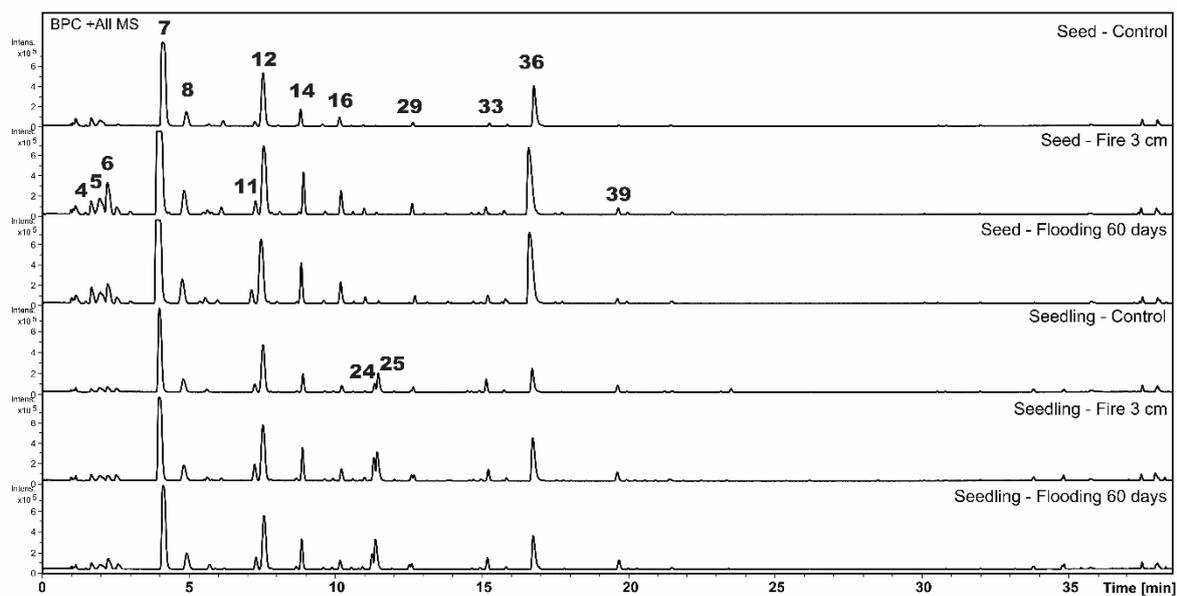


Fig. 2. Base Peak chromatogram recorded within the positive ion mode of seeds and seedlings of *Erythrina fusca* from different treatments to illustrate important compounds obtained by HPLC-DAD-MS/MS.

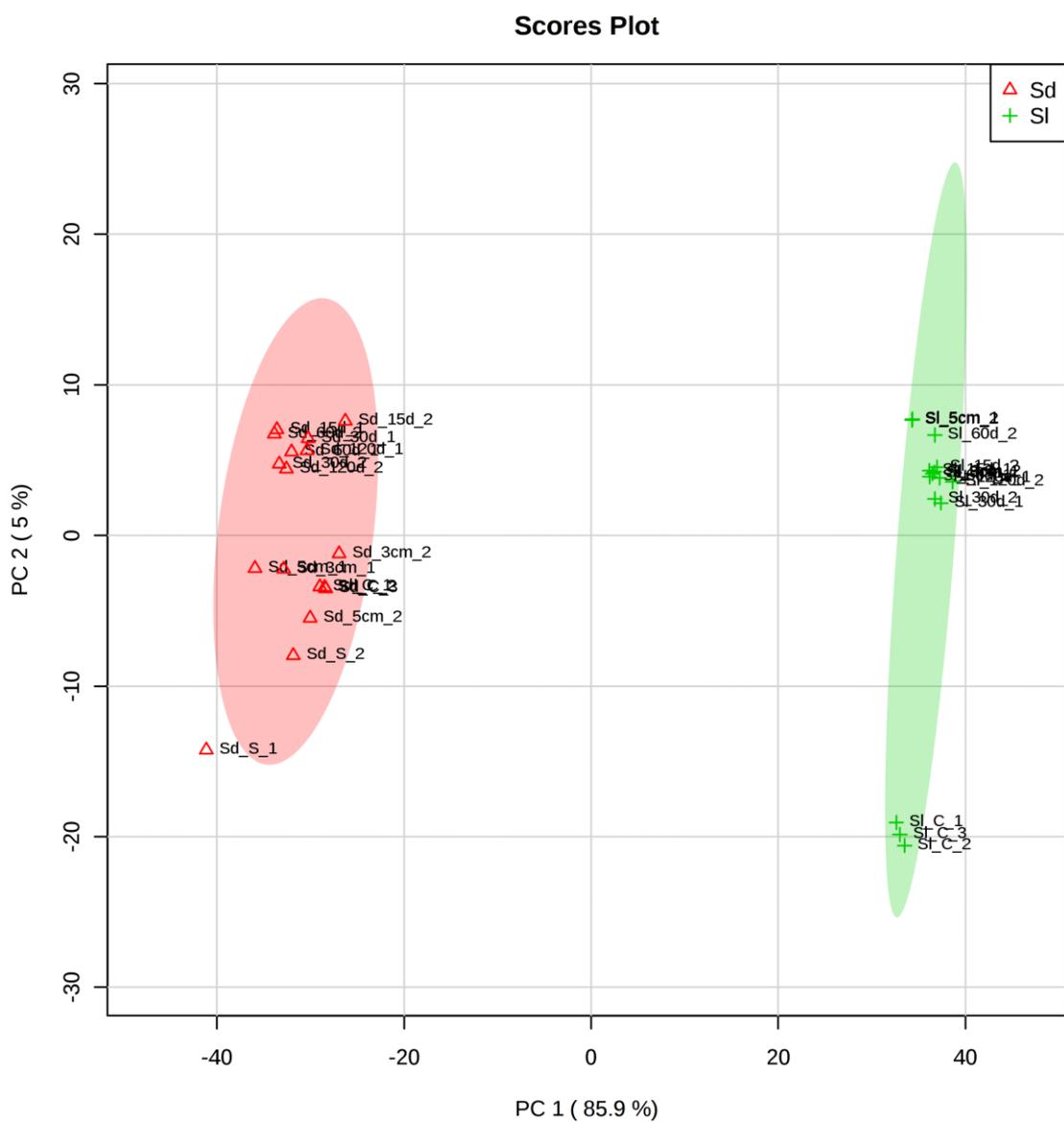


Fig. 3. Principal component analysis (PCA) of metabolites from seeds (Sd=triangle) and seedlings (Sl=cross) of all treatments. The first two components explain 89.6% of the variation (PC1:85.9% and PC2:5%) with a clear distinction between chemical composition of secondary metabolites of seeds (Sd) and seedlings (Sl) of *Erythrina fusca*.

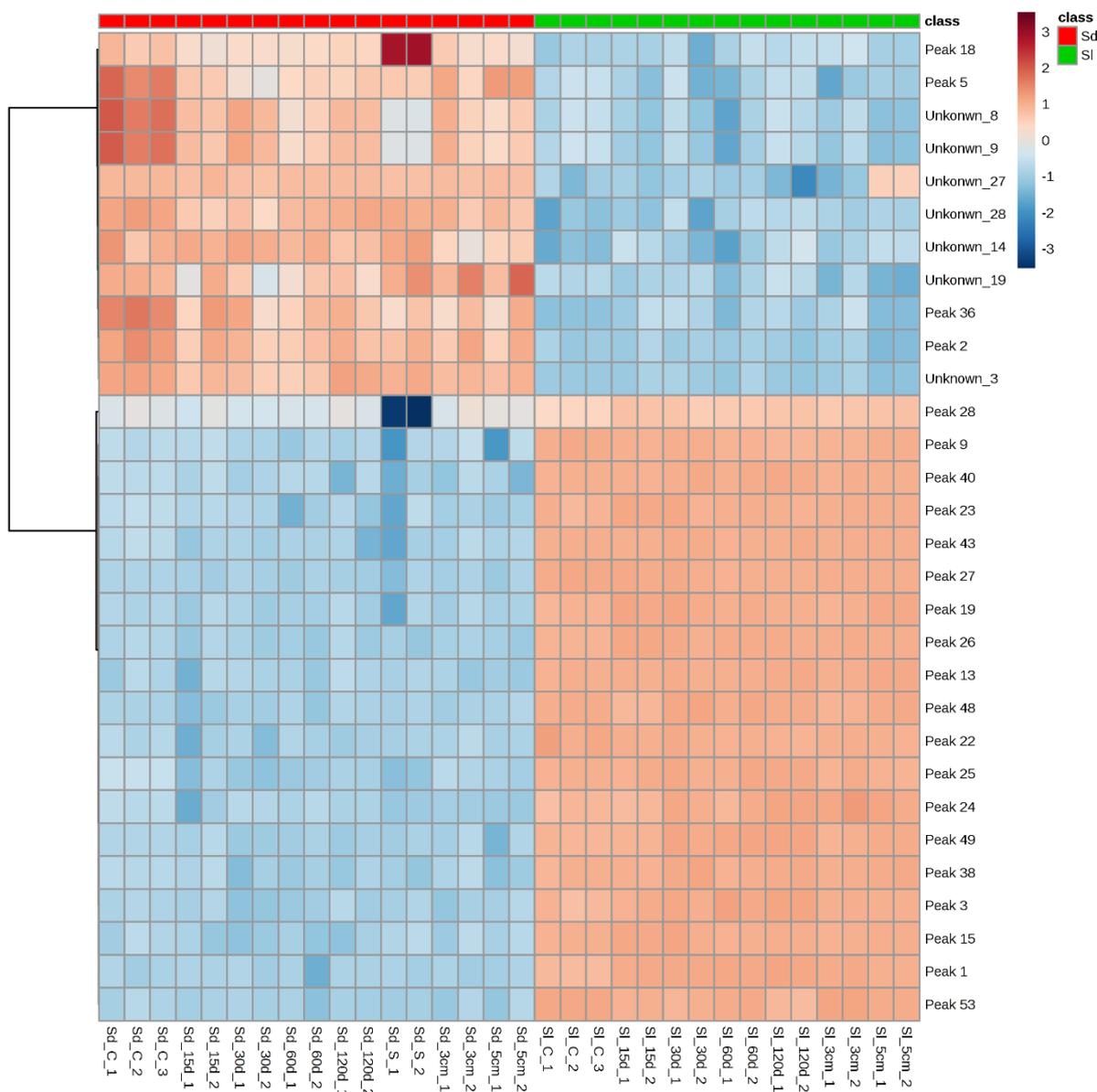


Fig. 4. Heatmap and hierarchical cluster of the compounds observed in seeds (Sd) and seedlings (Sl) of *Erythrina fusca*. The intensity of the compounds in each sample varies from more intense (more red) to less intense (more blue).

intensity in seedlings, while the compound **48** occurred exclusively in seedlings. Regarding the flavonoids, we observed that compounds **31** and **32** were transferred from seeds to seedlings, while the compounds **39**, **41** and **53** increased in seedlings and the compounds **40** and **43** appeared exclusively in seedlings, synthesized *de novo* from precursors in the seeds.

When we analyzed the responses of seeds and seedlings to the two stress factors studied, we observed that there were no significant differences between flood periods (15, 30, 60, or 120 days) (Fig. 5 and 6) or between the depths/temperatures of the fire experiment (3 and 5 cm) (Fig. 7 and 8), we observed differences only when we compared the samples submitted to stressors with the controls. The exceptions were seeds submitted to direct contact with fire, which presented a differentiated response in relation to buried seeds (3 and 5 cm).

The intensity of compounds differed between treatment samples and controls. For seeds submitted to both stress factors, two compounds appeared that were not present in the control seeds, the isoflavones daidzein (**44**) and its derivative acetyl-daidzin (**42**). Besides that, for seeds submitted to flooding experiments we observed a significant increase of the alkaloids **36**, **38** and **7** and the isoflavones (derivatives of daidzein) **41**, **39**, **34**, **32** and **31** (Fig. 9). For the seeds submitted to fire when buried (3 and 5 cm) we observed an increase in the alkaloid **6** and the isoflavone **32** (also derivative of daidzein) (Fig. 10).

The seeds submitted to direct contact with fire (surface of soil) showed a different o of compounds. We observed the absence of saponins **49**, isoflavone **41**, and alkaloid **28**, as well as a significant decrease of flavone **30** and isoflavone **31**. Besides that, we detected an increase of isoflavones **44** and **42** and alkaloid **18**. Another interesting response to the direct contact with fire was the significant increase of the acetylated compounds, peaks **18** and **42**.

For seedlings submitted to flooding, we observed a decrease of isoflavone **44**, pterocarpan **53**, and alkaloid **33** when compared with controls (Fig. 11). Meanwhile, we detected a significant increase in the intensity of alkaloids **3** and **26**. In addition, for seedlings submitted to fire treatments we observed a significant increase in the intensity of alkaloid **3** (Fig. 12).

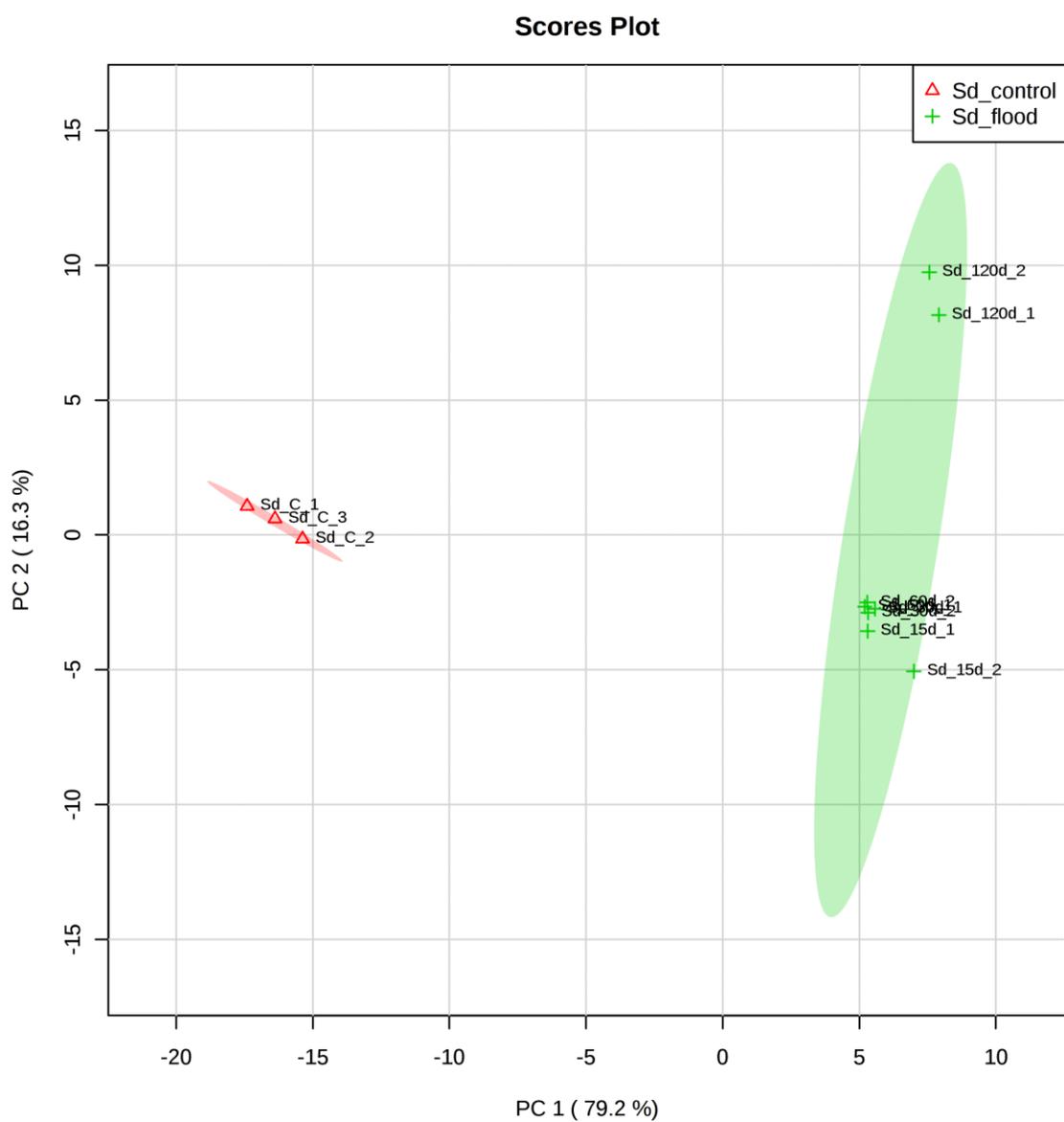


Fig. 5. Principal component analysis (PCA) of metabolites from control seeds (Sd_control=triangle) and all seeds from flood treatments (Sd_flood=cross) of *Erythrina fusca*. The first two components explain 95.5% of the variation and the metabolic profile of control seeds (Sd_control) and flood seeds (Sd_flood).

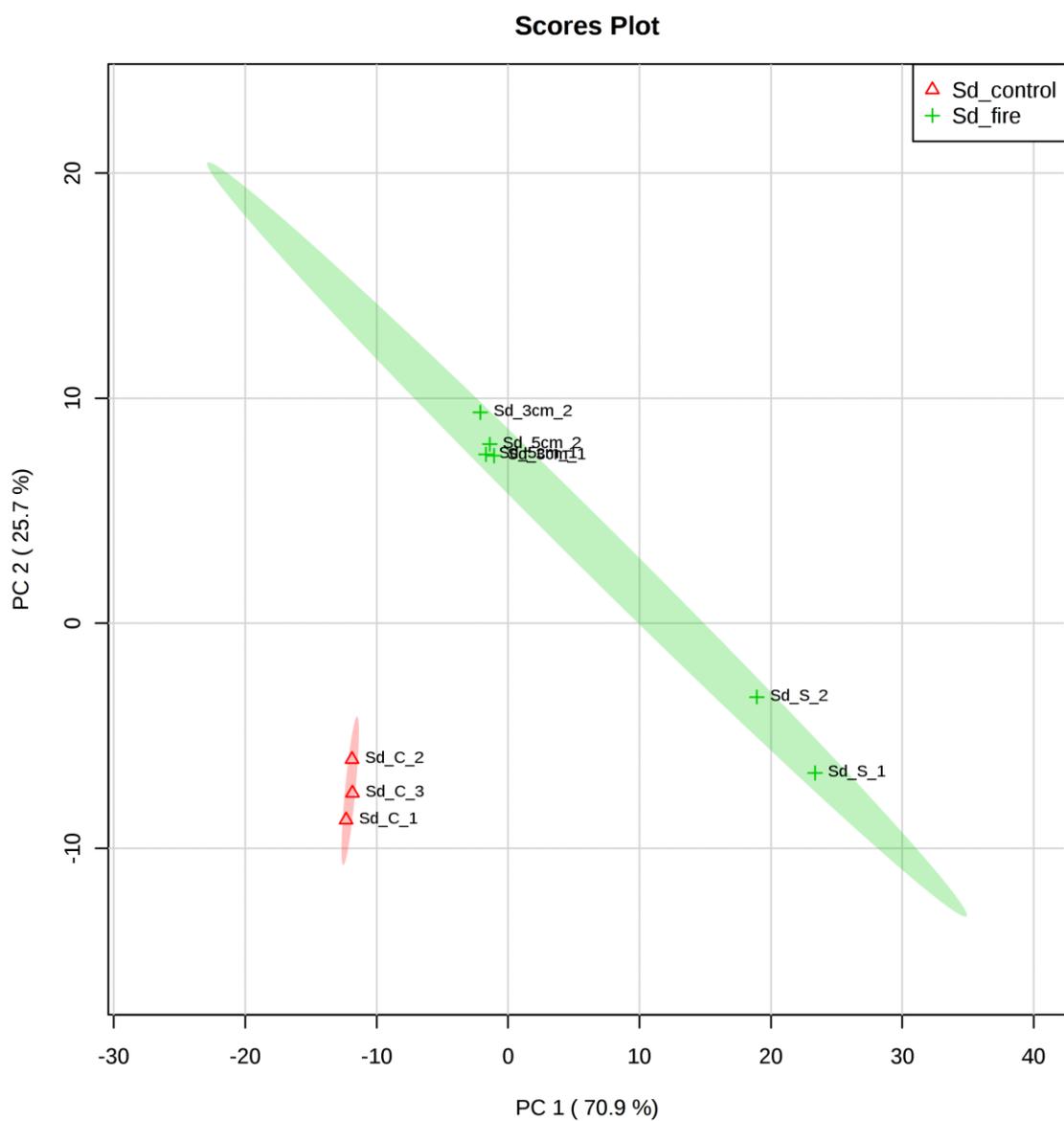


Fig. 6. Principal component analysis (PCA) of metabolites from control seeds (Sd_control=triangle) and seeds obtained from fire treatments (Sd_fire=cross) of *Erythrina fusca*. The first component (PC 1) explains 70.9% and the second component (PC 2) explains 25.7% of the difference between secondary metabolites of control seeds (Sd_control) and seed submitted to fire (Sd_fire).

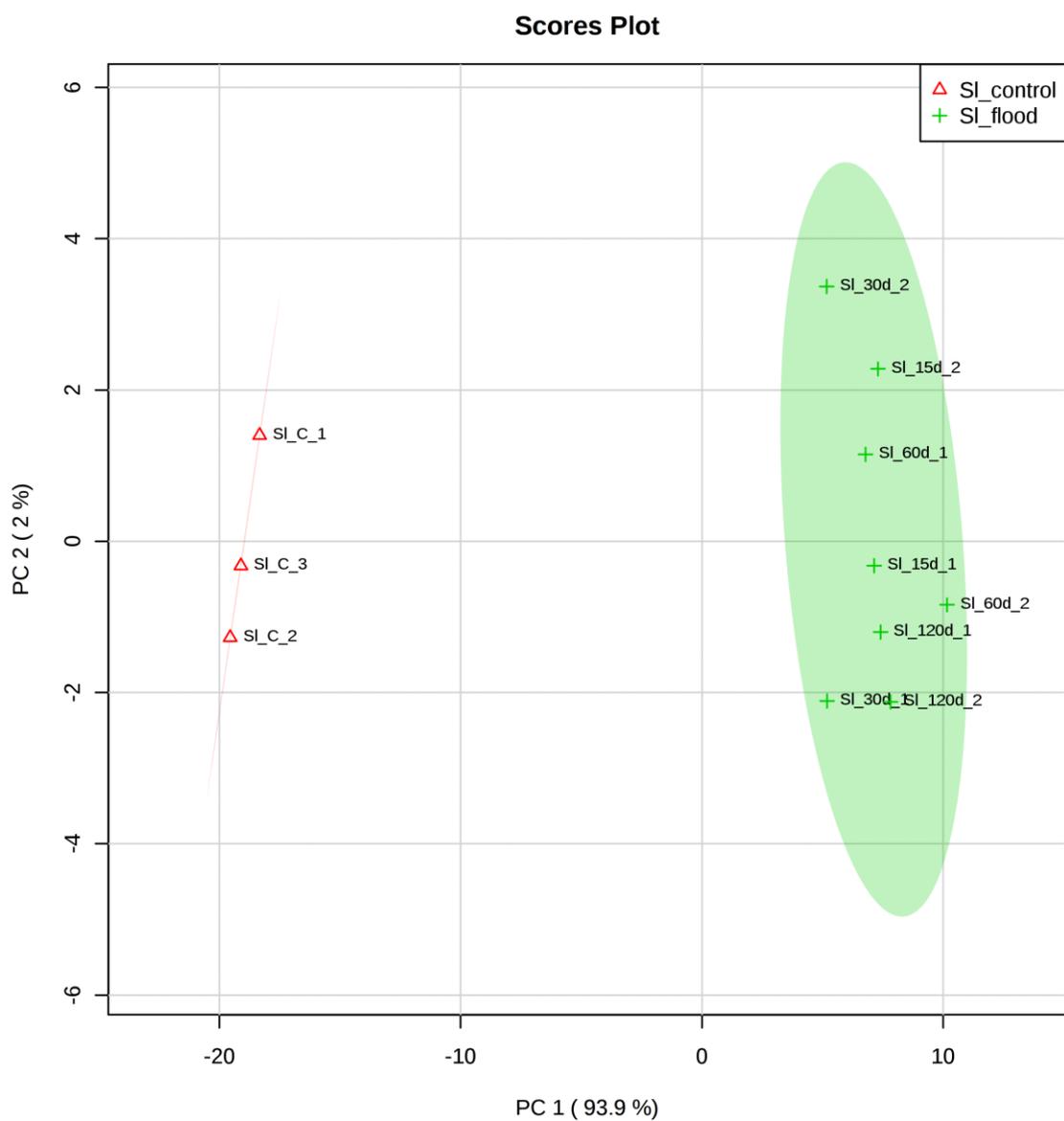


Fig. 7. Principal component analysis (PCA) of metabolites from control seedlings (SI_control=triangle) and all flood seedlings (SI_flood=cross) of *Erythrina fusca*. The PC 1 explains 93.9% of the variation and PC 2 expounds 2.0%. PCA demonstrated a clear discrimination between secondary metabolites profile of control seedlings (SI_control) and flooded seedlings (SI_flood).

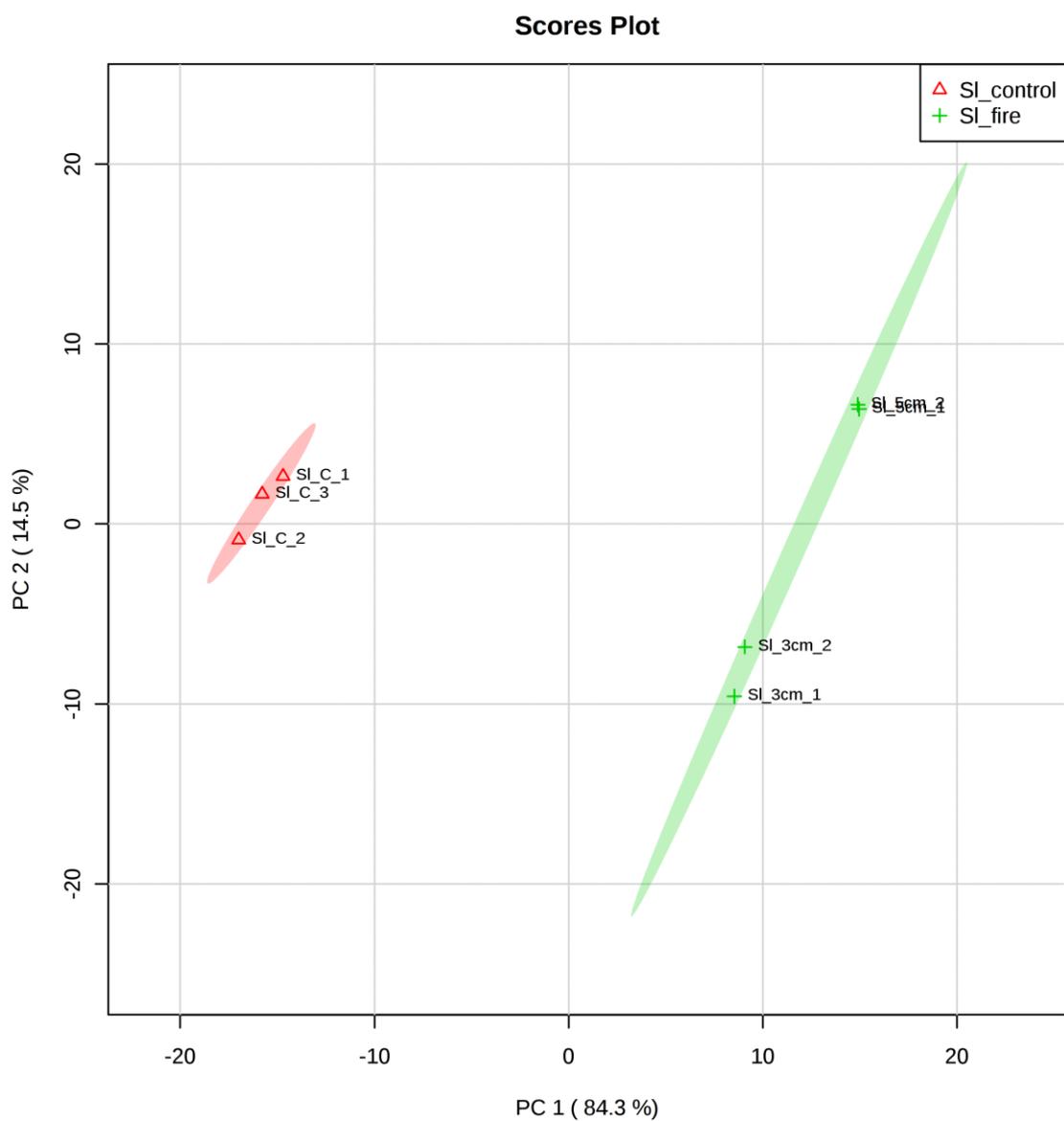


Fig. 8. Principal component analysis (PCA) of metabolites from control seedlings (SI_control=triangle) and seedlings of fire treatments (SI_fire=cross) of *Erythrina fusca*. The PC 1 explains 84.3% and PC 2 explains 14.5% of the variation between secondary metabolites of control seedlings (SI_control) and seedlings from fire treatment (SI_fire).

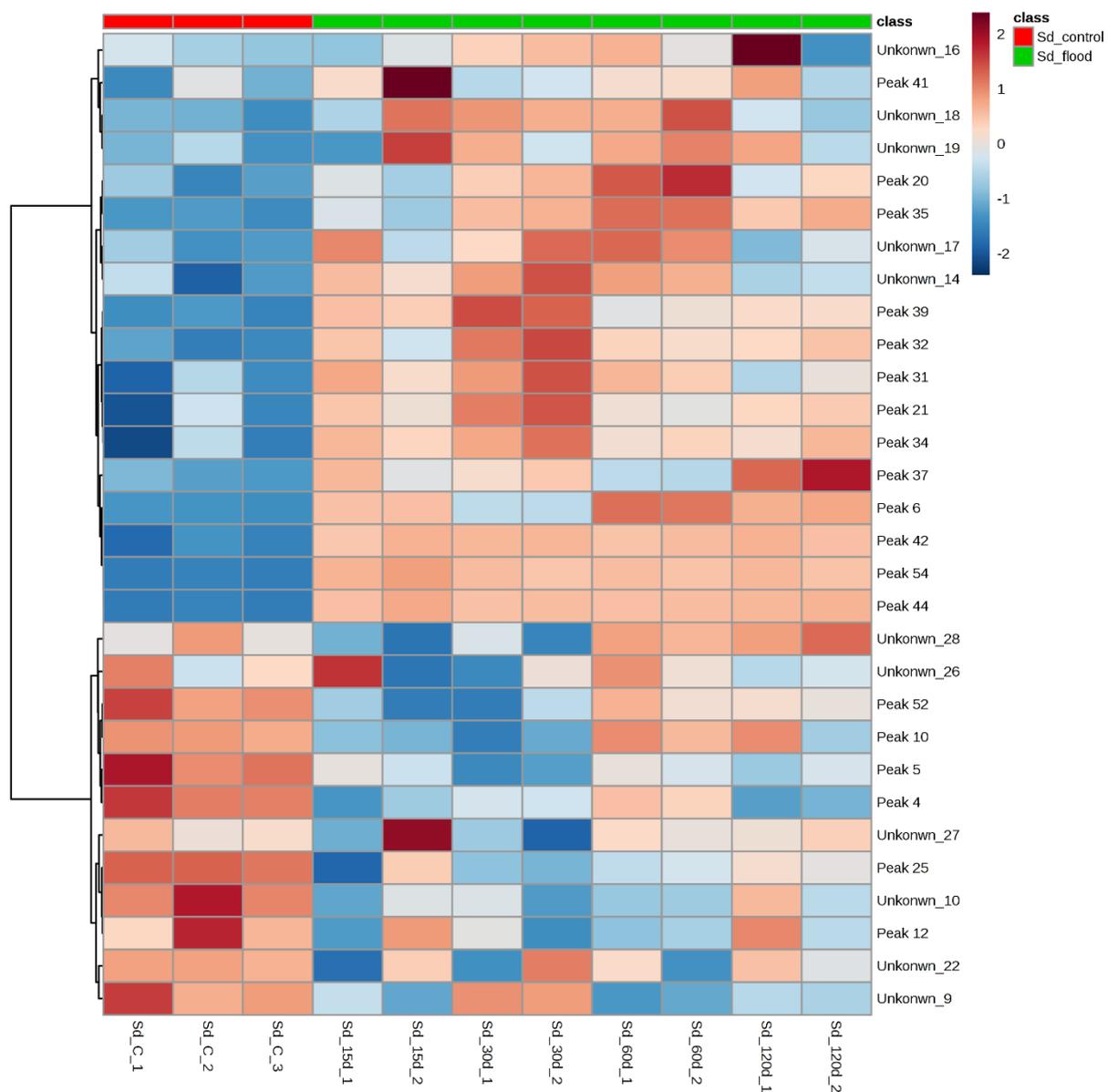


Fig. 9. Heatmap and hierarchical cluster of compounds acquired of control seeds (Sd_control) and flood seeds (Sd_flood) of *Erythrina fusca*. The intensity of the compounds in each sample varies from more intense (more red) to less intense (more blue).

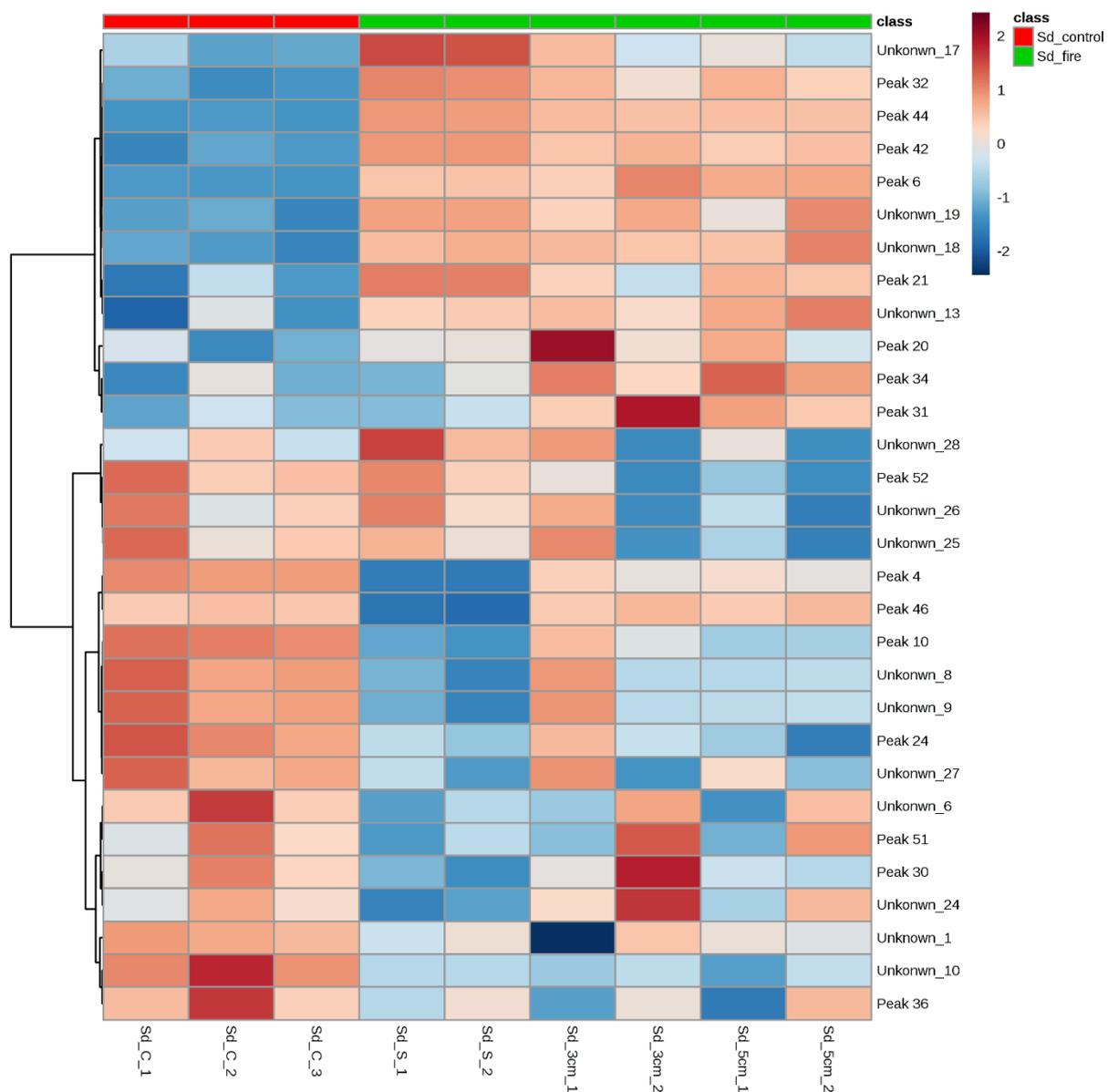


Fig. 10. Heatmap and hierarchical cluster of compounds obtained for seeds control (Sd_control) and seeds of *Erythrina fusca* submitted to fire stress (Sd_fire). The intensity of the compounds in each sample varies from more intense (more red) to less intense (more blue).

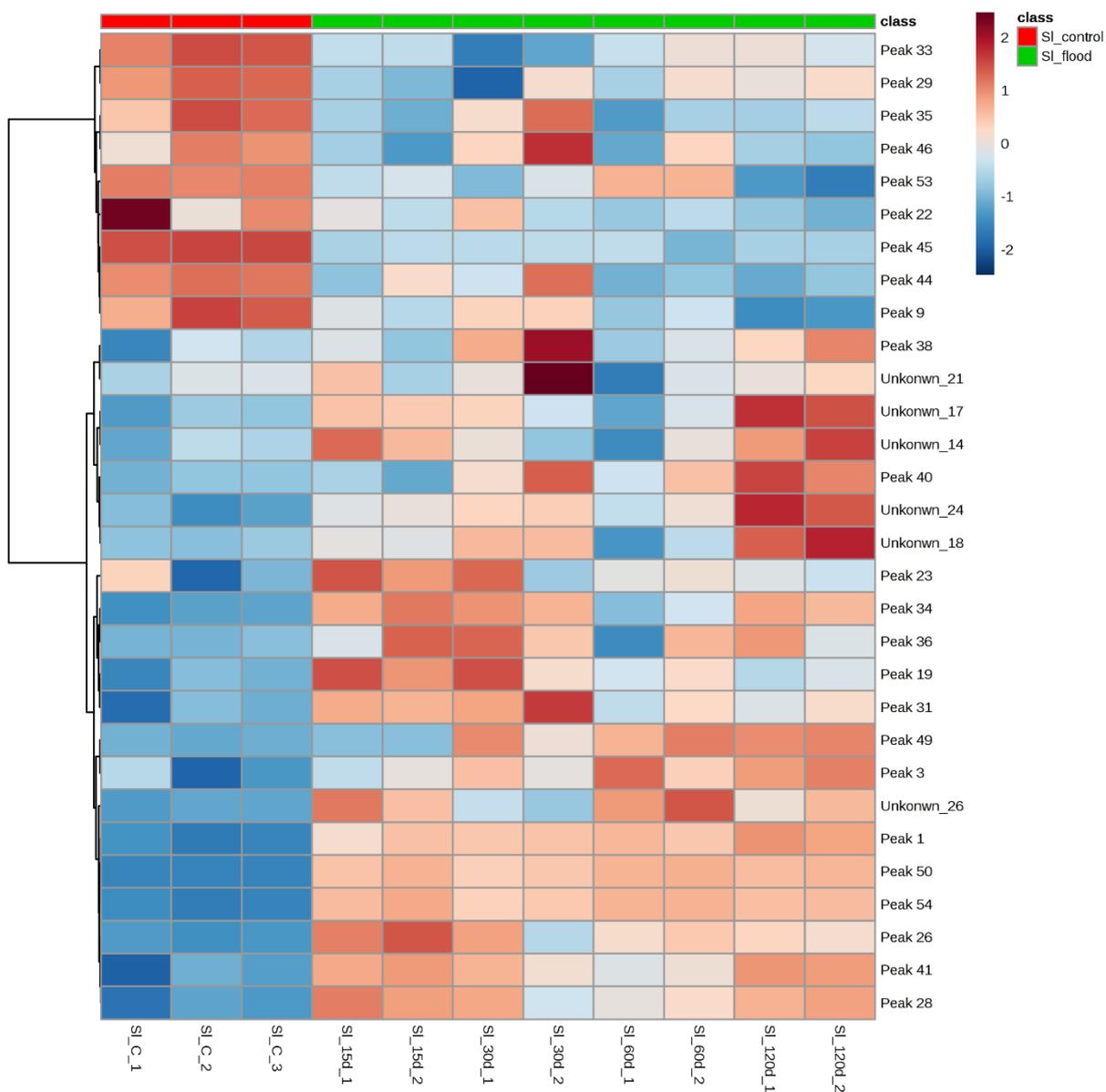


Fig 11. Heatmap and hierarchical cluster of compounds obtained from control seedlings (SI_control) and flood seedlings (SI_flood) of *Erythrina fusca*. The intensity of the compounds in each sample varies from more intense (more red) to less intense (more blue).

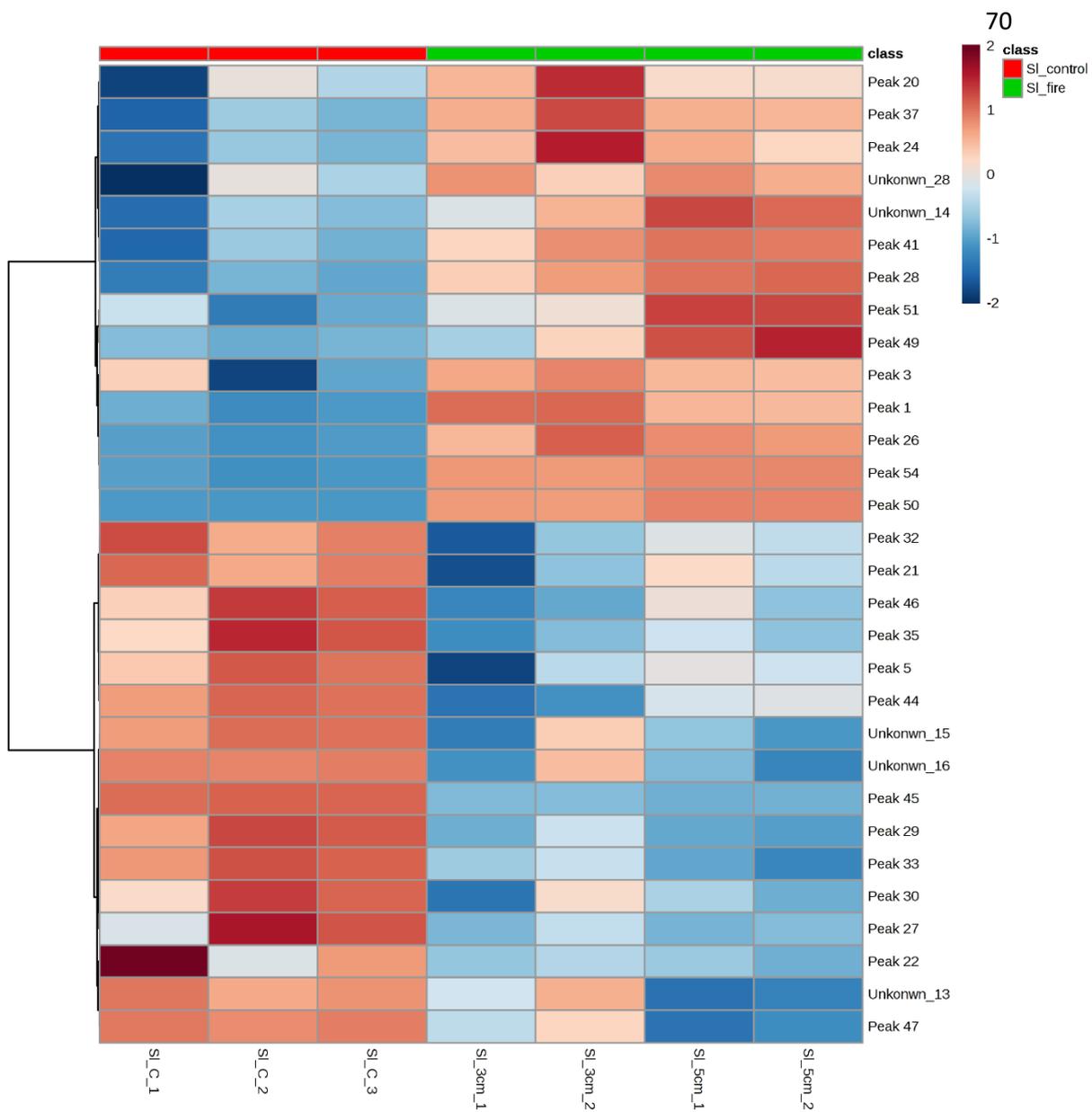


Fig. 12. Heatmap and hierarchical cluster of compounds obtained from control seedlings (SI_control) and seedlings submitted to fire stress (SI_fire) of *Erythrina fusca*. The intensity of the compounds in each sample varies from more intense (more red) to less intense (more blue).

Discussion

Flooding and fire simulation experiments

The increase in germination and seedling formation, in seeds submitted to 60 and 120 days of flooding, seems to be related to the long period the seed is subjected to flooding, due to softening of the integument, since the seeds of the genus *Erythrina* have integumentary dormancy. Therefore, for germination the seeds require an event to overcome dormancy. The exposure of seeds to flooding may help to overcome the dormancy of some annual wetland species, and the seeds can germinate right after the water table subsides (Crawford 2003). In the study region, *E. fusca* fruits at the beginning of flooding period, and seeds can remain for up to 120 days under water (unpublished data - see Chapter 1).

In our experiments with buried seeds, the temperatures did not release dormancy, what may have been because these temperatures were not high enough or long enough to break the dormancy of *E. fusca*, therefore, seed germination was not higher than observed in the controls. In some plant species with integument dormancy, the germination of seeds buried in the soil can increase after the passage of fire or treatment with high temperatures, as the dormancy may be overcome by the heat shock (Keeley & Fortheringham 2000). The levels of germination depend on the combination of intensity and duration that the seed is exposed to high temperatures (Keeley & Fortheringham 2000). The exposure of seeds on the soil surface to direct contact with fire led to a drastic reduction in the moisture content, reaching a critical level for their survival. Orthodox seeds, such as *Erythrina fusca*, can tolerate dehydration until 5%, while below this level can be deleterious (Roberts 1973). Thus, both exposure to fire (thermal threshold) plus dehydration seem to have been lethal to the seeds (Roberts 1973). The same pattern was found in a study with another Fabaceae species (*Mimosa bimucronata*)

when the seeds in direct contact with fire (on the soil surface) did not germinate, while buried seeds germinated (Menezes & Rossi 2011).

Metabolomic analyses

Monitoring metabolites during the germination process gave us a clear view of the translocation of metabolites and enzymatic activation throughout the plant development stages, with the generation of several new metabolites that may be correlated to tissue maintenance and defense.

Seeds need compounds for defense against pathogens and herbivores, as well as reserve compounds (Berenbaum 1981, Terras et al. 1995). Many compounds found in seeds are stored during the ontogeny process when the seeds receive nutrients and secondary metabolites from the mother plant, which are then accumulated in the cotyledons or endosperm as a source of energy for seed maintenance and germination (Chacón et al. 2013). For instance, the peak **3** is a reserve glycoside derived from the mother plant, which is significantly higher in seeds when compared with seedlings, most likely due to the consumption of this compound during the germination process of seeds.

Similarly, other compounds accumulated by seeds during the process of formation and maturation are transformed during germination. For example, the compounds **18** and **9** are similar and differ only by the presence of one extra hexoside in compound **9**. The compound **18** appears more intensely in seeds, while the compound **9** occurs exclusively in seedlings. Thus, during the germination process the compound **9** acquires an additional hexose, which makes it more polar and facilitates metabolite transportation. The compound **36** (erythraline) responds in the same way, as it is a non-glycosylated alkaloid received from the mother plant

during seed formation. This compound occurs with greater intensity in seeds, while in seedlings eight other alkaloids appear (which do not occur in seeds), perhaps this compound **36** is a precursor to the synthesis *de novo* of these exclusive alkaloids in seedlings.

The presence of these alkaloids in seeds and seedlings may be regulated by the same enzymatic role, or by parallel roles. For example, the compounds **18** and **28**, respond oppositely to the stress of direct contact with fire. For the seeds submitted to fire on the soil surface, the compound **18** was more intense, representing the metabolism of seeds at the time they underwent the stress. Therefore, as their metabolism stopped (because the seed died and probably enzyme denaturated), they remained with a higher intensity of that compound, while in the other seed treatment degradation or transformation of this compound was initiated. In addition, we observed a much lower intensity of the compound **28** in the samples submitted to the direct contact with fire, in this way, the synthesis of this compound must have also been stopped at the moment of seed death. Therefore, considering the response of this compound to the same treatment, we can suppose they belong to the same role.

We detected a major presence and intensity of alkaloids, saponins, and flavonoids in seedlings, which is probably due to the specific needs of this stage of plant development, as seedlings are more susceptible to the effects of herbivore attack and desiccation. Unlike seeds, seedlings of *E. fusca* do not have an integument as a physical barrier and are more vulnerable to attack by herbivores and pathogens, as well as to competition with other plants.

In this context, high intensities of secondary metabolites of some classes may confer greater resistance to seedlings. Among these classes, the flavonoids effectively participate in responses to stress factors with a significant role in plant resistance (Treutter 2006). They may confer protection during droughts (Tattini et al. 2004), attract N₂-fixing bacteria (Hungria & Stacey 1997), which are necessary in Fabaceae species, and act in the defense against

pathogens, principally fungi (Grayer & Harborne 1994) and herbivores (Feeny 1976, War et al. 2012). The alkaloids are compounds widely known to promote plant defense against biotic and abiotic stressors (Matsuura & Fett-Neto 2015). They repel herbivores for being toxic or having a bitter taste, interrupt protein functions, having antifeedant activity, and alter the central nervous system (War et al. 2012, Matsuura & Fett-Neto 2015). In addition, the saponins are compounds that act mainly in the defense of plants against pathogens, showing antimicrobial, antiparasitic, insecticide, and antifeedant activities (Osbourn 1996, Moses et al. 2014). Saponins are also known to have hemolytic potential (Sparg et al. 2004), negatively affecting the digestion capacity of proteins, animal reproduction, and the central nervous system (Francis et al. 2002).

We observed that the relevant compounds in seedlings showed higher solubility, many of them were polar (such as the alkaloids **3** and **9**), others were glycosylated (such as the flavonol **40**), linked with malonyl (as the isoflavone **43**) or naturally water soluble, as the saponins. This is an interesting pattern for plants, which need an efficient defense, because soluble compounds are easier to transport and the plant can direct these metabolites to the necessary region or can store these compounds in the vacuoles, reducing autotoxicity (Osbourn 1996, Mithöfer & Boland 2012).

When we analyzed the effects of stress factors on the composition of secondary metabolites, we observed an increase of defense compounds in seeds and seedlings submitted to stress treatments (flooding and fire) when compared with control seeds and seedlings. The greater intensity of defense compounds in samples submitted to stressors is a previous response for a possible attack, since plants under stress conditions are more susceptible to the action of pathogens, herbivores, or allelochemicals of other plants (Schoeneweiss 1975). For example, we observed an increased intensity of several alkaloids that are mainly related to

defense against herbivores in seeds and seedlings submitted to the stressors (War et al. 2012, Matsuura & Fett-Neto 2013).

Another outstanding example was the variation in the intensities of the compound daidzein (**44**) and its derivative acetyl-daidzin (**42**) in samples. Both compounds did not appear in the control seeds, but appeared in seeds submitted to the treatments (flooding and fire) and mainly in seeds submitted to direct contact with fire (soil surface). Conversely, for seedlings, these compounds appeared with greater intensity in the control seedlings than in those submitted to treatments. Thus, these compounds, which would be produced only in the seedling stage, began to be produced in the seeds, what indicates that the submission of seeds to these stress factors led to a deregulation in the synthesis role. We also observed an increased tendency of other daidzein derivative compounds for both seeds and seedlings submitted to stress factors (flooding and fire). The isoflavone daidzein is widely studied in species of Fabaceae, known for its antimicrobial defense potential (Graham 1991). This compound is the precursor of several other isoflavones (such as daidzin, glyceollin and genistein) and a significant increase of these compounds has already been observed in infected tissues (Graham 1991, Chacón et al. 2013).

In regards to seeds submitted to direct contact with fire (soil surface), we observed significant differences in the composition of secondary metabolites, with an increase in acetyl compounds (**18** and **42**), and a decrease in other metabolites, mainly flavonoids. Such a drastic difference in this treatment may be due to the deleterious low water content, which was lethal to the maintenance of seed metabolism.

We also detected decreased intensity or absence of some compounds, mainly in seeds submitted to the direct contact with fire (surface of soil) and seedlings submitted to both stress factors. However, we could not infer about the possible causes of this decrease, perhaps they

belong to metabolic pathways that have been deregulated by the occurrence of stress events, or their precursors have been used in the *de novo* synthesis of the most important compounds at that time of stress.

We observed a clear difference between the composition of secondary metabolites in seeds and seedlings of *E. fusca*. In the seeds, we observed the presence of reserve metabolites, as well as compounds of mid polarity, whereas in the seedlings we observed an increase of some compounds, more water soluble, which are easily transported facilitating the actions of defense, for example. In the flood experiment, we observed that the longer duration of period that a seed was flooded promoted higher germination and seedling formation rates, that is, flooding events may lead to a facilitation of dormancy overcoming in *E. fusca*. In contrast with flood, the exposure of seeds to fire did not show differences for percentages of germination and seedling formation. The exceptions were seeds submitted to direct contact with fire (soil surface), which presented a lethal decrease of moisture.

Despite differences in dormancy overcoming, germination, and seedling formation between the two stress factors, we observed that the metabolic responses were very similar for flood and fire, with an increase in the intensity of compounds that seem to be defense in both seeds and seedlings. Besides that, this similarity in metabolic changes of seeds and seedlings submitted to stressors shows that the response of this species is not selective. This more general response to both stressors may be interesting for plants susceptible to these factors, such as the plants that occur in the Pantanal. Moreover, considering that the metabolic responses to both events were very similar, and that we observed increased germination only in the seeds submitted to flood, we can infer that the observed metabolic changes are not related to the overcoming of dormancy, that is, dormancy of *E. fusca* is integumental and overcoming is facilitated by weakening the coat through prolonged contact with water.

Chapter 3

Dendroecology and population structure of *Erythrina fusca* Lour. (Fabaceae) monodominant stands

Abstract

Monodominant stands are vegetation types where more than half the number of arboreal individuals belong to only one species. Such pure formations are abundant resources, and can play very important roles in fauna maintenance. Therefore, it is essential to understand the climatic factors that influence species dominance. One way to interpret the dynamics of vegetation and growth of arboreal species is through tree-ring studies, accordingly, we aimed to study the structure of a monodominant population of *Erythrina fusca* Lour. (Fabaceae) and the distribution of individuals in different diameter classes. We estimated the age of this monodominant population and evaluated effects of climate on tree growth, all in an effort to obtain information for the protection of this species. The study was performed in the Pantanal subregion of Cáceres, Brazil. We obtained samples for dendrochronological analyses using destructive and non-destructive methods. To observe growth rings, we performed anatomical analyses. We then used TSAP-WINTM to count the tree-rings and build a chronology, correlating the results with climatic factors to test their influence on growth. We sampled 0.6 ha of the vegetation and separated individuals into diameter classes to estimate age based on the diameter/age ratio obtained through dendrochronological analyses. We observed well-defined growth rings with a layer of parenchyma with flat cells delimiting the rings. The only factor that influenced growth during the study period resulted from the effects of *El Niño*, which decreased precipitation from October through December. We estimated the maximum age of this stand as 54 years, with a reduction of young individuals in the last 9 years, what appears to be a response to decreasing in the minimum flood levels throughout this period.

This indicates a future decline in the number of individuals. These results allow us to propose measures of protection for these monodominant formations, which mainly involve avoiding further anthropic actions that could reduce flooding levels.

Keywords: *abobral*, annual rings, dendrochronology, monodominance, tree-rings

Introduction

In tropical areas some tree species can occur as monodominant stands, where more than half the total number of arboreal individuals belong to only one species (Connell & Lowman, 1989; Hart et al. 1989). Many features and events can determine monodominance, the most common being flood, fire, succession, fungal conditions, and soil features (Hart, 1990, Torti et al. 2001).

Knowledge about the factors that affect the dominance of a species can be gained through an understanding of its dynamics and structure. The study of tree-rings is one way to interpret both the dynamics of vegetation and estimate the growth rates of arboreal species (Worbes et al. 2003). The data provide clues to further understand how climate change can affect vegetation in the future, thereby arming researchers with the knowledge needed to formulate strategies to conserve these formations (Brienen et al. 2010). In tropical regions, the monodominance of a species is often associated with seasonal flooding (Campbell et al. 1986, Hart 1990). Some studies show that flood pulse and precipitation directly influence the growth and establishment of species (Junk et al. 1989, Schöngart et al. 2002). When we associate climatic variables with the wood increment patterns in some species, we can begin to understand how climate and natural events affect tree growth (Schöngart et al. 2004, Brienen & Zuidema 2006) and, perhaps, the establishment and growth of monodominant stands. One such natural event is *El Niño*, which, in the Pantanal, caused a significant reduction in precipitation from October to December (normally the rainy season), thus affecting the growth of different species, such as *Vochysia divergens* (Fortes 2006, Schöngart et al. 2011).

Environmental factors can also influence the establishment and development of individuals of different tree species. For example, the spatial arrangement of parent plants and

the interactions between their ancestral plants and the past environment affect the spatial structure and distribution of populations, therefore, the age structure of a current population reflects the conditions of recruitment and the mortality rates to which the populations were subjected (Hutchings 1997). The population structure of tropical species is expected to be a negative exponential, “J-reversed curve”, with several small and young individuals and field large and old ones (Meyer 1952).

The Pantanal is a favorable environment to study the occurrence and factors that influence species dominance, particularly because many monodominant species are found in its floodplains, such as *Tabebuia aurea* (Silva Manso) Benth. ex S. Morre (popularly known as “paratudal”), *Byrsonima cydoniifolia* A. Juss. (“canjiqueiral”), and *Copernicia alba* Morong ex Morong & Britton (“carandazal”), among others (Pott et al. 2011).

The monodominant stands of *Erythrina fusca* Lour. which occur in the Pantanal in the subregion of Cáceres, are an important resource in this subregion. Its flowers are fleshy, showy, and frequently visited by birds and pollinators. The dry legume fruit produces numerous seeds, which, during dehiscence, fall into the river and are normally ingested by fish (Pott & Pott 1994, Parrini & Raposo 2010). Furthermore, the roots present a particular arrangement that provides shelter for a variety of species, especially reptiles and mammals. Therefore, studies that report on the establishment and growth of a monodominant species in relation to environmental features are important for maintenance and conservation of these stands.

Normally, growth is negatively affected by inundation, therefore, the growth of woody species occurs principally during the terrestrial phase, when flooding and precipitation levels are lower, and stagnates during the aquatic phase, when the flooding and precipitation levels are higher (Worbes 1989, 1995, Schöngart et al. 2002). Our hypothesis is that the

establishment and growth of individuals of *E. fusca* in the monodominant stands in the Pantanal subregion of Cáceres is negatively influenced by higher flooding and higher precipitation levels, and positively influenced by the occurrence of *El Niño*, since this event can cause significant precipitation reduction. Therefore, in this study we aimed to build a chronology of *E. fusca* growth from monodominant stands in the Pantanal subregion of Cáceres, verify how the structure of the monodominant population is related to variations in inundation and climate, and evaluate effects of climate (local and global) and inundation on its growth. We intended to obtain data to support the conservation of these monodominant stands, considering their ecological importance in the study region.

Methods

Study area

The Pantanal is a vast floodplain crossed by the Paraguay River and its tributaries, which extends through Brazil, Paraguay, and Bolivia, with an area of approximately 140,000 km² (Adámoli 1986). This large wetland has a predictable and monomodal flood pulse, with low amplitude and long duration (Junk et al. 2014), and the structure and dynamics of the Pantanal are dependent on the fluctuation levels of the flooding (Junk et al. 1989, Nunes da Cunha & Junk 2014).

We conducted the study at Taiamã Ecological Station (TES) and the nearby areas of Sararé Island and the Jubran Private Reserve in the Pantanal subregion of Cáceres, where it is possible to find a sequence of floodplains covered by grasses and other herbaceous plants, riparian forests, and large monodominant stands of *Erythrina fusca*, locally known as *abobral*.

In this region, the climate is seasonal Aw in the Köppen classification, with a dry season from May to September and a rainy season from October to April. In the Pantanal subregion of Cáceres, high flooding levels almost coincide with the rainy seasons (Fig. 1). The average annual temperatures are around 26° C, ranging from minimum average of 20° C to the maximum of 31° C, and the average annual precipitation is 1227mm. We calculated these values from the data obtained from the Instituto Nacional de Meteorologia (INMET – Brazilian National Institute of Meteorology) and the Brazilian Navy.

In the subregion, the predominant soil is Eutrophic Gleysol (Fernandes et al. 2007), which remains periodically saturated by water stagnation or the occurrence of lateral flow in the soil. The striking feature of this soil type is its strong gleization due to a reduced

environment, where the water saturation throughout the year, combined with oxygen demand for biological activities, leads to low dissolved oxygen concentration in soil (Embrapa 2006).

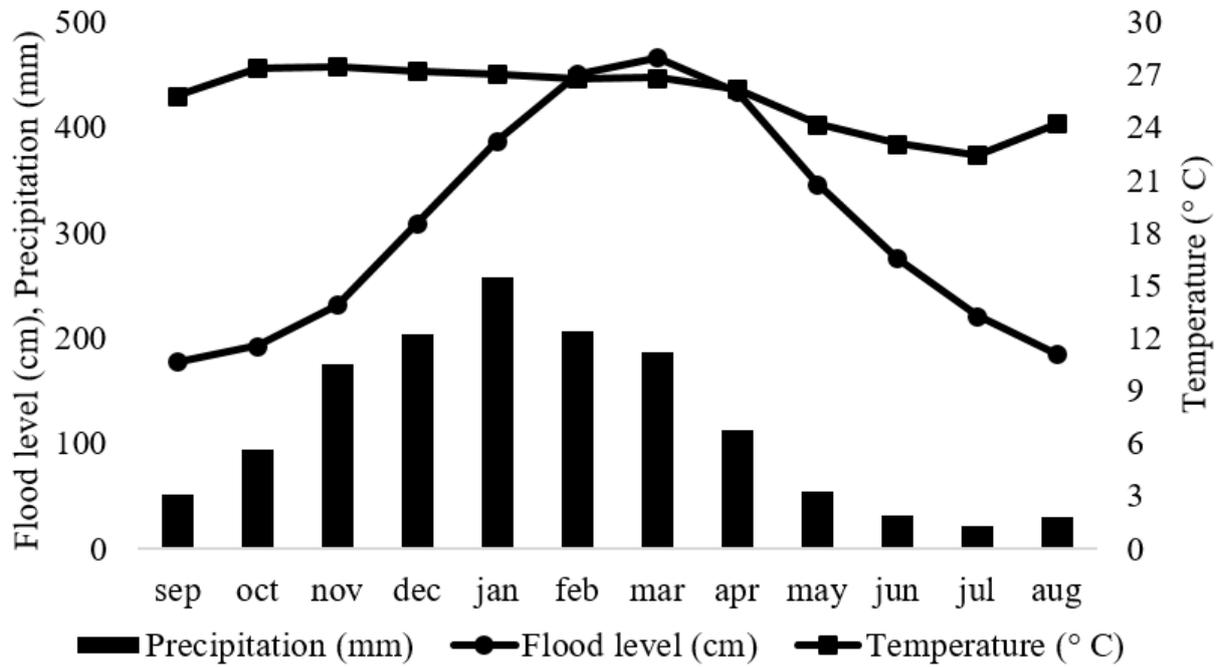


Fig. 1. Flood levels of the Paraguay River (line with filled circle), annual precipitation (bars), and temperature (line with filled square) between 1980 and 2014 in the northern Pantanal subregion of Cáceres, Mato Grosso, Brazil

Species description

Erythrina fusca Lour. (Fabaceae) is a tree species (20-30 meters high) with a globose treetop and short and branched trunk, prickly when young. The leaves are alternate, composite, trifoliolate, with leathery leaflets, glabrous. The inflorescence in the form of terminal racemes, with showy yellow-orange flowers (Lorenzi 2002). This is a deciduous species, which occurs naturally in riparian forests and blooms from May to September, with intense fruitification starting in November (Pott & Pott 1994, Lorenzi 2002).

These monodominant stands occur on the plains of the northern Paraguay River, in the Cáceres subregion, and along the banks of the Aquidauana River (Pott et al. 2011). In Cáceres subregion, there is a low-density vegetation with predominance of arboreal individuals of *E. fusca* (approximately 77% of the community), along with another 10 tree/shrub species (unpublished data - see Chapter 1). The herbaceous stratum is very homogeneous, dominated by grasses (Appendix 2 and Appendix 3), and soil that is covered by a histosol layer with leaf litter, mostly composed of *E. fusca* leaves (Appendix 7 and Appendix 8).

We collected and prepared a fertile sample of *E. fusca* according to herbarium techniques (Mori et al. 1989, Bridson & Forman 2004) and deposited the voucher in the CGMS Herbarium under registration CGMS 40967.

Collection, sample preparation, and analyses

In December 2015, samples for dendrochronological analyses were collected using the destructive method, whereby we injured the vascular cambium of 8 trees with different diameters. For this we used the Mariaux Windows method (Mariaux 1967), which starts by using a mechanical incision to remove a part of the bark from the tree to expose the vascular cambium for two years before the destructive collection (Appendix 9). In December 2016

using the non-destructive method (Worbes 1995), we extracted one or two cores of each stem using an increment borer (Pressler borer) of 5 mm in diameter. In the field, we measured the diameter at breast height (DBH) with a diametric tape and the height of all trees using a digital distance measurer.

We kept samples at room temperature until completely dried to avoid cracking. Then we polished the samples with sandpapers of different grains (80, 100, 180, 220, 320, 400, 600, and 1200) to show the growth rings and anatomical characteristics of the wood (Worbes 1995).

After the definition of the anatomical and growth ring characteristics, we counted and measured the width of the tree-rings. To do this we used a tree-ring measurement (LintabTM 6) with precision of 0.01 mm, associated with the software TSAP-WINTM Scientific (Time Series Analysis and Presentation), which is specific for time sequence analysis. We analyzed the samples at the Laboratório de Ecologia Vegetal of Universidade Federal de Mato Grosso do Sul (UFMS). The tree-rings were delimited and counted according to the classification of Coster (1927, 1928) adapted by Worbes (1995).

Anatomical procedures

The growth rings were delimited with anatomical analyses. For anatomical description and determination of tree rings, we used 1x1x2 cm wood samples of three *E. fusca* individuals. We boiled the wood samples in glycerin solution (50%) for 15 minutes to soften the wood. Afterwards, we prepared sections (longitudinal and cross planes) of about 25µm thickness using a sledge microtome (Leica SM2000R). The sections were bleached with sodium hypochlorite, washed in distilled water and acetic acid (1%), and stained with alcian blue and safranin, both in the concentration of 1% in water, proportion of 9:1 (Luque et al. 1996).

Macerations were prepared using wood segments dissociated in hydrogen peroxide and glacial acetic acid (1:1) and heated in oven at 60° for 12h. Isolated cells were stained with 1% Safranin or 12:25% basic fuchsin, in 50% ethanol and mounted in 50% glycerin solution. To detect lignified secondary wall we performed a histochemical test with acidic phloroglucin (phloroglucinol, HCl and ethyl alcohol) in which the lignified cell wall become violet-red (Berlyn & Miksche 1976).

We analyzed and photographed the sample under a microscope. The descriptions of the wood constituents followed the guidelines of IAWA Committee (2004). We identified and delimited the growth rings using the stereoscopic microscope at 40X.

Growth modeling

After we measured the width of the rings, we generated the individual radial increase rates, from which we obtained the rates of mean diameter increment (MDI) in millimeters. The cumulative diameter growth curves were built, which were fitted to the DBH obtained in the field (Brienen & Zuidema 2006). Through the adjustments of the cumulative diameter growth curves, we obtained the relationship between age and diameter of individuals (Stahle et al. 1999, Schöngart et al. 2007, Schöngart 2008). Based on the individual cumulative diameter growth curves, we calculated the mean diameter growth curve, which was fitted to a sigmoidal regression model (Schöngart et al. 2007, Schöngart 2008). The relationship between DBH and the height of trees sampled (H) was adapted to a non-linear regression, according to Schöngart et al. (2007) and Schöngart (2008). The regression models were produced using the software Xact (SciLab).

Population structure

We sampled monodominant stands of *E. fusca* in eight areas, distributed throughout the TES (over 115 km²) and in each area we established three 50x5 m plots with a minimum distance of 20 m between them, totaling 0.6 ha. We allocated the plots in order to best show different elevations and respective flooding durations of the Paraguay River levees. The monodominant stands of *E. fusca* occurred only in the northwestern subregion of the Pantanal, thus we distributed the plots in the study area between the coordinates 16°50'58.2"S 57°28'25.7"W and 16°52'57.4"S 57°30'22.2"W. Trees with circumference at breast height (CBH) was ≥ 15 cm were included in the sampling. Bifurcated stems were only included if at least one had a circumference ≥ 15 cm. We did not find individuals below 15 cm of CBH. We transformed the data of CBH into DBH (diameter at breast height) before performing analyses. We also measured the height of trees with a digital distance measurer.

Using the population sampling data, we constructed a correlation of diameter with height of the individuals, adapted to a nonlinear regression (Schöngart et al. 2007). In addition, using the values of DBH and the age of each sample we estimated the age of the individuals included in the population sample.

We also distributed the individuals in classes of diameter, with intervals of classes defined by Spiegel's formula (1976) constituted by A/K , where A represents the breath of data (diameter) and K the algorithm of Sturges: $K = 1 + 3,3 \log N$, where N is the number of individuals sampled. Considering the relationship between diameter/age of individuals sampled for dendrochronological analyses, we estimated the age of all the individuals sampled from the population.

Considering that, fewer individuals in larger diameter classes are expected in tropical forests (Meyer 1952), we were unable to perform a direct analysis between the environmental factors and the number of individuals in each diameter class. Therefore, we used the

distribution of individuals in the first five diameter/age classes (classes with more than five individuals, totaling the last 35 years), to build series containing maximum and minimum values of flooding levels and total precipitation data for the years comprised into each class. To verify differences in the levels of these factors between age groups we performed analysis of variance followed by the Tukey 5% test using R software and the package agricolae (Mendiburu 2016).

We determined the duration of flooding of each plot, first we measured from the ground to the highest water mark left by the last inundation on tree trunks inside the plots to obtain the flooding level of each plot. Then, we obtained an average of the water marks per plot and compared these values with the highest level recorded at the hydrometric gauge of Cáceres during that year of sampling. Considering that the overall topography is very flat and there are no tributaries between the gauge and the sampled area, we considered variation in water level to be the same. Thus, we used ten years of data to calculate an average period of flooding for each area.

We observed that the monodominant stands of *E. fusca* in the Pantanal subregion of Cáceres were subjected to different periods of flooding, ranging from 42 to 117 days per year (unpublished data - see Chapter 1). Therefore, to observe the differences in the distribution of individuals in diameter and age class submitted to different flood periods, we divided the plots in two parts (i.e. half the total length of the inundation gradient) based on the results obtained by Damasceno-Junior et al. (2005) and Arruda et al. (2016). Therefore, half of the plots were in the first category (flooding between 42-78 days per year) with 62 individuals, while the second category (flooding between 79-117 days per year) presented 91 individuals

To compare the number of individuals between the diameter classes in both flood periods (42-78 and 79-117) we used a chi-square test, using R software (R Development Core Team).

We repeated this test to compare the number of individuals between the first two classes for each of the periods, we disregarded the other classes in this comparison due to the expected natural decrease in the largest diameter classes.

Environmental variables

We used the weather data corresponding to the period from 1981 to 2014. We obtained the precipitation data from KNMI Climate Explorer (https://climexp.knmi.nl/select.cgi?id=someone%40somewhere&field=cru_pre). We obtained the data on daily flood level of the Paraguay River in the study subregion from the Brazilian Navy (Marinha do Brasil - <http://www.mar.mil.br/ssn-6/>). The records of *El Niño/La Niña* events (*El Niño 1+2*, *El Niño 3*, *El Niño 3.4* and *El Niño 4*), Pacific Decadal Oscillation (PDO), and Southern Oscillation Index (SOI) were obtained from the database available on the National Oceanic Atmospheric Administration (NOAA http://www.esrl.noaa.gov/psd/gcos_wgsp/Timeseries/TNI/).

Chronology and correlations with climatic variables

We used standard dendrochronological techniques to cross-date the time series of different individuals and combine these time series with a main chronology (Stokes & Smiley 1968). We used the software TSAP-WINTM to describe the similarity between individual curves. To obtain the similarity measures we used Student's T-values (Baillie & Pilcher 1973) and the percentage of parallel run to indicate the year-to-year agreement in the oscillation of two curves within the overlapping interval (Schweingruber 1988).

We tested the significance of these relationships between climatic factors and chronology of *E. fusca* using correlation matrices in the software STATISTICA (StatSoft). We built the graphics using R software (R Development Core Team).

Results

Anatomical description and determination of growth rings

The wood of *Erythrina fusca* is whitish and poorly lignified. The pores are distinct to the naked eye, form semi-porous rings, with a row of larger pores at the beginning of the growth layer, which are sparsely distributed and small sized toward the end of the layer. The pores are in dendritic pattern, with solitary (most) and multiple clusters. The axial parenchyma is visible to the naked eye and is confluent and in bands.

The growth layers are distinct and the beginning is marked by the distribution of the semiporous rings and the presence of parenchyma and few fibers. The rest of the layer shows a decrease in pore size and number, the end of the layer is marked by little (or no) pore and a band of parenchyma with flattened cells (Fig. 2 and 3). When looking at the samples with Mariaux window marks we noticed that soon after we made the scars, new rings started, so the beginning of the growth of a new layer occurred between the months of December and January.

Growth modelling

The ages of sampled individuals of *E. fusca* ranged from 6 to 34 years. The relation between age and DBH of the trees was highly significant ($r^2 = 0.84$, $p < 0.001$, Fig. 4). The maximum diameter increment rate of *E. fusca* was observed at 7 years (1.6 cm year^{-1}), after this period, the rate decreased linearly.

Diameter was highly related ($r^2 = 0.93$, $p < 0.001$) with height of individuals. The same was observed for individuals in the population structure samples ($r^2 = 0.74$, $p < 0.001$, Fig. 5), described by a nonlinear regression model.



Fig 2. Macroscopic detail of the *Erythrina fusca* stem disk collected in the Pantanal subregion of Cáceres, Mato Grosso, Brazil. The arrows indicate the delimitations of growth layers, with a band of parenchyma flat cells.

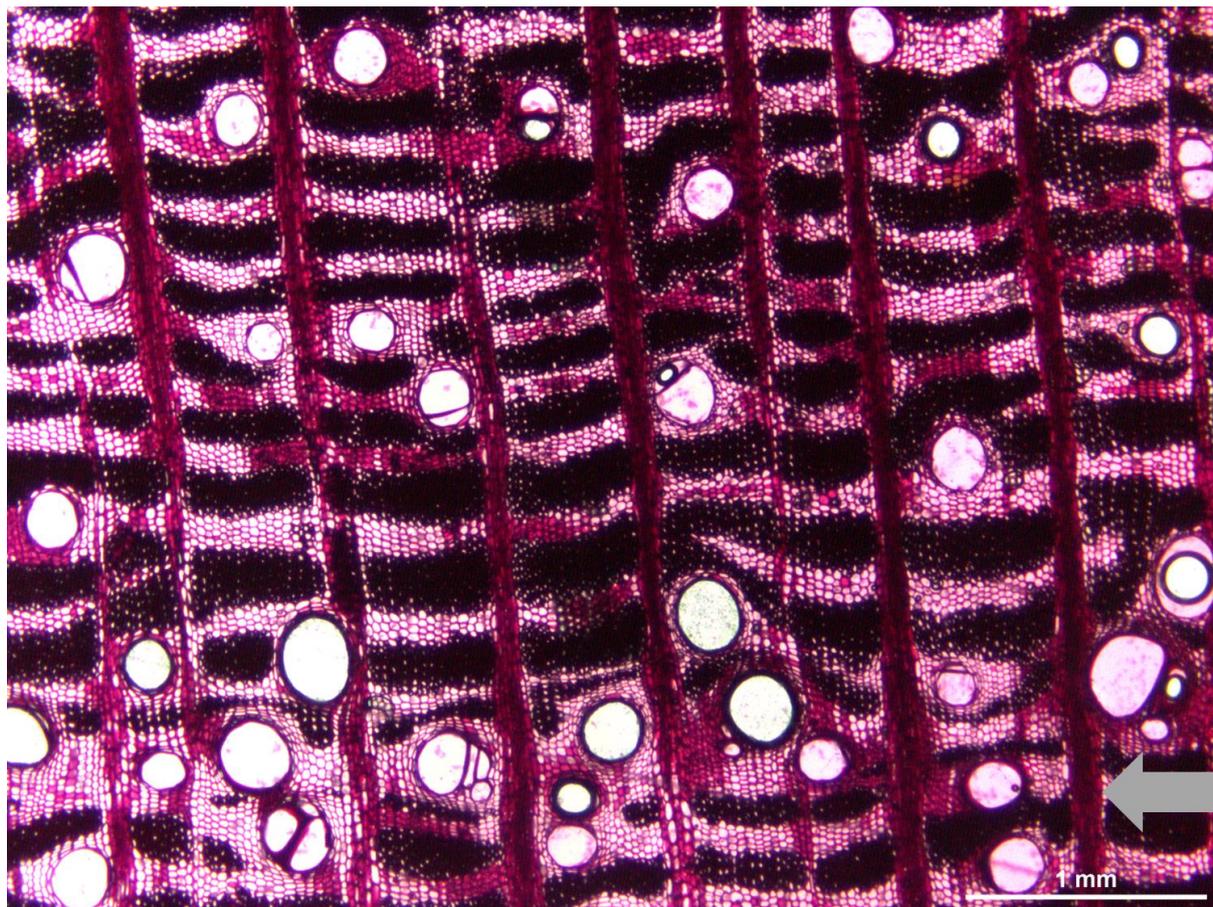


Fig 3. Microscopic detail of *Erythrina fusca* wood collected in the Pantanal subregion of Caceres, Mato Grosso, Brazil. The gray arrow indicates the region of the beginning of the growth layer, with a higher concentration of parenchyma and large pores.

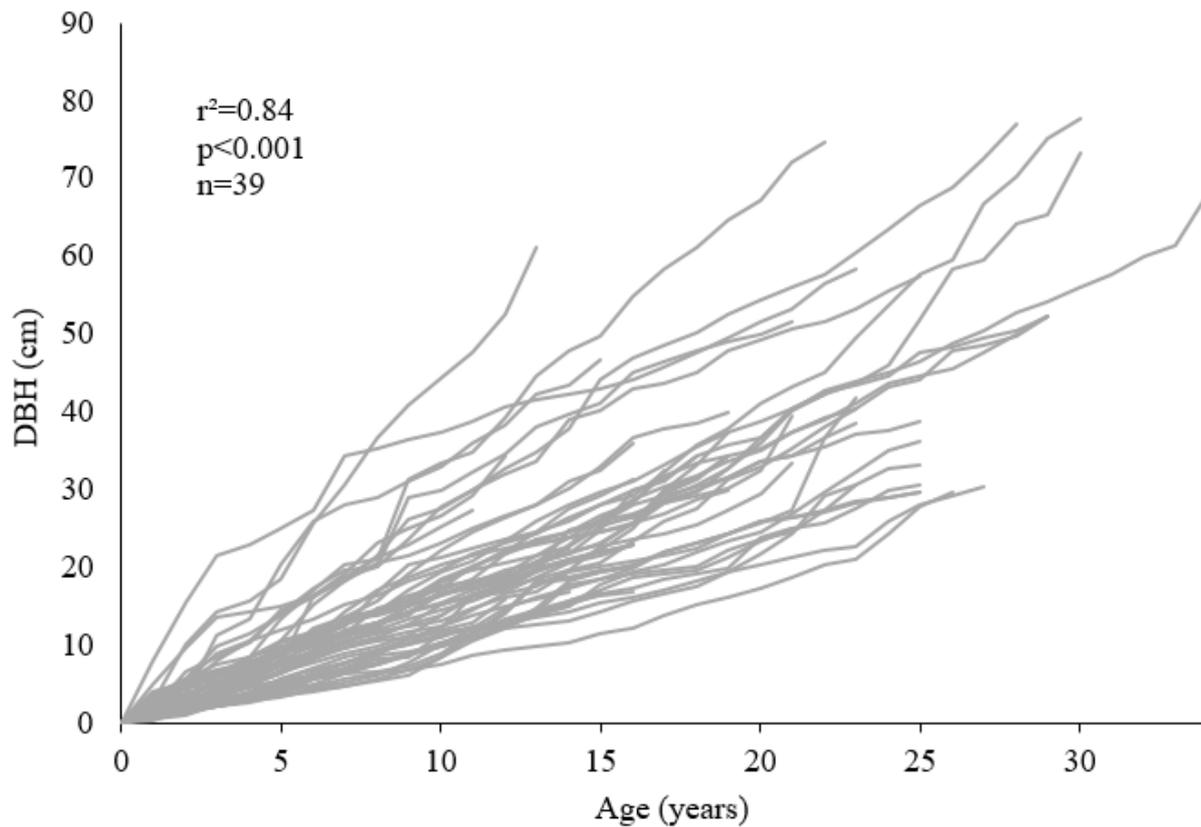


Fig. 4. Relation between diameter growth at breast height (DBH) and age of individuals of *Erythrina fusca* in the Pantanal subregion of Cáceres, Mato Grosso, Brazil. Lines represent the individual growth in diameter of each sample.

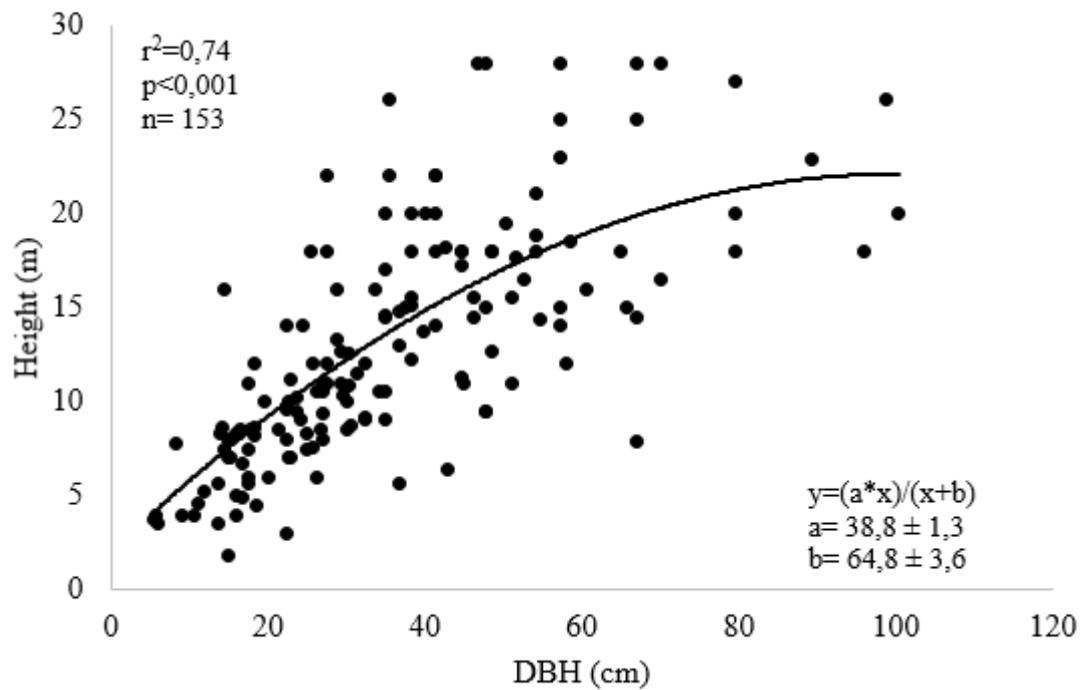


Fig. 5. Relationship between diameter at breast height (DBH) and height of 153 individuals of *Erythrina fusca* in the Pantanal subregion of Cáceres, Mato Grosso, Brazil, adjusted with a non-linear regression analysis

Population structure

We observed that areas with an annual mean of 42-78 days of flooding (Fig. 6a) showed a lower amount of individuals ($\chi^2=4.17$, $p=0.04$) in the first class of diameter compared with areas subjected to 79-117 days of flooding (Fig. 6b). Besides, only for areas with 42-78 days of flooding, we observed a significantly lower quantity of individuals at the first diameter class (individuals smaller than 16.97 cm and with less than 9 years) when compared with the next classes ($\chi^2=5.53$, $p=0.02$). The other classes follow the expected pattern of reverse J and most of individuals established between the middle and the end of the 1990s. For the older classes we observed a pronounced decrease in number of individuals, with a maximum age of 54 years.

We did not observe differences between the levels of precipitation and maximum flooding between the last 35 years evaluated, but we observed a significant decrease in the minimum flooding levels in the last 9 years (average of 0.84), which corresponds to first-class diameter, when compared to the previous 26 years (averages of 2.20, 2.04, 1.38, and 1.21 for classes 5, 4, 3 and 2 respectively).

Chronology and correlations with climatic variables

The construction of an average chronology was possible for the period of 1981 to 2014 (Fig. 7). We did not observe correlation between the width of the rings and the precipitation and flooding data from the study subregion, and we did not observe correlation with the PDO or SOI data, indicating that the growth of *E. fusca* is not affected by these factors in this subregion. However, we observed a negative correlation between the width of the rings obtained in our mean chronology with data from the regions with *El Niño*: *El Niño* 1+2 and *El Niño* 3.4 ($r = -0.40$ and $r = -0.41$ $p < 0.05$, respectively), (Fig. 8a and 8b).

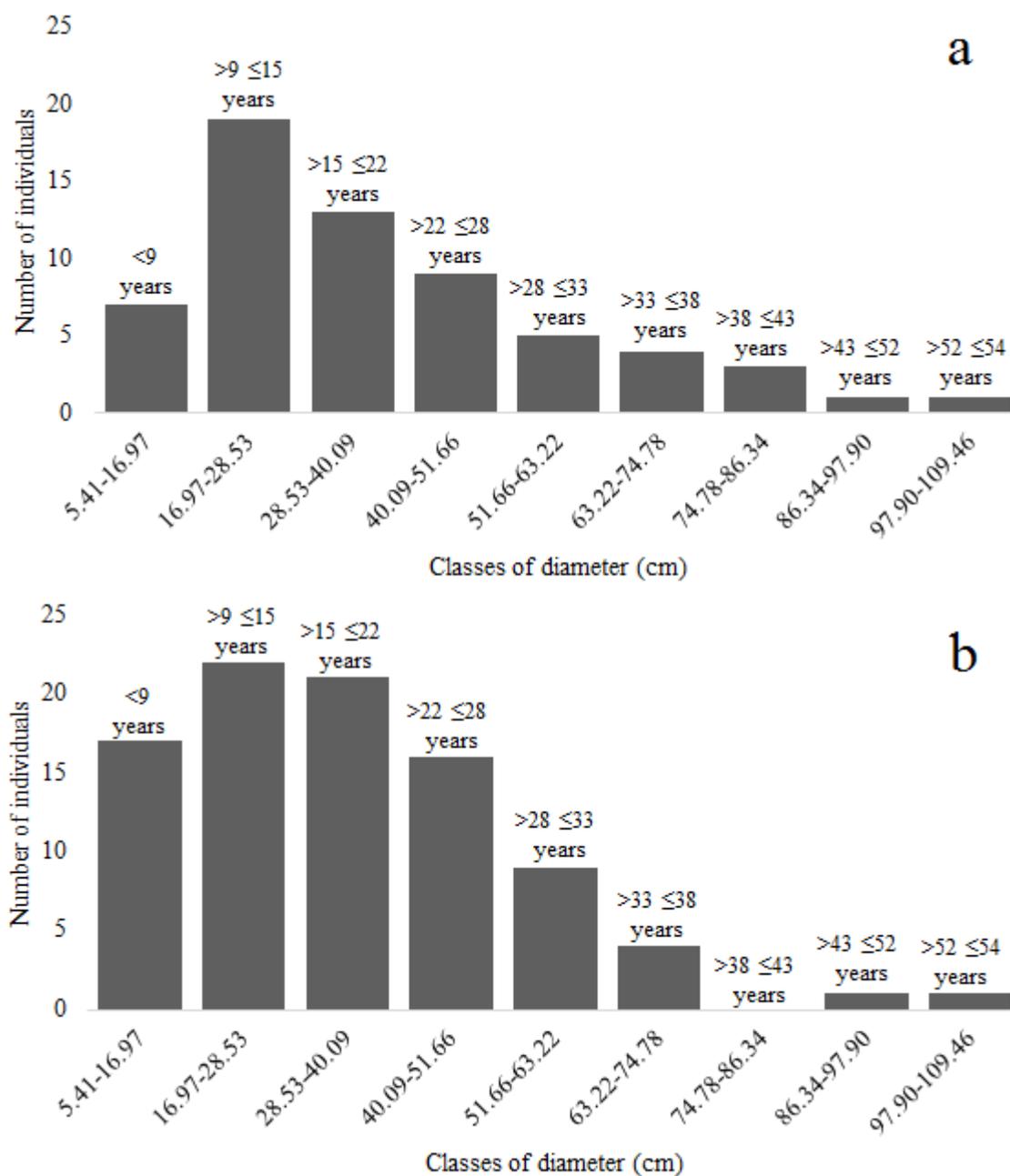


Fig. 6. Number of *Erythrina fusca* individuals collected in the Pantanal subregion of Caceres, Mato Grosso, Brazil, distributed in diameter classes (DBH) and estimated age between classes, a) individuals occurring in areas where the flood period varies between 42-78 days/years, b) individuals occurring in areas where the flood period varies between 78-117 days/years.

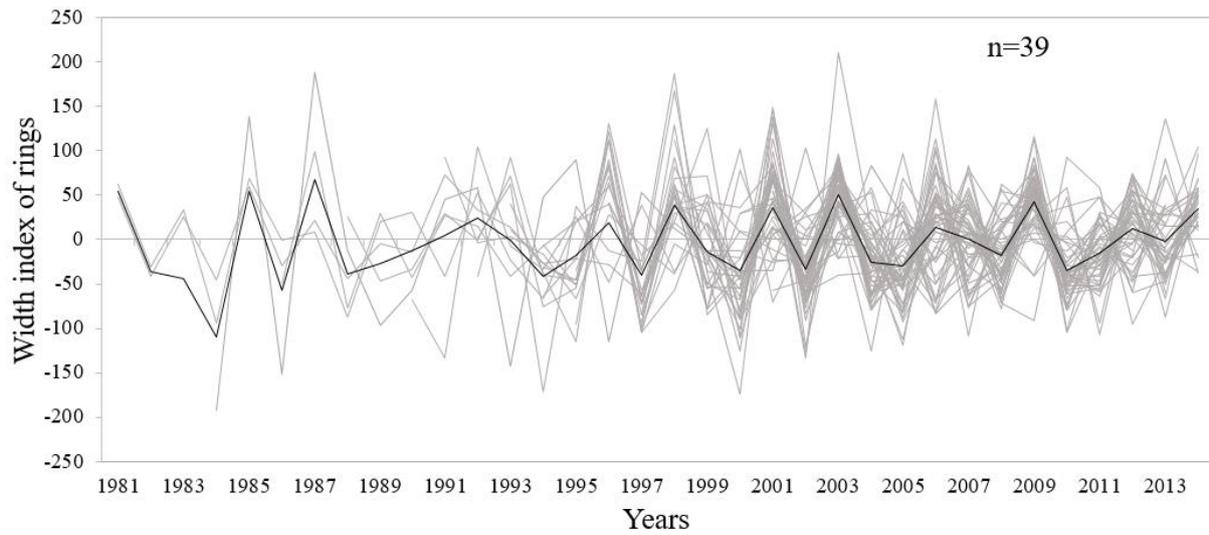


Fig. 7. Indexed chronology of annual rings of *Erythrina fusca* trees sampled in an area of monodominance in the Pantanal subregion of Cáceres, Mato Grosso, Brazil. The gray curves represent the individual indexed curves and the black curve represents the average chronology.

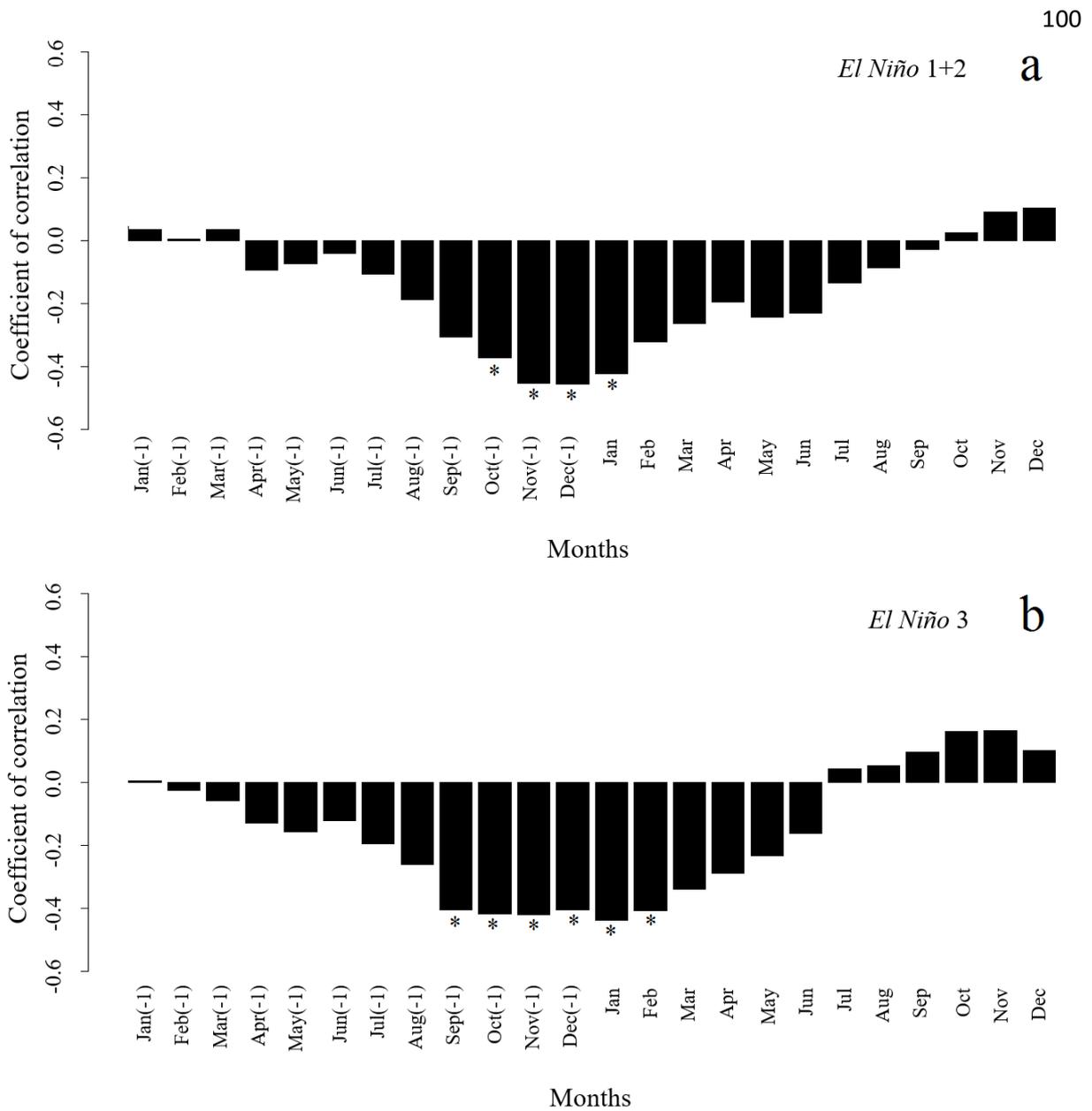


Fig. 8. Correlations between the anomaly indices in the *El Niño* 1+2 (a) and *El Niño* 3 (b) regions and the *Erythrina fusca* ring width index sampled from a monodominant stand in the Pantanal subregion of Cáceres, Mato Grosso, Brazil. Months from the previous year are indicated by (-1). Bars with * represent significant correlations for each month ($p < 0.05$).

Discussion

The positive relationship between diameter and height of *Erythrina fusca* in the study region allowed us to extrapolate the structure of the population, as we observed a decrease in diameter of individuals from the seventh year onwards, and we noticed that the height of most individuals in monodominant population was uniform.

The dendrochronological analyses showed that the individuals of *E. fusca* were young, and the oldest sample we found was estimated to be 34 years. Although young, the individuals sampled were large, what is probably due to the rapid increase in diameter of the species, especially in the first years of life, which is an expected pattern of this pioneer species.

When analyzing the population as a whole, we observed that, regardless of the period that individuals remain flooded (42-78 or 79-117 days of flooding), the majority is younger than 22 years, with few individuals over 40 and maximum estimated age of 54 years. This low longevity of individuals is probably due to the wood's characteristics, since it has poorly lignified fibers with a large amount of parenchyma. In the field, we observed that the trees of *E. fusca* break easily due to drastic environmental events such as storms and after such breakage, the stems disintegrate rapidly. In addition, we observed that the wood of the species is often attacked by wood feeder insects, which may influence its low strength and durability.

In relation to tree diameter distribution, the expected pattern for tropical forests is a reverse J-shaped distribution of individuals in diameter classes, with most individuals in the younger classes and few individuals in the older classes (Meyer 1952). However, our results showed that the first diameter class (5.41-16.97 cm), with an estimated age of less than 9 years, presented fewer individuals than the other classes. When we analyzed the climatic history of the region, we observed that in the last 9 years there was a decrease in the minimum

flood level and, consequently in the number of days these areas remained flooded. This leads us to believe that longer periods of flooding favor the establishment of *E. fusca*, which was apparent when we observed that areas subject to longer flood periods (79-117 days, Fig. 5b) showed a greater number of individuals in this first class of diameter when compared with areas subject to shorter periods (between 42-78 days) (Fig. 5a). This is also supported by the increased seed germination under longer flooding (unpublished data - see Chapter 2).

Conditions greatly affect tree growth, thus conspecific individuals can present variable growth rates depending on the conditions they are exposed to (Worbes 1989, Schöngart et al. 2005). When we analyzed growth, the formation of new layers seemed to start between the months of December and January, during which time precipitation and flooding begin to increase in the subregion. This is an unusual pattern, since for most species the wood growth seems to occur mainly during the terrestrial phase, and is stagnant during the aquatic phase (Worbes 1989, 1995, Schöngart et al. 2002). Despite the evidence that growth starts during the period of increased flooding and precipitation, there was no direct correlation between these two factors, that is, although the flood influences the establishment of the species (Worbes 1989), *E. fusca* growth does not seem to have a direct correlation with this factor.

Meanwhile, the events of *El Niño* showed a negative impact on the growth of *E. fusca* trees. The correlation of *El Niño* 1+2 data with the increment of rings occurred mainly during the months of October-December of the previous year and January of the current year, while for *El Niño* 3 were from September-December of the previous year and January-February of the current year. This period coincides with the months when precipitation and flood levels increase, and February is the month of maximum flood levels in the subregion. Analyses of time series of data obtained by Schöngart et al. (2004) indicated that during the last two centuries the severity of the *El Niño* effects has increased significantly. The events of *El Niño*

significantly reduce precipitation in the Pantanal during the period of October-December, which for *Vochysia divergens* also resulted in a reduction in the diameter increment (Fortes 2006, Schöngart et al. 2011). Thus, although we did not observe a direct response of the precipitation data with the growth of *E. fusca*, we observed a response regarding the effects of *El Niño* on precipitation.

We conclude that, from the factors analyzed, only *El Niño* has effects on the growth of the species in the study region, which is probably due to decreased precipitation at a time when levels should be higher (October-December). From the construction of a reliable chronology, we were able to estimate that the individuals found in this monodominance of *E. fusca* in the Pantanal subregion of Cáceres are quite young, with a maximum age of 54 years, but we could not infer about the age of the entire population, since it may have established long before that and individuals can be replaced. Besides that, we observed a reduction in the number of young individuals, which appears to be a response to the decrease in the minimum flood level in the study region over the past few years. This indicates a future trend that this monodominant population will undergo a decline, since in less floodable areas there was a drastic decrease in the number of young individuals. From these results, proposals aimed at protecting these formations can be supported, especially those preventing anthropic actions that lead to an even greater reduction in flood levels, such as dams for hydroelectric power and harbors, and deforestation of the river headwaters.

Chapter 4

Allelopathic potential of *Erythrina fusca* Lour. (Fabaceae), a monodominant species of the Brazilian Pantanal

Abstract

Erythrina fusca is a monodominant species in the Pantanal wetland, growing aggregate and represents more than half of the arboreal individuals in some areas. Many factors can influence the dominance of some species, such as allelopathy. We hypothesized that *E. fusca* has allelopathic potential, so we aimed to evaluate the effects of extracts on germination and development of *Lactuca sativa*. We collected leaves, bark, roots, and seeds during the dry season in the Pantanal subregion of Cáceres, Brazil. We tested 14 treatments, four replicas each, consisting of: negative control (water with buffer); positive control (2,4-Dichlorophenoxyacetic acid, 1% in distilled water), and three concentrations of each extract from leaves, bark, roots, and seeds of *E. fusca* (5, 1 and 0.2 mg.ml⁻¹). We calculated the percentage of germination, germination speed index, the percentage of normal seedlings and the normal seedlings speed index. Moreover, to evaluate the effects of the same treatments described above on growth of *L. sativa*, we subjected seedlings to the same treatments, measured root and aerial part length. Extracts from different parts of *E. fusca* showed an inhibitory activity on germination and formation of normal lettuce seedlings. Leaf extracts at 5 mg.ml⁻¹ caused a reduction in all analyzed parameters, while bark, roots (5 mg.ml⁻¹), leaves, and roots (1 mg.ml⁻¹) caused reduction in all analyzed parameters, but in lower magnitude. We also observed a significant reduction in root length induced by the highest concentration of all extracts (5 mg.ml⁻¹). Afterwards, we observed that all parts of *E. fusca* had adverse effects on the germination and/or development of *L. sativa*, and leaves had the highest potential. Considering that *E. fusca* presents allelopathic potential and that this monodominant

stand occurs in areas subject to flooding, which facilitates the leaching of secondary metabolites into the soil, this seems to favor the dominance of this species.

Keywords: allelopathy, monodominance, Leguminosae, Pantanal.

Introduction

In tropical regions, some species grow aggregate and present more than 50 % of the total number of trees belonging to a single species, such formations are called monodominant stands (Connell & Lowman 1989, Hart et al. 1989). Several factors seem to be commonly related with monodominance, such as flood patterns, fire events, conditions and characteristics of the soil, among others (Hart 1990, Torti et al. 2001, Peh et al. 2011). Another mechanism that may influence dominance of some plant species is allelopathy, since this process can affect plant-plant and plant-environment interactions (Hart 1990, Macías et al. 2008). According to Rice (1984), allelopathy refers to any direct or indirect effect which one plant species performs on another, and secondary metabolites as flavonoids, saponins, and alkaloids carry out this action.

Erythrina fusca Lour. (Fabaceae) is the most widespread species of the genus *Erythrina* (Russo & Baguion 1997). It is one of some monodominant tree species found in the Brazilian Pantanal. It occurs frequently in riparian forests, mainly in the Amazon region and Pantanal, but is only monodominant in the plains of the northern Paraguay River, in the Cáceres subregion of the Pantanal (Lorenzi 2002, Pott et al. 2011).

The genus *Erythrina* is rich in secondary metabolites, such as tetracyclic alkaloids (erythrina alkaloids), terpenoids, flavonoids (especially pterocarpanes), coumarins, and saponins (Tanaka et al. 2002, Yenesew et al. 2003 Juma & Majinda 2004, Dao et al. 2009, Pérez et al. 2015). Studies of metabolites from *E. fusca* confirmed the presence of flavonoids, such as pterocarpanes (Innok et al. 2009, 2010).

In the field, we observed that these monodominant communities of *E. fusca* present low species richness for both arboreal and shrubby vegetation, and that the herbaceous stratum is composed almost exclusively of grasses. Thus, this low occurrence of other woody

species, combined with the presence of potential allelopathic compounds in the genus *Erythrina*, lead us to believe that *E. fusca* may have a potential adverse effect on the establishment of other species.

Therefore, we hypothesized that *E. fusca* has allelopathic potential, which may be retarding and/or inhibiting seed germination and development of other species. Furthermore, the aim of this study was to evaluate the effects of leaf, bark, root, and seed extracts on germination and development of *Lactuca sativa* L. (Asteraceae, lettuce).

Methods

Study area and plant material

Erythrina fusca is a deciduous species that blooms from May to September with fructification occurring in November (Lorenzi 2002). This species is monodominant in the Pantanal subregion of Cáceres, which is a low-density vegetation with a predominance of arboreal individuals of *E. fusca*, as well few individuals of other arboreal species. The herbaceous stratum is very homogeneous, dominated by grasses (Appendix 2 and Appendix 3), with soil covered by a histosol layer with leaf litter, primarily composed of *E. fusca* leaves (Appendix 7 and Appendix 8).

We collected leaves, bark, roots, and seeds during the dry season (November 2013), at Taiamã Ecological Station, between the coordinates: 16°50'58.2"S 57°28'25.7"W and 16°52'57.4"S 57°30'22.2"W. The regional climate is Aw according to the Köppen classification, with two seasons: dry season from May to September and rainy season from October to April. The average precipitation is 1227 mm, and the average annual temperatures are around 26 °C, we calculated these values from the data obtained from National Institute of Meteorology (INMET). We prepared a fertile sample using herbarium techniques (Bridson & Forman 2004, Mori et al. 1989) and the voucher was deposited in the CGMS Herbarium under registration CGMS 40967.

Plant extraction

We dehydrated plant material in an air-dry oven at 50°C for 24 hours. We briefly ground and homogenized each plant part separately in a Willey-type mill. The seed extract was prepared through percolation, using ethanol:water (7:3), with 3 days of extraction, 20 drops per minute. Other plant tissues were submitted to a pressurized fluid extractor (DIONEX® - ASE 150),

using a mixture of ethanol:water (7:3), with temperature of 130°C, static extraction time of 4 minutes, 150% volume wash, five cycles of extraction and 100 seconds of purge. The extracts were concentrated in a rotary evaporator, lyophilized and maintained at -20°C until tests were performed.

Bioassay and analysis

For the germination experiment, we used Petri dishes (9 cm in diameter) containing two sheets of filter paper, previously autoclaved. We solubilized and prepared all extracts in MES buffer (2-morpholinoethanesulfonic acid) 10 mM, pH 6.0.

The experimental design was completely randomized with 14 treatments consisting of MES buffer solution (negative control); solution of 2,4-Dichlorophenoxyacetic acid (2,4D) 1 % diluted in distilled water (positive control) and three concentrations of each extract of leaves, bark, roots and seeds of *E. fusca* (5, 1 and 0.2 mg.ml⁻¹). We used four replicas for each treatment, with 25 seeds of *Lactuca sativa* L. (Asteraceae; lettuce) and 2 ml of the solution for the respective treatment. We kept all dishes in BOD at 25°C and photoperiod of 12 hours. Every 12 hours during 7 days, we counted germinated seeds (seeds with at least 2 mm of radicular protrusion) and, on the last evaluation day, we counted the normal seedlings (well-developed, complete, proportionate, and healthy seedlings). We calculated the percentage of germination (G), germination speed index (GSI), the percentage of normal seedlings (NS) and the normal seedling speed index (NSSI). GSI and NSSI were calculated by adapting the formula according to Maguire (1962), for example, $GSI = G1/N1 + G2/N2 + \dots + Gn/Nn$, where G1, G2, Gn = number of seeds germinated in each count day (from first to last day) and N1, N2, Nn = number of days from first to last count day (Maguire 1962, Labouriau & Valadares 1976).

We also considered growth as a parameter to measure allelopathy potential. To evaluate the effects of the same treatments described above on growth of seedlings of *L. sativa*, we first germinated the seeds in autoclaved Petri dishes, which contained two sheets of filter paper moistened with 2 ml of buffer solution MES. After 3 days, we selected the normal seedlings, and transferred them to dishes containing the 14 treatments described above (4 replicates of 25 seedlings each). After 4 days, we measured the root and aerial part length of seedlings.

We used R software (R Development Core Team) to analyze the results with a two-way analysis of variance (plant part and concentration of the extracts), which was followed by a Tukey test (5%).

Results

Effects of leaf, bark, root and seed extracts on germination and seedling formation

We observed that extracts from different parts of *Erythrina fusca* presented an inhibitory activity on the germination and formation of normal lettuce seedlings, as well as on the germination and seedling speed indexes (Fig. 1). Leaf extract at a concentration of 5 mg.ml⁻¹ caused a reduction in all analyzed parameters, which were similar to the positive control (2,4-D). Bark and root extracts at 5 mg.ml⁻¹ concentration and leaf and root extracts at 1 mg.ml⁻¹ concentration also reduced all analyzed parameters, but with lower magnitude. Furthermore, seed extracts at 5 mg.ml⁻¹ concentration reduced only germination parameters, not seedling parameters.

Seed and bark extracts at concentrations of 1 and 0.2 mg.ml⁻¹, as well as leaf and root extracts at a concentration of 0.2 mg.ml⁻¹ did not present allelopathic activity on germination and normal seedling formation of *L. sativa*.

Effects of leaf, bark, root and seed extracts on developing of root and aerial part.

We observed a reduction of aerial part length caused by leaf extract at 5 mg.ml⁻¹, which is statistically similar to 2,4D control, but does not significantly differ from the other treatments. On the other hand, root length of lettuce exhibited a significant reduction in the highest concentration (5 mg.ml⁻¹) of all extracts with a similar mean to that observed for the 2,4-D control, but only seeds 5 mg.ml⁻¹ differing significantly from the negative control (Fig. 2).

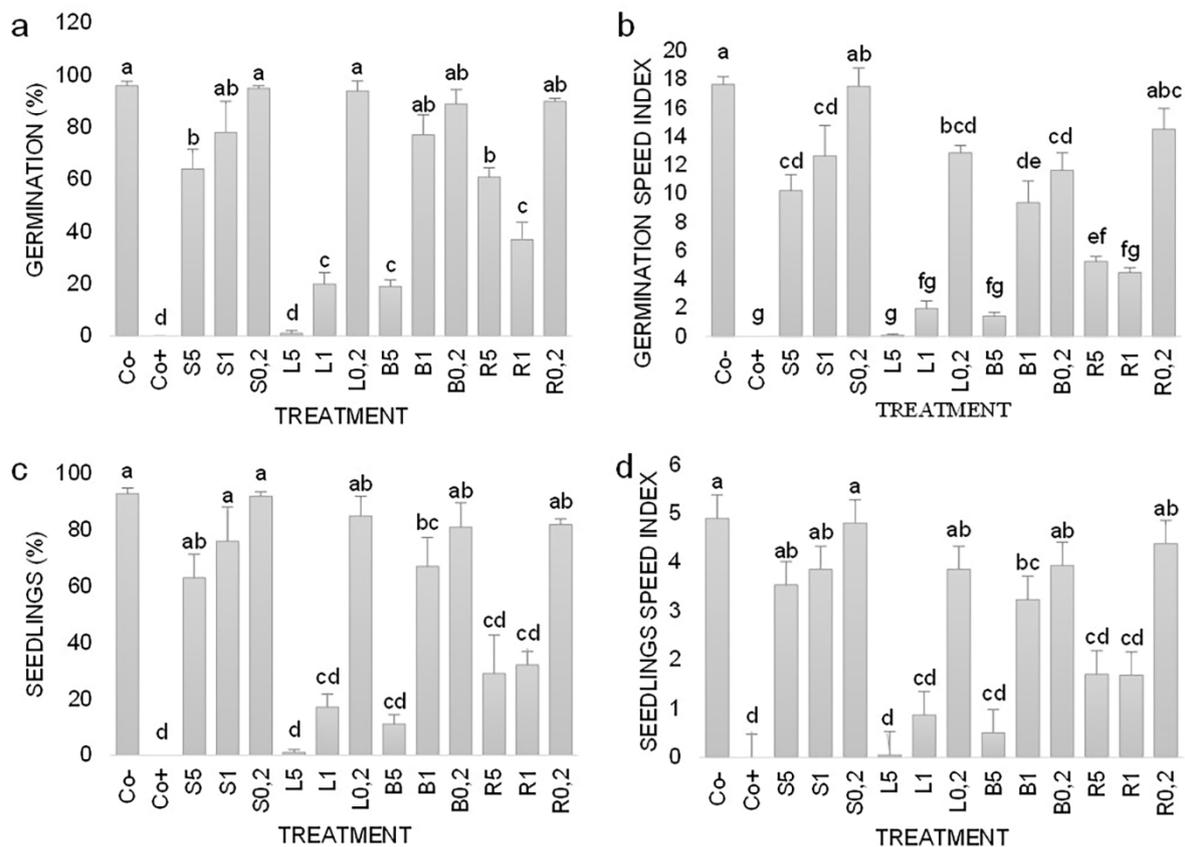


Fig. 1. Results of percentage of germination (a), germination speed index (b), the percentage of normal seedlings (c) and the normal seedling speed index (d), with default error of *Lactuca sativa* submitted to controls with water (Co-) and 2,4D (Co+) and the different concentrations of extracts of *Erythrina fusca*: seeds 5 mg.ml⁻¹ (S5); seed 1 mg.ml⁻¹ (S1); seed 0.2 mg.ml⁻¹ (S0.2); leaf 5 mg.ml⁻¹ (L5); leaf 1 mg.ml⁻¹ (L1); leaf 0.2 mg.ml⁻¹ (L0.2); bark 5 mg.ml⁻¹ (B5); bark 1 mg.ml⁻¹ (B1); bark 0.2 mg.ml⁻¹ (B0.2); root 5 mg.ml⁻¹ (R5); root 1 mg.ml⁻¹ (R1); root 0.2 mg.ml⁻¹ (R0.2). Different letters express statistically different means. Data is expressed as percentage means from experiments with four replicates of 25 seeds each. Different letters in the columns express differences detected with Tukey test ($p < 0.05$).

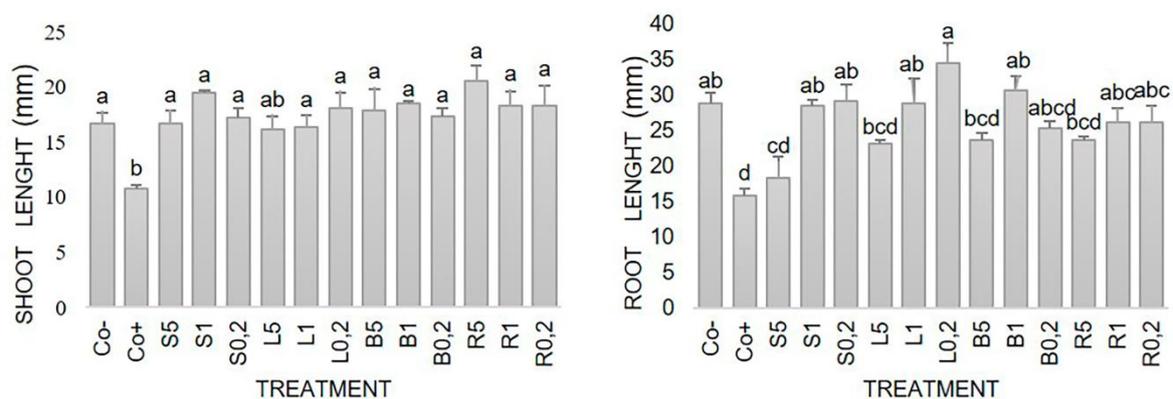


Fig. 2. Results of length of aerial part (LAP) and length of roots (LR), with default error of *Lactuca sativa* submitted to controls with water (Co-) and 2,4D (Co+) and the different concentrations of extracts of *Erythrina fusca*: seed 5 mg.ml⁻¹ (S5); seed 1 mg.ml⁻¹ (S1); seed 0.2 mg.ml⁻¹ (S0.2); leaf 5 mg.ml⁻¹ (L5); leaf 1 mg.ml⁻¹ (L1); leaf 0.2 mg.ml⁻¹ (L0.2); bark 5 mg.ml⁻¹ (B5); bark 1 mg.ml⁻¹ (B1); bark 0.2 mg.ml⁻¹ (B0.2); root 5 mg.ml⁻¹ (R5); root 1 mg.ml⁻¹ (R1); root 0.2 mg.ml⁻¹ (R0.2). Different letters express statistically different means. Data is expressed as percentage means from experiments with four replicates of 25 seeds each. Different letters in the columns express differences detected with Tukey test ($p < 0.05$).

Discussion

The tests of allelopathy allowed us to infer about the relationship between species that co-occur, and using the bioindicator *Lactuca sativa* is a great method for testing allelopathic potential, since it has a rapid life cycle and is highly sensitive to the action of allelochemicals (even in low concentrations) (Ferreira & Aquila 2000), as all stages of development are well known. Meanwhile, the germination and development patterns are not known for many wild species and it is difficult to infer about the actual effects of potential allelopathic effect on these wild plant species. Therefore, the results of the experiment using *L. sativa* are quick and easy to understand, because if we regulate all external factors and compare percentages of germination and/or development of *L. sativa* with controls (negative and positive), any change in this trait could be attributed to the adverse potential of the tested extracts. Furthermore, considering that *E. fusca* affects the development of *L. sativa* seeds, we can infer about its probable effect on wild eudicots.

We observed that different parts of *E. fusca* have allelopathic potential in varying degrees. The presence of allelopathy is a common in species of Fabaceae (Oliveira et al. 2008, Cândido et al. 2010, Aguilera et al. 2015, Id et al. 2015). Studies with *Erythrina velutina* (Centenaro et al. 2009, Oliveira et al. 2012) and *E. speciosa* (Soares et al. 2002) showed that different parts of the plants affect the germination and development of *L. sativa* seeds. The differential toxicity between leaves, bark, roots and seeds for *E. fusca* may be due to a differentiated pattern of allelopathic substances, which may vary in composition and concentration.

Those species of *Erythrina* are excellent sources of secondary metabolites, such as tetracyclic alkaloids, flavonoids, coumarins, and saponins (Tanaka et al. 2002, Yenesew et al. 2003, Juma & Majinda 2004, Innok et al. 2009, 2010, Pérez et al. 2015). These secondary

metabolites are essential for plant defense and signaling, are representative of the chemical composition of the soil, where they participate in different interactions. Also, these metabolites are known as classes with allelopathic activity (Rice 1984, Ferreira & Aquila 2000). Thus, the observed allelopathic potential of *E. fusca* may be related to such secondary metabolite compounds.

According to Ferreira and Aquila (2000), metabolites vary in different parts and tissues of the plant, in concentration, location, and composition and can be excreted into air, soil, or leached. In this study, we observed that among the four analyzed parts, the leaves of *E. fusca* presented the highest presence of allelopathic potential. The monodominant stands of *E. fusca* showed a histosol with higher levels of organic matter, a layer of leaf litter, and very homogeneous rotten plant matter mostly composed of *E. fusca* leaves. In addition, we observed a superficial water table in these areas, suggesting that the soil essentially remains waterlogged, even in the dry season, which may allow the compounds present in the litter to persist there. In this way, the continuous leaching of the litter, mainly composed of *E. fusca* leaves, may influence the germination and development of other species.

For these compounds to be effective in nature, they must be produced in large quantities and released to the soil through active secretion by the rhizosphere or by leaching of leaves, bark, roots or seeds. Thus, taking into account the particularities of each type of soil, the concentrations must be sufficiently high in the soil to reach inhibitory levels (Wink & Latz-Brüning 1995). Our results corroborate this, since for all parts of the plant at concentration 5 mg.ml^{-1} we observed an effect on growth and development of the roots of *L. sativa*, which means that, at high concentrations, all tissues of *E. fusca* have allelopathic potential.

Thus, we observed that all parts tested of *E. fusca* had adverse effects on the germination and/or development of *L. sativa*. The leaves had the highest potential, and this species has intense leaf loss and deposition in the litter, which may increase the leaching and release of allelopathic compounds in the soil. Thus, considering the presence of allelopathic potential and that this monodominant stand occurs in areas subject to flooding, which provides more favorable conditions for the release of compounds into the soil, the dominance of *E. fusca* seems to be facilitated by the inhibition of development of other species.

General conclusions

We observed a robust relationship between edaphic conditions, flooding duration and the dominance of *E. fusca*. The incidence of dominant stands of *E. fusca* at Taiamã Ecological Station in Pantanal subregion of Cáceres appears to be significantly related to low fertility and higher levels of sulfur in the soil and less affected by duration of flooding.

We found that dormancy, germination, and seedling formation of *E. fusca* are affected by flooding and fire and we observed that the metabolic responses were very similar for both stress factors, with an increase in the intensity of defense compounds in seeds and seedlings.

We detected that *El Niño* events effected growth of *E. fusca* in the study region, which may have been due to a decrease in precipitation level caused by *El Niño*. We also perceived a reduction of young individuals, which seems to be a response to the decreased flooding level in this region over the past few years.

We also observed that the leaves of *E. fusca* have a high allelopathic potential, which is an interesting information considering that this species has intense leaf loss and deposition in the litter. Such events can provide more favorable conditions for the release of compounds into the soil, thus, the dominance of *E. fusca* seems to be facilitated by the inhibition of development of other species.

We conclude that the dominance of *E. fusca* has a strong relationship with higher flood levels, some soil characteristics, and other environmental and intrinsic characteristics, which may influence its occurrence, establishment, germination and release of allelopathic compounds.

Therefore, changes in these environmental factors, to which this monodominance is strictly linked, may be detrimental to the occurrence of this important plant formation. The conservation of this formation depends on the maintenance of environmental conditions, thus,

proposals aimed at protecting these formations can be supported, preventing anthropic actions especially that lead to an even greater reduction in flood levels, such as deforestation of the river headwaters, dams for hydroelectric power and harbors.

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Appendix



Appendix 1: General view of a monodominant stand of *Erythrina fusca* on the Paraguay River, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 2: Internal view of a monodominant stand of *Erythrina fusca* on the Paraguay River, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 3: Internal view of a monodominant stand of *Erythrina fusca* on the Paraguay River, Pantanal subregion of Cáceres, Mato Grosso, Brazil, evidencing the abundance of grasses and other herbaceous species.



Appendix 4: The inflorescence of *Erythrina fusca* with yellow-orange flowers. Paraguay River, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 5: Inflorescences of *Erythrina fusca* on the Paraguay River, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 6: Young individuals of *Erythrina fusca* associated with floating histosol, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 7: Prop roots pattern of *Erythrina fusca* and litter layer in monodominant stands, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 8: Litter layer in monodominant stands of *Erythrina fusca*, Pantanal subregion of Cáceres, Mato Grosso, Brazil.



Appendix 9: Mariaux Windows in stem of *Erythrina fusca* in monodominant stands, Pantanal subregion of Cáceres, Mato Grosso, Brazil.