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Exploring reproductive biology in *Eucalyptus globulus* clones under distinct climatic conditions

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Abstract
Despite the great economic benefits, one of the major threats of introducing species into exotic ranges is their ability to regenerate and spread to novel environments. For example, *Eucalyptus globulus* plantations have been established in many parts of the world, using seedlings or clonal material, the latter often from advanced generation tree improvement programs. However, major concerns exist about this species' ability to invade non-native areas. Therefore, it is necessary to study whether breeding programs affect this species' reproductive biology to assess the risk of invasiveness of these improved trees. In this sense, this work compared the *E. globulus* phenological behaviour and reproductive structures production of three clonal and one seedling-based stand across two distinct climatic zones (mesic and xeric) in Portugal. Through binocular observation, four different stands (seedlings descending from open-pollinated crosses and three clonal-stands) were monitored every three weeks for different reproductive phenological stages, namely the presence of flowers and capsules. Clonal stands presented a shorter flowering season, less abundant reproductive structures, and higher phenological variability across sites than seedling-based stands. Phenological patterns and fructification abundance also varied between the three clonal stands. These results suggest that clones with decreased reproductive ability can be chosen during the breeding process, lowering the dispersal risk while enhancing productivity. Thus, understanding the phenological behaviour of *E. globulus* is critical to more sustainable agroforestry by reducing the spread risks.

Keywords Exotic species · Flowering · Phenology · Reproduction · Tree improvement

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1 Introduction

Planted forests have reached nearly 300 million ha worldwide [1] due to the ever-increasing demand for essential forest products such as wood, fibre and fuel. Exotic species occupy approximately 19% of these planted forests [2], some based on genetically improved clones selected for better growth and wood properties [3]. However, despite their economic and social benefits, exotic plantations frequently raise concerns about their potential for naturalisation and dispersal, which could affect ecosystem functioning and biodiversity [4–7].

Eucalyptus globulus Labill. (Tasmanian blue gum) is one of the world's most widely planted forest species due to its excellent wood properties for the pulp and paper industry, rapid growth, and adaptability [8]. It is native to Australia, specifically Southern Victoria, Tasmania, and the Bass Strait Islands [9], with up to 13 geographical races and 20 subraces described [10]. This species was introduced into Portugal around 1829 [11] and now covers approximately 850.000 ha, making it one of the country's dominant forest types [12]. Since 1966, pulp companies in Portugal have been working on *E. globulus* genetic improvement programs based on local landrace populations and provenances introduced from Australia [11]. These programs aim to improve tree growth, adaptability, and wood quality [13]. As a result, wood productivity has increased between 25 and 50% [14], and trees are more resilient to biotic and abiotic stresses [15]. However, reforestation with genetically improved trees has been mainly carried out on land managed by pulp companies, specifically through clonal plantations [16], and many eucalypt plantations in Portugal are still based on unimproved seeds.

The phenological development of *E. globulus* takes about one year, from flower bud initiation to seed set, with trees producing flowers at four years old [17, 18]. Flower buds form from the current season's shoots, and their maturation result in the formation of flowers that are fully receptive to bird and insect pollination, particularly bees [19, 20]. Eucalypt flowers are bisexual, but the breeding system is a mixed mating system with preferential outcrossing [21], with flowers potentially pollinated by many male parents, mostly from nearby trees with synchronised flowering [22]. Outcrossing is potentiated by protandry mechanisms, which cause the stigma to become fully receptive a few days after the flower's pollen matures and sheds [22]. Fruits are woody capsules with triangular valves on top [21] that change colour from green to brown as they mature over several months [22]. *Eucalyptus globulus* is a serotinous species that stores capsules for several years in the canopy while attached to tree branches, forming a canopy seed bank [23] critical for seedling recruitment and dispersal events [24].

Temperature and moisture significantly impact eucalypt flowering [21], which is especially relevant as climate change affects air temperature and precipitation patterns [25]. Furthermore, as reproductive structures require favourable environmental conditions, the reproductive phase is extremely vulnerable to climate change [26], which affects the timing of phenological events [27].

Concerns have been raised about *E. globulus*'s ability to regenerate and establish outside planted areas and become invasive [28–32]. However, while quantitative studies have shown that its dispersal capacity is limited [33–35], regeneration can be abundant, particularly after fire [36–38]. Even if the intrinsic invasive capacity of *E. globulus* is limited, the large areas occupied by this species in some parts of the world raise concerns. Furthermore, as areas planted with improved trees are expanding, it is critical to investigate the impact of tree breeding programs on *E. globulus* reproductive biology due to their eventual effects on regeneration and invasiveness capacity, to better understand how to handle propagules and protect ecosystems near plantations. This concern was raised by Águas et al. [39], who suggests a higher potential for greater fertility in these improved trees. To address these issues, the aim of this study was to identify the influence of stand type (clonal and seedling-based trees) and climate (mesic and xeric conditions) on the timing of the phenophases and production of reproductive structures of *E. globulus*.

2 Materials and methods

2.1 Study sites

This study was conducted in two sites located in different climatic regions in Portugal: Coimbra (40º10′19.6″ N, 8º33′58.3″ W, 65 m altitude) and Pegões (38°39′29.16″ N, 8°37′16.23″ W,

85 m altitude) (Fig. 1). The Coimbra site is cooler and wetter than Pegões. Coimbra's mean annual maximum and minimum temperatures are 21.6 ℃ and 10.8 ℃, respectively, while in Pegões, it is 22.3 ℃ and 11.5 ℃, respectively.

Fig. 1 Study site location: Coimbra (mesic site) and Pegões (xeric site)

Mean annual precipitation differed by 200 mm, with 872.9 mm and 672.5 mm in Coimbra and Pegões, respectively. According to Köppen-Geiger Climate Classification, Coimbra is classified as Csb, a warm-summer Mediterranean climate, while Pegões is classified as Csa, a hot-summer Mediterranean climate [40].

Both sites have a seasonal wet winter/dry summer pattern, with summer months presenting total mean precipitation of 50.8 mm in Coimbra and 18.9 mm in Pegões. August is the hottest month at both sites, with a mean difference of almost 2.5 ℃ (28.6 ℃ and 31.1 ℃, mean maximum temperature). As mentioned, winter precipitation (from December to February) is higher in Coimbra (315.7 mm compared to 250.8 mm), and January is the coldest month with 5.8 ℃ mean minimum temperature registered for Coimbra and 6.7 ℃ for Pegões [historical data 1990–2018, CRU-TS 4.03 [41] downscaled with WorldClim2.1 [42]. Hereafter, Coimbra will be referred to as a mesic site and Pegões as a xeric site. Climatic conditions (monthly total precipitation and mean temperatures) during our study were obtained from the closest meteorological stations and are presented in Fig. 2.

Both mesic and xeric sites exhibited similar weather seasonality with higher precipitation and lower temperatures in autumn and winter (October to February) and low rainfall and higher air temperatures in summer (June to August). The xeric site had higher maximum temperatures throughout the year, with the highest values recorded in July 2020 (32.6 ℃ in the xeric site and 31.4 ℃ in the mesic site). The beginning of 2019 was particularly dry at both sites compared with 2020 and 2021. However, winter 2019 in the mesic site was marked with considerable precipitation, 835.2 mm between October 2019 and April 2020, while the xeric site registered 392.8 mm. In terms of temperature, the minimum monthly values were registered in the xeric site, with the lowest temperatures recorded in January months in both sites: 4.8 ℃, 6.4 ℃ and 3.5 ℃ in the xeric site and 6.0 ℃, 7.2 ℃ and 5.5 ℃ in the mesic site. There was almost no summer precipitation in the xeric site (2.0 mm), while in the mesic site, the total precipitation of the three summer months was 92.3 mm.

2.2 Stand description

Both sites were under similar management regimes, with all stands located close to each other, with the same planting spacing (2 × 4 m) and having the same sun exposure. All stands are intensively managed with periodic understory clearings. The four different stands studied included one with plants grown from open-pollinated seedlings (uncontrolled crosses seedlings, SB) and three from clonal-origin, namely clones C1, C2 and C3. While SB stands have many different, non-improved genotypes, the clones are selected genotypes. Whereas clone C1 is a second-generation selection, a cross between a Portuguese landrace base parent and a "Strzelecki Range" (southern Australia origin) base parent, clones C2 and C3 are second-generation selections from unrelated crosses between Portuguese landrace base parents. Portuguese landrace mainly originated from southern and eastern Tasmania races [43, 44].

At the beginning of the study, stands at both sites have around six to nine years of age. The Perimeter at Breast Height (PBH) was measured in each tree (Table 1).

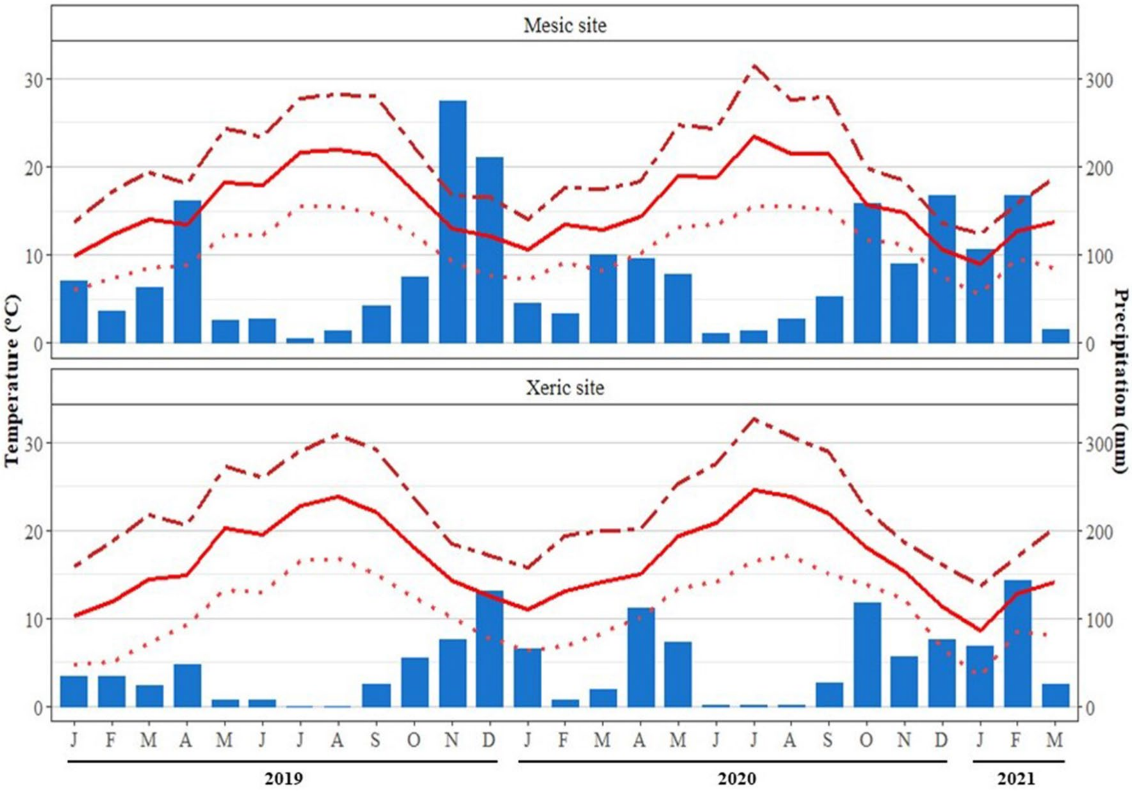

Fig. 2 Climatic conditions for each study site. Monthly average temperature [◦C] (dotted line—minimum temperature; filled line—mean temperature; traced line—maximum temperature) and monthly total precipitation [mm] (bars) in the two study sites (mesic and xeric) from January 2019 to March 2021 by nearby meteorological stations

Table 1 *Eucalyptus globulus* stand description. Characterisation of the four stands (seedling-based, SB, and clones C1, C2 and C3): tree cut rotation (Rotation), plantation date and Perimeter at Breast Height (PBH) at the beginning of the study (mean ± SE, in cm)

Site	Stand	Rotation	Plantation date	PBH (cm)
Mesic	**SB**	1	2012	63.8 ± 3.4
	C1	1	2012	57.7 ± 2.5
	C2	1	2012	54.8 ± 2.3
	C3	1	2012	52.2 ± 1.8
Xeric	**SB**	3	2010	88.7 ± 8.8
	C1	1	2013	50.4 ± 3.0
	C2	1	2013	59.6 ± 4.1
	C3	1	2011	65.6 ± 2.6

2.3 Data collection

At each site, 12 *E. globulus* trees from the four stands (clones C1, C2 and C3 and SB) were monitored every three weeks between 22 October 2019 and 17 March 2021. However, due to CoViD-19 legal restrictions, monitoring was interrupted on 6 March 2020 and resumed on 8 June 2020, resulting in four missed monitoring visits.

In each stand, trees were randomly selected along the plantation edge, where flowering is generally more abundant [45]. Each tree's genetic identity was confirmed in the clonal stands based on Simple Sequence Repeat (SSR) nuclear markers genotyping (viz [46]). Selected trees were interspersed, and each tree was regularly monitored for different reproductive phenological stages, namely the presence of flowers and capsules, through binocular observation. According to their position on the branch, capsules were assigned to different years of maturation, with older capsules remaining more basal. Since the phenological development of *E. globulus* take place between two different years [47], capsules that matured in 2018 were classified as 2018 capsules even though the flower buds that originated them appeared in 2017. The same principle was applied to the capsules maturated in 2019, 2020, and 2021. Once 2018 and 2019 capsules were present at the start of the study, only the maturation of 2020 and 2021 capsules was recorded. Following Barbour et al. [45], each reproductive stage was scored as a count on a logarithmic scale, with class 0 (absence of the stage), class 1 (1 to 10 reproductive structures), class 2 (11 to 100), and class 3 (≥ 101).

2.4 Data analysis

Statistical analysis was performed by transforming observation dates to julian days (jd), starting on 22 October 2019, corresponding to jd 295 and ending on 17 March 2021, corresponding to jd 806, by adding 365 days for each new year [48]. Among the 12 studied trees of each stand in each site, only those that presented reproductive structures were considered to compare the initiation, end and duration of each phenological stage between sites through the Wilcoxon-Mann–Whitney test and among stands in each site with the Kruskal–Wallis test with the Bonferroni adjustment method to adjust *p* values to multiple comparisons. Due to CoViD-19 legal restrictions, the end of 2020 flowering was not completely recorded. However, considering that *E. globulus* flowering in Portugal occurs primarily between November and March [47] and that flowers were in a declining phase on 6 March (the last record before the interruption), we assumed that the end of the flowering stage occurred on the next expected monitoring date (27 March) for statistical purposes.

The effect of stand type and site on each reproductive stage's intensity (scored in categorical classes) was evaluated using a Generalized Linear Mixed Model (GLMM) with Poisson and negative binomial families. For flower abundance, data of the two years of production were considered, with sampling year as a random factor, along with the monitored tree to account for these parameters' effect on the studied variables. Also, differences in reproductive stage intensity were evaluated between sites through the Wilcoxon-Mann–Whitney test and among stands in each site with the Kruskal–Wallis test with the Bonferroni adjustment method to adjust *p* values to multiple comparisons. Statistical analysis was performed using packages *agricolae* [49], *lme4* [50], *rstatix* [51] and *stats* in R software version 4.2.2 [52].

3 Results

3.1 General phenology pattern

3.1.1 The influence of genetic background

Clonal trees' phenological behaviour showed some differences comparing with seedling-based (SB) trees. Although no formal statistical analysis was performed to compare the percentage of trees showing reproductive structures in each stand, many trees did not produced any reproductive structure at both sites, particularly in clonal stands. For example, clonal C3 trees did not develop any reproductive structures in both sites throughout the monitoring period (or capsules matured in 2018 and 2019 were observed). Furthermore, clonal C2 trees did not produce flowers in the first year and exhibited the lowest frequency of the stands in the second year: seven out of twelve in the mesic site and five out of twelve in the xeric site. Moreover, in 2020, only seven SB trees and ten C1 trees produced reproductive structures in the xeric site (see Table 2). Also, it was observed in all stands that some trees produce flowers, but these do not transit to capsules. During the study, capsules from four seasons of maturation remained in the canopy at both locations, and only clonal C2 trees lost all their 2018 capsules in the mesic site (data not shown).

Differences in the initiation and duration of reproductive stages were observed when comparing clonal and SB trees within each site (Table 2). The onset of flowering (or anthesis) occurred in late autumn (end of November to early December), with capsules maturing throughout winter (from December to mid-February). In the mesic site, a delay in the onset of flowering of almost one month (end of January to early February, $p < 0.01$) was observed when comparing C1 and SB trees. In the second year, the same pattern of delayed emergence of flowers along with capsules in the clones was detected, but now for both C1 and C2 ($p < 0.001$), with clone C1 presenting again a later and shorter flowering ($p < 0.001$). In the xeric conditions, the phenological behaviour of SB and C1 trees was very similar in the first year, except for the flowering period, which was longer for the C1 clone ($p < 0.05$). In the second year, there was a much shorter flowering period, half of the duration of the other two stands ($p < 0.05$), in clone C2.

Table 2 Initiation and duration (mean ± SD) of reproductive phenological stages (flowers and capsules) for *E. globulus* seedling-based (SB) and clonal-based (clones C1, C2 and C3) stands during the first year (2019-2020) and the second year (2020-2021) in the mesic and xeric sites

			Phenological stages				
			Flowering			Fruit development	
First-year (2019–2020)							
Site	Stand	N	P	Initiation date	Duration (days)	P	Initiation date
Mesic	SB	12	8	9 Jan ± 25^{a}	64 ± 22^{a}	8	27 Feb ± 47^{a}
	C1	12	12	9 Feb ± 13^{b}	42 ± 11^{b}	12	6 Mar ± 10^{a}
	C2	12	0	–	–	0	–
	C3	12	0	–	–	0	–
Xeric	SB	12	6	9 Jan ± 19^{a}	53 ± 25^{a}	5	28 Jan ± 18^{a}
	C1	12	9	9 Jan ± 13^{a}	81 ± 19^{b}	9	24 Jan ± 13^{a}
	C2	12	0	–	–	0	–
	C3	12	0	–	–	0	–
Second-year (2020–2021)							
Site	Stand	N	P	Initiation date	Duration (days)	P	Initiation date
Mesic	SB	12	12	26 Nov ± 32^{a}	102 ± 37^{a}	12	25 Dec ± 16^{a}
	C1	12	8	13 Jan ± 15^{b}	51 ± 19^{b}	8	12 Feb ± 0^{b}
	C2	12	7	14 Dec ± 17^{a}	62 ± 29^{b}	7	2^{t} Jan ± 25^{c}
	C3	12	0	–	–	0	–
Xeric	SB	12	12	7 Dec ± 32^{a}	82 ± 37^{a}	12	9 Jan ± 36^{ab}
	C1	12	12	1 Dec ± 28^{a}	86 ± 44^{a}	12	20 Dec ± 30^{a}
	C2	12	4	25 Dec ± 11^{a}	32 ± 12^{b}	2	12 Feb ± 0^{b}
	C3	12	0	–	–	0	–

Different letters indicate significant differences between initiation and duration among different stands in each site. N represents the total number of trees monitored; P represents the number of trees that developed a specific stage. The absence of phenological stages is marked with "-"

3.1.2 The influence of climate

The phenological behaviour of clonal stands differed more between the two sites than that of SB trees (Fig. 3). Seedling-based trees only had statistically significant site differences for the end of the flowering period, which ended earlier in the xeric site, although only in the first year ($p < 0.01$). Clone C1 showed the highest contrast in reproduction events between sites. For both years, flowering and capsule maturation occurred later ($p < 0.01$) in the mesic site. Clonal C2 trees produced reproductive structures only in the second year of monitoring, without statistically significant differences between flowers and capsule appearance.

3.2 Abundance of reproductive structures

The abundance of flowers and capsules was affected by genetic background, site and its interaction. The generalized linear mixed model showed that SB trees produced higher amounts of all reproductive structures than clonal stands, whereas, between clones, clone C1 presented a higher abundance of structures than C2 trees (Table 3).

In the first year of monitoring, the abundance of reproductive structures in the mesic site was similar between SB and clonal C1 trees. However, a higher abundance of flowers in SB trees was observed on 20 January compared with C1 trees, and a higher abundance of capsules was observed in SB trees on 20 February. Nonetheless, the number of capsules produced by SB and clone C1 was similar. In the second year, SB trees had a greater abundance for all phenological structures, whereas clonal C1 and C2 trees did not show differences in most of the monitoring period (Fig. 4).

On the contrary, under xeric conditions, during the first year of monitoring, clonal C1 trees produced more flowers and capsules, reaching a pick of flowering on 20 January 2020, while in the second year, SB and clonal C1 trees produced similar values, with clone C2 produced fewer reproductive structures.

Between studied sites, clonal C1 trees produced more structures under xeric conditions, except for the 2020 capsule' production; when producing, clone C2 presented more structures in the mesic site.

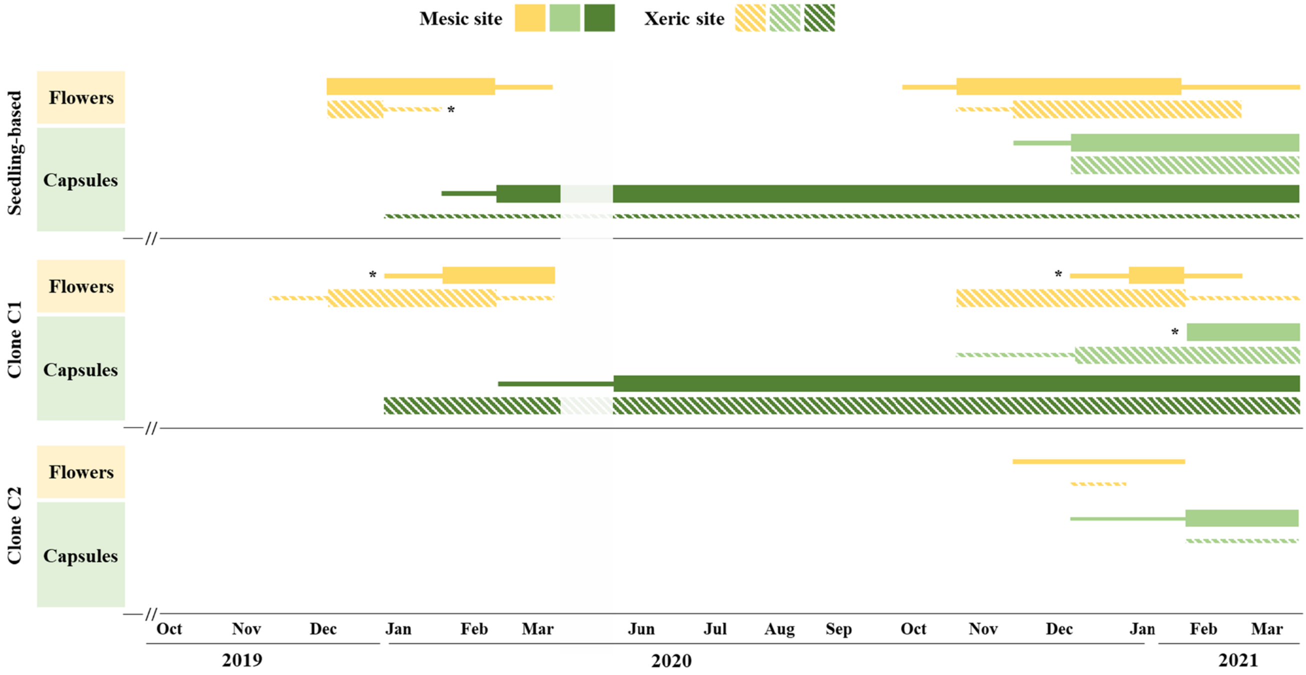

Fig. 3 Phenophase diagram. Comparison of flowers and capsules from seedling-based and clonal-based (C1 and C2) *E. globulus* trees in the mesic and xeric sites from October 2019 to March 2021. White shaded bars represent the interruption due to CoViD-19 legal restrictions. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown. Frequencies are indicated by lines (when 25–50% of the studied trees presented phenological structures) and filled bars (when over 50% of the studied trees presented phenological structures). Results for the mesic site are displayed in full lines and bars, while results for the xeric site are displayed in striped lines and bars. Significant differences between sites are denoted with "*"based on data presented in Supplementary Table 1

Table 3 Generalized linear mixed model (GLMM) using *Stand* (Seedling-based, Clone C1or Clone C2), *Site* (Mesic or Xeric) and their interaction to model *E. globulus* flower and 2020 and 2021 capsule production. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown

		Estimate	Std. Error	z value	Pr(>|z|)
Flowers	(Intercept)	− 0.628	0.13	− 4.800	***
	Clone C1	− 0.833	0.19	− 4.308	***
	Clone C2	− 1.656	0.25	− 6.724	***
	Xeric site	− 0.477	0.18	− 2.673	**
	Clone C1 * Xeric site	1.221	0.26	4.613	***
	Clone C2 * Xeric site	− 0.898	0.50	− 1.791	ns
2020 capsules	(Intercept)	− 0.227	0.14	− 1.577	ns
	Clone C1	0.133	0.09	1.387	ns
	Clone C2	NA	NA	NA	NA
	Xeric site	− 0.649	0.12	− 5.450	***
	Clone C1 * Xeric site	0.729	0.15	4.911	***
	Clone C2 * Xeric site	NA	NA	NA	NA
2021 capsules	(Intercept)	− 0.415	0.15	− 2.702	**
	Clone C1	− 1.303	0.30	− 5.948	***
	Clone C2	− 1.836	0.26	− 5.120	***
	Xeric site	− 0.053	0.20	− 0.267	ns
	Clone C1 * Xeric site	1.848	0.36	5.083	***
	Clone C2 * Xeric site	− 1.804	0.59	− 3.059	**

Coefficients of the model, standard errors, the z statistic and the associated probabilities are presented. ns = non-significant, * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001). NA – not applicable data

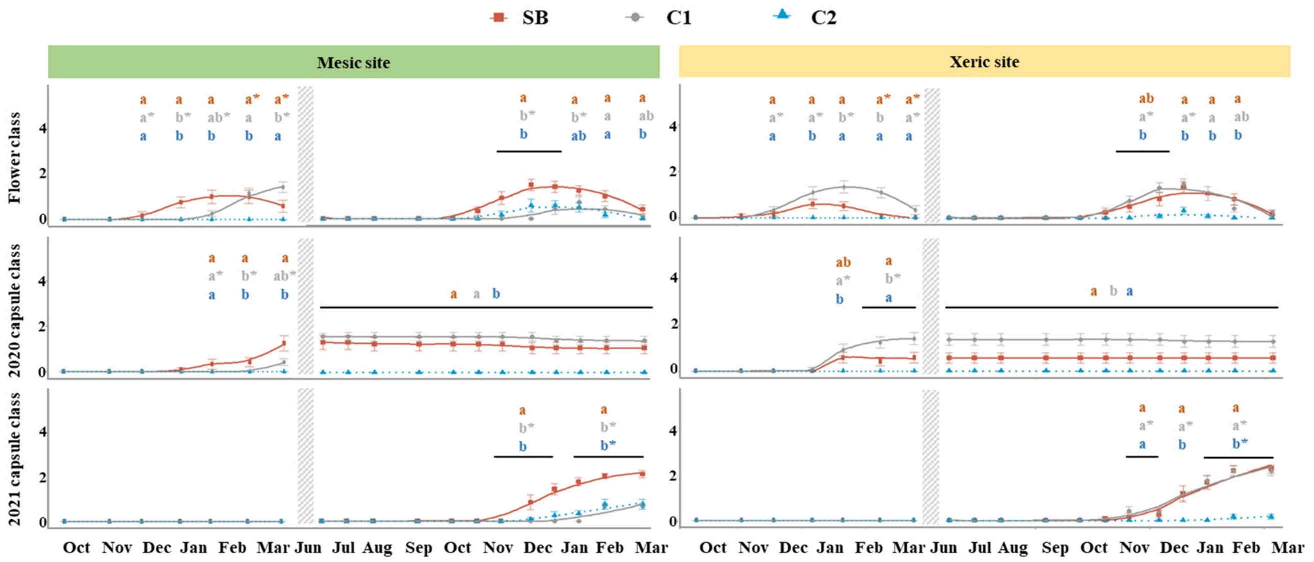

Fig. 4 Abundance of reproductive structures. Comparison of the abundance of flowers, and 2020 and 2021 capsules from seedling-based (SB) and clonal-based (C1 and C2) *E. globulus* stands in the mesic and xeric sites from October 2019 to March 2021. Vertical shaded bars represent the interruption due to CoViD-19 legal restrictions. Clone C3 did not exhibit any phenological structure during the monitored period, so data are not shown. Different letters indicate significant differences regarding tree stand within each site; "*" indicate differences between study sites for each phenological class in each tree stand. Horizontal black lines represent the same pattern of differences within sampling dates

4 Discussion

Eucalyptus globulus genetically improved clonal-based stands showed clear differences in the onset, persistence and abundance of reproductive phenological structures compared with unimproved plantations.

In Portugal (likewise many other countries), many eucalypt plantations rely on advanced generation genetically improved clonal forestry. These stands are more vigorous and uniform than their unimproved seedling-based (SB) counterparts. However, these clonal-based stands appear to have less abundant flowering, a slightly delayed onset, and a shorter duration, resulting in lower capsule production. These differences suggest that clonal-based forestry would have lower reproductive fitness, though these differences are likely clone-specific: while clone C3, one of Portugal's most commonly deployed materials, failed to produce reproductive structures in both study sites and years, clone C1 outperformed unimproved trees in the drier environment. On the other hand, clonal C2 trees flowered at the same time as SB trees only in the second year of monitoring, showing the shortest flowering period. Indeed, it seems to occur a specific behaviour for each tree as well as an inter-annual variation as already described for *E. regnans* [53].

Precocity and phenology are strongly genetically controlled (e.g. [54, 55]), which could explain the flowering differences observed between clones C1 and C2. A longer flowering period was recorded for C2 trees under colder and wetter conditions and a particularly high inter-site variability for C1 trees, reflecting a delay in the flowering period of more than 40 days in the mesic site. This could be explained by the clone origins: clone C2 is derived from Southern Tasmanian base parents and adapted to colder conditions with no dry season. In contrast, clone C1 has Strzelecki sub-race ancestry, adapted to a warmer region in southern Australia [56]. Indeed, xeric conditions seemed to be associated with an earlier flowering initiation in clonal C1 trees, ultimately triggering earlier bud maturation and operculum shedding. In addition, following this information on clonal origins, it is also verified in terms of reproduction capacity that drier conditions favour the abundance of reproductive structures in clone C1, whereas clone C2 has greater success in mesic conditions. However, regardless of location characteristics, clonal trees had lower reproductive output than SB trees.

The differences in flowering between some of the clonal- and the seedling-based stands or the short flowering period of clonal trees could result in poor synchrony between the two stand types. In the former, this would be expected to lead to higher self-pollination rates (viz [45, 57]), resulting in lower seed set [58] and poorer viability and development of the progeny [57, 59, 60]. However, under drier conditions, differences in the timing and duration of phenological stages were less apparent. Temperature is known to influence *Eucalyptus* flowering behaviour [61–63], with early flowering usually found in warmer sites [64], explaining the more homogenous flowering pattern observed in the xeric site.

Observations of up to three-year-old capsules in both SB and clonal trees have also been described for *E. regnans* [65, 66]. Older capsules gradually decreased over time, especially in the xeric site (data not shown), most likely due to more rapid leaf fall and subsequent branch death, a well-known strategy for coping with a longer summer season [67]. Understanding the demography of capsule dehiscence and seed storage in the canopy and how it varies with stand and site conditions is critical to determining plantation regeneration potential, particularly after a fire [38].

Due to its importance in the paper industry, increased planting of *E. globulus* will raise concerns about its spread, invasion, and adverse impacts on ecosystems. Therefore, planting sterile genotypes or clones with low seed production will be crucial to limit dispersal events and should be a criterion of choice when selecting a genotype to deploy. This claim is noteworthy because clones C1 and C2 have similar numbers of seeds and germination rates as SB trees (data not shown), indicating that a reduced output of capsules reduces the probability of dispersal. However, since our findings revealed large variability in the reproductive behaviour of clones, new plantings should be continuously monitored to ensure that trees produce less seeds, not posing a threat.

Unfortunately, pandemic constraints hampered the continuous monitoring of the phenological behaviour of the studied trees. Notwithstanding, results pointed out different reproductive behaviour, especially among clones, including an inter-annual variation and an extreme of non production. Future long-term studies should consider these phenological differences between clonal and SB trees. They are important to better interpret reproduction output over time, specifically seed viability and germination, ultimately clarifying the potential dispersal risk of genetically improved clonal stands *versus* unimproved stands.

5 Conclusions

This study indicates a different reproductive behaviour of clones compared with unimproved *E. globulus* stands, which may significant impact the dispersal dynamics of this exotic species, a concern often raised by conservationists [68]. Seedling-based (SB) stands produced more flowers and capsules and behaved more consistently across climatic conditions. On the contrary, clonal-based stands were more variable, presenting significant inter-annual differences, shorter flowering seasons, less abundant reproductive structures and higher phenological variability across sites relative to SB plantations. It is relevant to denote that one of the studied clones did not produced any reproductive structures nor present remaining capsules; considering the tree cutting cycles performed in Portugal (i.e., 10–12 years), even if a later in age flowering could be occurring, it will avoid seed production. These results are consistent with the hypothesis that the broader genetic basis ascribed to SB stands may result in greater reproductive fitness across different climatic conditions, whereas clonal trees may be more sensitive and less plastic [69]. A better understanding of genetically improved clones' phenological behaviour may reduce regeneration risks and naturalization from this species in introduced ranges, providing an effective tool for sustainable management.

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Author contributions AA and JGJ conducted fieldwork. All authors contribute to the conceptualisation and methodology design. AA, JGJ and SC analysed the data. AA led the writing and all the authors made substantial contributions to the writing. All authors have read and agreed to the published version of the manuscript.

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Data availability Further data and details are provided in the Supplementary Material file. The full dataset used in this study is available to the corresponding author upon request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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