



Germination niche of co-occurring threatened native and alien species: a case study in *Lindernia procumbens* and *L. dubia*

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Abstract

Plant invasions have a negative impact on biodiversity and lead to species loss. Although studies on traits promoting invasiveness have been largely developed, comparative studies on germination traits of closely related native versus alien invasive species, which also take into account threatened species, are lacking. We analysed the germination requirements of two congeneric *Lindernia* species inhabiting paddy field in Northern Italy. Germination tests were conducted exposing seeds of both species across a broad range of temperature and conditions (i.e., cold-wet stratification, dark condition, alternate temperature, gibberellic acid). The invasive *L. dubia* showed higher and faster germination, occurring over a broader range of temperatures compared to the native threatened *L. procumbens*, along with a lighter non-deep physiological dormancy. These results show a competitive advantage of the alien species already in the early stages of plant regeneration and provide a comprehensive overview of the germination requirements, necessary for the development of future conservation and management plans.

Keywords Alien plants · Germination · *Lindernia* · Threatened species · Invasive species

Introduction

Despite efforts to halt invasions, invasive species affect negatively the richness, the abundance and the functional diversity of native communities (Vilà et al. 2011), increasing the extinction risk of native species. Traits promoting invasiveness include high growth rate and specific leaf area (Kaushik et al. 2022), long and early flowering periods (Pyšek and Richardson 2008; Colautti et al. 2006). In addition, seed traits may strongly contribute to invasion in the new region, with alien species showing high seed dispersal over a broader range of environmental conditions (Pyšek and Richardson 2008) and larger soil seedbanks compared to natives (Gioria and Pyšek 2016). Comparative germination studies of closely related native and invasive species

contribute to identifying traits related with invasiveness since this approach minimizes biases associated with phylogenetic distance and habitat affinities (Gioria and Pyšek 2017). Nevertheless, when it counts for threatened species in view of plant conservation, a few studies investigated differences in germination traits, mostly reporting contrasting results (e.g., Paulů et al. 2017).

In our study we have investigated the germination requirement of the congeneric species *Lindernia procumbens* (Krock.) Philcox and *L. dubia* (L.) Pennell [Linderniaceae]. *L. procumbens* is native to the temperate to tropical zones of Eurasia, included in the Annex IV of the 92/43/EEC “Habitat” Directive and Annex I of Bern Convention. The species is classified as “Least Concern” (Bilz et al. 2011) on the European Red List but is locally threatened, being included in several national Red Lists due to its scattered distribution, the fragmented habitat and the intense agricultural activities that include the use of chemicals. Last but not least, the species suffer a competitive disadvantage with the invasive congeneric *L. dubia* (L.) Pennell, a North American species native to catchments of the Mississippi River (Šumberová et al. 2012), accidentally introduced in France in 1850 by ships on the Loire river banks and further spread through Europe (Simons and Jansen 2018). The

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life forms and ecological preferences of the two species are similar. They are short-lived annuals, reproduce exclusively by seeds and co-occur in periodically flooded habitats such as river floodplains, wet meadows and paddy fields. Previous studies stated that *L. dubia* displays a robust growth, being able to colonize a broader range of vegetation types and to reproduce over longer time during the growing season compared to *L. procumbens* (Šumberová et al. 2012). In our study we investigated the germination requirement of both species to deepen the information on 1) seed germination across a broad range of temperature and conditions (i.e., cold-wet stratification, dark condition, alternate temperature) 2) the presence of physiological dormancy. We hypothesised that seeds of *L. dubia* show higher and faster germination, wider temperature range and are less dormant compared to *L. procumbens*. By shading light on the regeneration niche of both species we hope to contribute to better-informed management decisions of the invasive species and to the development of conservation plan for the threatened species.

Material and methods

Germination tests

Considering the limited occurrence and scarce data availability of *L. procumbens* distribution, seeds of both species were collected in July 2019 in a paddy field (i.e., one population) where both species co-occur (Pavia Province, N-Italy). Seeds were manually cleaned and stored in the Seedbank of the University of Pavia under dry room conditions (15°C and 15% RH) for 3 months in darkness to preserve their viability until germination tests started

(i.e., on 30th October 2019). Both fresh and cold-wet stratified seeds (at 0°C—dark condition for three months) were exposed to five constant temperatures (10, 15, 20, 25, 30°C) with 12/12 day/night photoperiod; fresh seeds were also exposed to alternate temperature regime (i.e., 5/15 and 15/25°C), with light provided at higher temperature. Germination tests were conducted sowing three replicates of 20 seeds each on 1% distilled water agar held in 60 mm diameter Petri dishes using temperature and light controlled condition (photosynthetically active radiation 40–50 $\mu\text{mol}\mu\text{mol mm}^{-2} \text{ss}^{-1}$). Germination plates were checked for radicle emergence (> 1 mm) daily in the first week, then at weekly interval until germination stopped (i.e., no emergence after 6 weeks). Once cotyledon emergence was completed, seedlings were removed from the Petri dishes. At the end of the experiments, non-germinated seeds were cut-tested and mouldy and empty seeds were counted as inviable to calculate final germination percentage (FGP).

On 19th February 2020, further tests were conducted exposing seeds to gibberellic acid (250 mg/l, GA₃) and at dark condition at the optimal germination temperature previously found (30 °C). Sowing method and photoperiod were the same as described above.

Data analysis

The effect of temperature and cold-wet stratification on seed germination was investigated using a generalised linear mixed model (*glmer*) with binomial error structure and *logit* link function where temperature (5 levels), species (2 levels), stratification (2 levels: i.e., presence/absence) and their interactions were tested against seed germination (model a, Table 1). Similarly, the effects of alternate vs.

Table 1 *Glmer* results on the effects on seed germination of a) temperature, cold-wet stratification, species and their interactions, b) high alternate and c) low alternate temperature, species and their interaction, c) GA₃, species and their interaction, and e) differences between species in MGT. For all models, *L. dubia* was taken as a reference level for species comparisons, thus estimates refer to *L. procumbens* difference from the latter. Significant differences ($p < 0.05$) are highlighted in bold, marginally significant values are highlighted in italic

Model	Fixed factors	Estimate	Std. Error	z value	P value
a)	Temperature	0.307	0.035	8.808	< 0.001
	Stratification	4.817	0.868	5.547	< 0.001
	Species	−6.459	2.322	−2.781	< 0.010
	Temperature × Stratification	−0.241	0.039	−6.184	< 0.001
	Temperature × Species	0.062	0.079	0.781	0.434
	Stratification × Species	1.138	0.780	1.447	0.148
b)	Species	−3.077	1.050	−2.930	< 0.010
	Alternate 15/25	−0.466	0.412	−1.132	0.258
	Alternate 15/25 × Species	0.338	1.484	0.227	0.820
c)	Species	−0.223	1.428	−0.156	0.870
	Alternate 5/15	3.308	1.045	3.165	< 0.010
	Alternate 5/15 × Species	−3.379	1.768	−1.910	0.056
d)	Species	−3.729	1.043	−3.575	< 0.001
	GA ₃	2.220	0.460	4.785	< 0.001
	GA ₃ × Species	0.572	1.153	0.496	0.619
e)	Species	0.235	0.108	2.168	0.096

constant temperatures (2 levels) on germination was analysed at high (i.e., 15/25 vs 20 °C) and low (i.e., 5/15 vs 10 °C) temperature with species as a fixed factor (model b and c, respectively; Table 1). Replicates were considered as random factor. The effect of GA₃ (2 levels, i.e., application/no application) was analysed using species as fixed factor (model d; Table 1). The effect of dark condition was not analysed since darkness inhibited seeds germination. In all models, the interaction and fixed factor combination was chosen taking into account the Akaike information criterion (AIC; Bozdogan 1987); models assumptions were tested with the Dharma package (Hartig and Lohse 2022). Finally, Mean Germination Time (hereafter, MGT), was calculated as follow:

$$MGT = \sum \frac{nT}{N}$$

where n is the number of seeds that germinated at time T , T are the days between the beginning of the test and the measurement and N is the total number of seeds that germinated. Differences in MGT between species were assessed for the germination experiments with a minimum FGP of 10%, where the variable species was considered as a fixed factor (model e; Table 1). Statistical analyses were performed with R 3.3.0 (R Core Team 2022).

Results

Both species germinated at the highest temperature (30 °C) without cold-wet stratification. *L. dubia* reported a significantly higher germination (81%) compared to *L. procumbens* (14%) at its optimum (Table 1). While seeds of *L. procumbens* germinated only at 30 °C, *L. dubia* germinated over a wider temperature range (from 15 to 30 °C; Fig. 1A).

Cold-wet stratification overall increased seed germination. However, it decreased seeds germination at the warmer temperatures (25, 30 °C), while increasing it on both species at cooler conditions (15, 20 °C) (Table 1a, Fig. 1B). Darkness completely inhibited seeds germination of both species. Alternate low temperature (5/15 °C) promoted germination, but only in *L. dubia* (38% FGP). *L. dubia* FGP was lower, but not significantly, at high alternate temperature (15/25 °C) than at constant temperature (20 °C; 27% vs 36%, respectively) while *L. procumbens* FGP was significantly lower (0 and 2% FGP both at constant and alternate temperature, respectively; Table 1b and Table 1c). Seeds germination was significantly improved by GA₃, particularly in *L. dubia* (Table 1d, Fig. 1C). Because of the low germination of *L. procumbens* (< 10% in most experiments), differences in MGT were estimated at 30 °C. *L. dubia* germinated on average one day before *L. procumbens*, requiring 3 days, but this difference was only marginally significant (Table 1e).

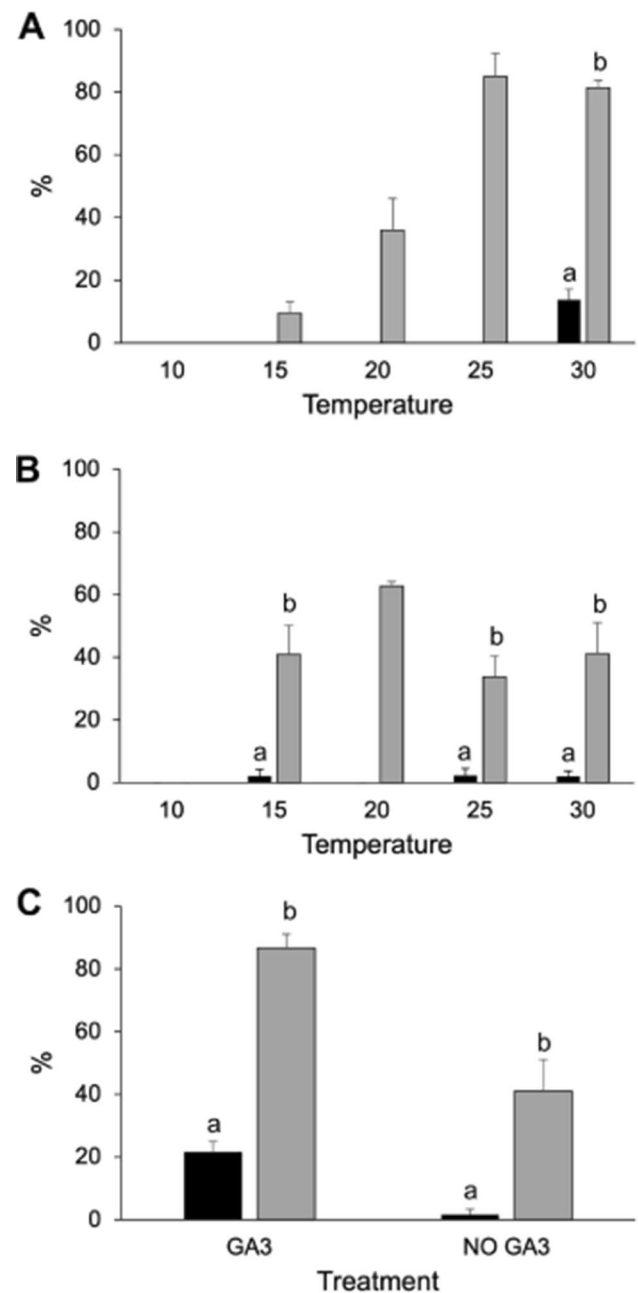


Fig. 1 Mean (+ES) FGP of *Lindernia dubia* (grey bars) and *Lindernia procumbens* (black bars) exposed at different temperatures on A) fresh and B) cold-wet stratified seeds and at C) GA₃ application. Letters indicate significant differences between species according to the Tukey's post-hoc HSD tests

Discussion

Germination behaviour of plants influences their fitness, persistence, evolutionary potential and the biotic environment, with differences between native and non-native congeners representing a mechanism that has contributed to the naturalization and invasiveness of many alien species (Gioria

and Pyšek 2017). According with the hypothesis tested, the invasive *L. dubia* showed higher germination, occurring over a broader range of temperatures compared to the native threatened *L. procumbens*. These results echo previous studies investigating germination traits associated with invasiveness (e.g., Colautti et al. 2006; Pyšek and Richardson 2008; Gioria and Pyšek 2016; Gioria and Pyšek 2017).

Warm temperatures are capable of promoting the success of invasion by increasing the probability of germination of invasive species compared to native species (Trotta et al. 2023). However, the results observed in our experiment contrast with this general pattern, but echoes previously observed germination behaviours in *Lindernia* (see Hrivnák et al. 2016), with both species being defined as thermophilic (Nobis et al. 2010). Furthermore, our study confirmed the traits associated with the invasion success of *L. dubia*, displaying a more robust growth, ability to colonize a broader range of wetland vegetation types (i.e., the phytosociological classes of *Littorelletea uniflorae*, *Phragmitetetea*, *Bidentetetea tripartitae* and *Isoëto-Nanojuncetetea*, with the latter shared with *L. procumbens*; Simons and Jansen 2018) and reproducing over longer time during the growing season compared to *L. procumbens* (Šumberová et al. 2012). These traits can also be explained by the different size and reproductive potential of the species, with *L. dubia* being usually bigger and producing more flowers and fruits than *L. procumbens* (Šumberová et al. 2012). Our results also highlight typical traits of invasiveness related to fast mean germination time, which facilitate access to resources and niches in the environment (Gioria and Pyšek 2017). Early germination is indeed a major factor in the success of several invasive species (Laube et al. 2015), demonstrating that competition mitigation or avoidance through the exploitation of vacant germination niches is a better strategy than superior competitive ability in the colonization of habitats where the species coexist (Keller and Shea 2021). At the same time, the delayed germination of *L. procumbens*, together with the limited germination cues, could be a strategy to reduce mortality risk associated with both unfavourable post-germination conditions and the effects of inter- and intra-specific competition among neighbouring species (Donohue et al. 2010).

Cold-wet stratification promoted the germination of both species over a wider temperature spectrum, albeit at lower germination percentage, while dark condition did not have any relevant effect on seeds germination. Interestingly, while the warm alternate temperature (i.e. 25/15°C) does not affect the seed germination of the two species, the cold alternate temperature (15/5°C) improves germination compared to the constant associated regime (10°C) only for the invasive species. This result suggests that the germination of the invasive *L. dubia* may occur earlier in

the vegetative season than that of native *L. procumbens*, thus (once again) providing a competitive advantage over space and nutrients resources. . Seeds germination was improved by GA₃, suggesting a non-deep physiological dormancy in both species (Baskin and Baskin 2014). A certain proportion of fresh seeds may thus enter in primary or secondary dormancy to prevent the germination of all dispersed seeds, leading to the formation of a soil seed bank (Fenesi et al. 2014). The formation of a persistent seed bank is considered more important for the persistence of annual than perennial species (Gioria et al. 2020), with a short life cycle being often associated with naturalisation and invasiveness (Funk et al. 2016; Pyšek et al. 2017), as for *L. dubia*. Although *L. procumbens* have been demonstrated to display a large soil seed bank (Šumberová et al. 2012), its sensitivity to warm temperature outlines a fluctuation in the size of population strictly related with the climate. However, the increased temperature foreseen by climate change in the upcoming years, jointly with the increased frequency of heat waves, could determine an increase of the population size of the species (Nobis et al. 2010); further studies are necessary to better understand *L. procumbens* population dynamic.

By providing a comprehensive overview of the germination requirement of *L. procumbens* we hope to contribute to a conservation plan for this threatened species that is currently not available in Italy and also mandatory at EU level according to the Habitat Directive. Conservation activities for the species could be applied easily in agroecosystems (i.e., organic paddy fields), where the species is repeatedly found. However, specific measures and protocols must be developed considering both the short life-cycle and the size of the seeds, which are dust-like, that can hardly envisage the cultivation in nursery and the subsequent translocation through planned interventions.

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Author contributions SO, FP, AM contributed to the study conception and design. FP and SL collected the data. AC and FP supervised and performed the statistical analysis. AC wrote the first draft of the manuscript. All authors contributed substantially to the revisions and approval of the final manuscript.

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Data availability Data analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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References

- Bilz M, Kell SP, Maxted N, Lansdown RV (2011) European Red List of Vascular Plants. Publications Office of the European Union, Luxembourg
- Bozdogan H (1987) Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. *Psychometrika* 52:345–370. <https://doi.org/10.1007/BF02294361>
- Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG (2010) Germination, post-germination adaptation, and species ecological ranges. *Annu Rev Ecol Evol Syst* 41:293–319. <https://doi.org/10.1146/annurev-ecolsys-102209-144715>
- Fenesi A, Albert Á-J, Ruprecht E (2014) Fine-tuned ability to predict future competitive environment in *Ambrosia artemisiifolia* seeds. *Weed Res* 54:58–69. <https://doi.org/10.1111/wre.12048>
- Funk JL, Standish RJ, Stock WD, Valladares F (2016) Plant functional traits of dominant native and invasive species in Mediterranean-climate ecosystems. *Ecology* 97:75–83. <https://doi.org/10.1890/15-0974.1>
- Gioria M, Pyšek P (2016) The legacy of plant invasions: changes in the soil seed bank of invaded plant communities. *Bioscience* 66:40–53. <https://doi.org/10.1093/biosci/biv165>
- Gioria M, Pyšek P (2017) Early bird catches the worm: germination as a critical step in plant invasion. *Biol Invasions* 19:1055–1080. <https://doi.org/10.1007/s10530-016-1349-1>
- Gioria M, Pyšek P, Baskin CC, Carta A (2020) Phylogenetic relatedness mediates persistence and density of soil seed banks. *J Ecol* 108:2121–2131. <https://doi.org/10.1111/1365-2745.13437>
- Hartig F, Lohse L (2022) DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. <https://CRAN.R-project.org/package=DHARMa> Accessed 14 February 2024
- Hrivnák R, Kochjarová J, Šumberová K, Schmotzer A (2016) Alien wetland annual *Lindernia dubia* (Scrophulariaceae): the first recently mentioned localities in Slovakia and their central European context. *Biologia* 71:281–286. <https://doi.org/10.1515/biolog-2016-0039>
- Kaushik P, Kumar Pati P, Khan ML, Khare PK (2022) Plant functional traits best explain invasive species' performance within a dynamic ecosystem - A review. *Trees for People* 8:100260. <https://doi.org/10.1016/j.tfp.2022.100260>
- Keller JA, Shea K (2021) Warming and shifting phenology accelerate an invasive plant life cycle. *Ecology* 102:e03219. <https://doi.org/10.1002/ecs.3219>
- Laube J, Sparks TH, Bässler C, Menzel A (2015) Small differences in seasonal and thermal niches influence elevational limits of native and invasive *Balsams*. *Biol Conserv* 191:682–691. <https://doi.org/10.1016/j.biocon.2015.08.019>
- Marushia RG, Cadotte MW, Holt JS (2010) Phenology as a basis for management of exotic annual plants in desert invasions. *J Appl Ecol* 47:1290–1299. <https://doi.org/10.1111/j.1365-2664.2010.01881.x>
- Nobis A, Nobis M, Piotrowicz K, Kącki Z, Dajdok Z (2010) *Lindernia procumbens* in Poland: the relationship between weather conditions and the occurrence of the species. *Biodiv Res Conserv* 17:39–46. <https://doi.org/10.2478/v10119-010-0005-3>
- Paulů A, Harčariková L, Münzbergov Z (2017) Are there systematic differences in germination between rare and common species? A case study from central European mountains. *Flora* 236–237:15–24. <https://doi.org/10.1016/j.flora.2017.09.004>
- Pyšek P, Richardson DM (2008) Traits associated with invasiveness in alien plants: where do we stand? In: Nentwig W (ed) *Biological invasions, Ecological Studies*. Springer, Berlin, pp 97–125
- Pyšek P, Pergl J, Essl F, Lenzner B, Dawson W, Kreft H, Weigelt P, Winter M, Kartesz J, Nishino M, Antonova LA, Barcelona JF, Cabezas FJ, Cárdenas D, Cárdenas-Toro J, Castaño N, Chacón E, Chatelain C, Dullinger S, Ebel AL, Figueiredo E, Fuentes N, Genovesi P, Groom QJ, Henderson L, Inderjit, Kupriyanov A, Masciadri S, Maurel N, Meerman J, Morozova O, Moser D, Nickrent D, Nowak PM, Pagad S, Patzelt A, Pélser PB, Seebens H, Shu W, Thomas J, Velayos M, Weber E, Wieringa JJ, Baptiste MP, van Kleunen M (2017) Naturalized alien flora of the world: species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. *Preslia* 89:203–274. <https://doi.org/10.23855/preslia.2017.203>
- Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I, Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M, Wingfield MJ, Richardson DM (2020) Scientists' warning on invasive alien species. *Biol Rev* 95:1511–1534. <https://doi.org/10.1111/brv.12627>
- R Core Team (2022) R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria (<https://www.R-project.org/>)
- Simons ELAN, Jansen MGM (2018) Ecology of naturalized invasive species *Lindernia dubia* (L.). Pennell in the Netherlands *Gorteria* 40:1–10
- Šumberová K, Lososová Z, Ducháček M (2012) Distribution, habitat ecology, soil seed bank and seed dispersal of threatened *Lindernia procumbens* and alien *Lindernia dubia* (Antirrhinaceae) in the Czech Republic. *Phyton* 52:39–72
- Trotta G, Vuerich M, Petrusa E, Hay FR, Assolari S, Boscutti F (2023) Germination performance of alien and native species could shape community assembly of temperate grasslands under different temperature scenarios. *Plant Ecol* 224:1097–1111. <https://doi.org/10.1007/s11258-023-01365-7>
- Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecol Lett* 14:702–708. <https://doi.org/10.1111/j.1461-0248.2011.01628.x>

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