


ORIGINAL ARTICLE

Germination ecology of *Phytolacca americana* L. in its invasive range

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Abstract

Invasive species are a worldwide problem, and the germination process is useful to understand the characteristics that allow alien species to be invasive and their projected response to global climate change. *Phytolacca americana* is one of the most invasive plants in Italy, and we tested, for different populations (from different altitudes) how light, temperature, and cold stratification affect seed germination. According to our analyses, *P. americana* produces an exceptionally high number of seeds that may potentially survive in soil for extended periods. Seeds subjected to cold stratification and exposed to warmer temperatures, both in light and darkness, exhibited faster germination, with a higher germination rate and a shorter T50. Seeds collected at the highest elevation (337 m a.s.l.) have germinated in all tested thermal conditions, albeit with a lower germination percentage and a longer T50 compared with seeds collected at lower elevations (5 and 50 m a.s.l.) and tested under warm and moderate temperatures. In general, *P. americana* seems to adapt to moderate-warm temperatures (at low elevations) and moderate-cool temperatures (at highest elevations) and appears to increase germination with seeds exposed to cold stratification. These results, in a scenario of climate change, show that the invasiveness of *P. americana* may increase in the future.

KEYWORDS

ecological adaptation, germination, invasive alien species, logistic model

1 | INTRODUCTION

Invasive alien species are a worldwide problem having a negative impact on ecosystems and representing a component of human-induced environmental change (Keller et al., 2011). Globalization has promoted the transfer of organisms between different continents and facilitated the

exchange of allochthonous (i.e., introduced) species (Van Kleunen et al., 2015), dramatically increasing introduction rates, leading to the erosion of biogeographical barriers, reducing biodiversity richness, and increasing the homogenization in several environments (Daru et al., 2021).

Humans have introduced thousands of species in regions outside their native ranges, and several of them

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have become permanent components of the local fauna and flora (Seebens et al., 2018). More than 50% of alien plant species were introduced for ornamental and horticultural purposes (Kowarik, 2011; Pepe et al., 2020; Pergl et al., 2016). Almost two-thirds (62.8%) of the established plant species in Europe were introduced intentionally, and the percentage of introduced species that become invasive exceeds 50% (Keller et al., 2011; Lambdon et al., 2008). Europe nowadays has more than 300 invasive terrestrial plant species (Vilà et al., 2010; Vilà & Ibàñez, 2011).

The effects of invasive species on environments depend on biological and ecological species characteristics (tolerance thresholds, species adaptability, polymorphism, generalism, fitness, and dispersion), ecological compatibility among invasive species, and the vulnerability of ecosystems (Dullinger et al., 2013). Seed production and germination, associated with the initial phase of plant establishment, are key processes that determine the outcome of invasion into new regions (Moravcova et al., 2006) and can help us to understand the dynamics of invasion (Pyšek et al., 2008). Understanding seed production is useful for unraveling reproduction mechanisms and developing preventive measures for weedy plants (Zeng et al., 2021). Germination in invasive species typically occurs rapidly, it is highly synchronous, and exhibits broad environmental tolerance (Díaz-Segura et al., 2020; Gioria & Pyšek, 2017). Germination time is associated with successful naturalization and invasiveness in South African Iridaceae species and some introduced herbaceous species in North America (Schlaepfer et al., 2010; Van Kleunen & Johnson, 2007). Early germination is a crucial trait, as it allows species to gain access to resources and space while reducing competition during the initial stages of establishment (Gioria & Osborne, 2014; Godoy et al., 2009; Pyšek & Richardson, 2007). Several invasive plants have been reported to experience positive effects on growth and fecundity due to early germination (Dickson et al., 2012; Engelhardt & Anderson, 2011).

The European Commission, with the EU Regulation 1143/2014, addressed the issue of invasive alien species to counteract their effect on ecosystems. All species listed under the Alien Species of Union Concern require measures from all EU countries to prevent, detect, eradicate, and manage the associated biodiversity risks. One of the species included in this list is *Phytolacca americana* L., a polycarpic perennial herb native to North America. In its native range, it is a pioneer plant for open and disturbed areas (Pepe et al., 2020).

In Southern Europe, it has been cultivated as a garden plant since the seventeenth century (Steinmetz, 1963). It has been widely introduced into central Europe, the Mediterranean Basin, and East Asia (Balogh & Juhász, 2008;

Orrock & Damschen, 2005; Pepe et al., 2020). The species has been classified as harmful due to its negative impact on ecosystems (e.g., in South Korea, Min, 2014 and in Italy, Lazzaro et al., 2020). The effects include competition with native plants for space, light, and nutrients, as well as toxicity for animals and production of allelopathic compounds that can affect soil microbial community and other organisms. Indeed, *P. americana* altered arthropod community structure and repels many earthworm species (Campana et al., 2002; Dumas, 2011; Orwig & Foster, 1998; Schirmel, 2020). Another effect of *P. americana* on abiotic conditions is the accumulation of potassium in the soil, forming reserves of the element in the environment (Dumas, 2011). This species is highly distributed in Italy, but its effects and impacts are not fully known; the “European Risk Assessment” for *P. americana* is not complete (Armesto et al., 1983; Dumas, 2011). The plant is favored in propagation by temperatures around 20°C and is rarely found in places where temperature is below −15°C for prolonged periods in winter (Balogh & Juhász, 2008). Additionally, it exhibits physiological dormancy in seeds, with optimal germination conditions ranging between 10°C and 30°C (Pepe et al., 2020), and seeds are considered orthodox, as their moisture content (MC) is lower than 15% at maturity (SID, Kew Gardens). Moisture content also affects the speed of metabolic processes and, consequently, seed longevity (Bacchetta et al., 2006). Orthodox seed production is a characteristic of plants that grow in disturbed environments such as roadsides because these seeds can remain dormant until the propitious time to germinate. On the other hand, recalcitrant seeds do not form soil seed banks, as they cannot remain viable without internal water, losing vitality in a short time (Bacchetta et al., 2006). Therefore, the production of seeds that can persist in soils for prolonged periods emerges as a crucial survival strategy (Bacchetta et al., 2006), enabling species to adapt to different ecological conditions and significantly enhancing the survival of their seedlings (de Lima et al., 1997). *Phytolacca americana* is mainly found in highly anthropized environments, as allochthonous species are frequent in areas where humans introduce them, either accidentally or for ornamental purposes. Anthropic areas have a high environmental heterogeneity that can satisfy the ecological needs for a wide range of species. The ripening period for the species is November (Pignatti et al., 2017).

Southern Europe may be increasingly suitable for *P. americana* establishment under a climate change scenario (over the next 30–50 years) due to increased temperature and length of growing season and both increased summer and winter temperatures would benefit the species. Increased precipitation and CO₂ levels, as a result of climate change, could also be beneficial (Aislabie & Cefas, 2020).

The aim of this study was to assess whether populations from different elevations, and consequently with individuals grown at different temperature and rainfall conditions, exhibit distinct germination patterns in response to different temperatures, light availability, and cold stratification.

2 | METHODS

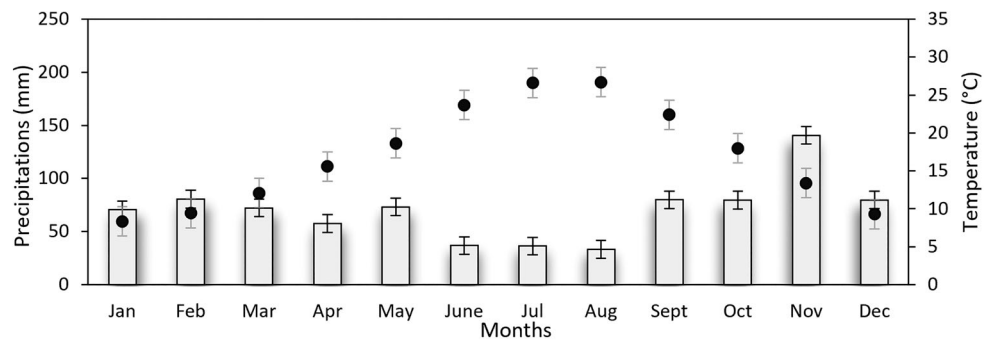
2.1 | Field sampling

Phytolacca americana seeds were collected in November 2020 from three sites in Rome and its province: the Botanical Garden of Sapienza University of Rome (BG,

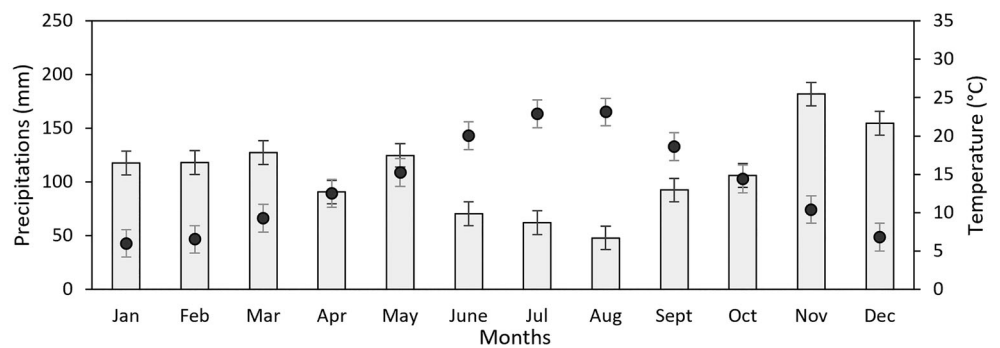
41°53'53" N, 12°28'46" E, 50 m a.s.l.), Zagarolo (ZAG, 41°50'38"76 N – 12°48'41"04 E, 337 m a.s.l.), and Ostia (OS, 41°44'51.511" N – 12°16'24.732" E, 5 m a.s.l.). The sites have an average distance between them of about 40 km.

The values considered for the different collection sites were the mean air temperature (°C) and the mean of monthly precipitation for 30 years (mm) (Figure 1). The climate diagrams, according to Bagnouls and Gausson (1953), show that the ZAG site is characterized by higher rainfalls and lower temperatures than the BG and OS sites. Sampling of individuals was carried out according to the protocols of Bacchetta et al. (2006). Twenty individuals were sampled in each site, ensuring a distance among individuals of at least 100 m for genetic variability of the

(a) Botanical Garden of Rome



(b) Fiumicino



(c) Zagarolo

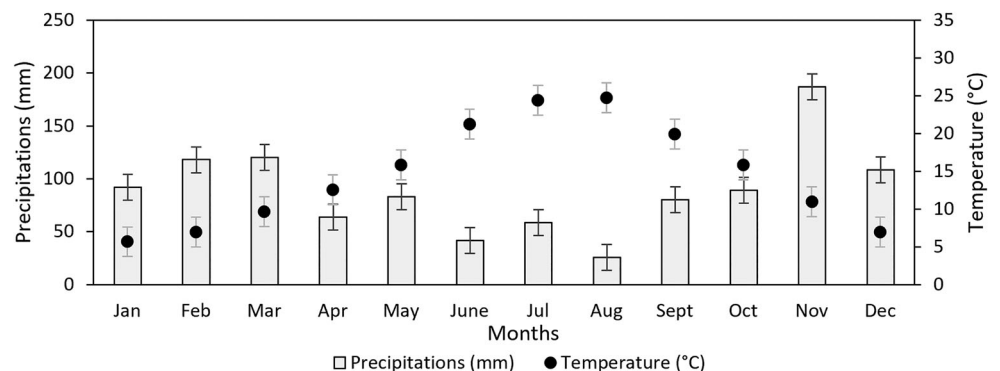


FIGURE 1 Climate data were taken from the regional meteorological website (Regione Lazio, <https://www.arsial.it/>) for the period 1990–2020.

Umbrothermal barplot according to Bagnouls and Gausson (1953) for monthly mean (and standard deviation) temperature (°C) and monthly mean precipitation for 30 years (mm) at (a) the Botanical Garden of Rome (BG), (b) Ostia (OS), and (c) Zagarolo (ZAG). Data were provided by “Agenzia Regionale per lo sviluppo e l’Innovazione dell’Agricoltura nel Lazio.”

□ Precipitations (mm) ● Temperature (°C)

collection. Two racemes were collected from each healthy individual plant of comparable size and exposure.

Fruits were sampled at the end of the ripening period reported for the species, which is November (Pignatti et al., 2017). The fruits were dark purple in color and had reached maximum size. The number of completely immature, partially immature and mature fruits was counted to evaluate possible differences among different sites. Fruit color and the ratio between fruit diameter and length of tepals were chosen as parameters to distinguish the three stages of maturity: Dark purple fruits were classified as mature if their fruit diameter was twice the length of tepals; green fruits were classified as partially immature if their size was equal to the length of tepals; and the remaining fruits were classified as completely immature. Fruits were postripened for 2 days in open containers at the Germplasm Bank of the Botanical Garden of Rome. The process occurred in a controlled environment, shielded from heat and bright light sources, maintaining room temperature 20°C–30°C and relative humidity below 40%. This postripening method, following the protocols of Bacchetta et al. (2006), aimed to standardize fruit ripeness levels. It allowed all fruits to reach a uniform stage of maturity, addressing variations observed in seeds collected at different maturity stages by employing a postmaturation period to ensure uniformity in seed maturity. At the end of the postripening phase, 40 fruits were randomly taken for each site, and the number of seeds for each fruit was counted.

2.2 | Seed morphometric traits

Seed morphometric traits included major axis (A, mm), minor axis (a, mm), seed fresh mass (SFM, g), seed dry mass (SDM, g), and MC (%).

For axis measurements, four containers were used, for each maturity level and each site, containing 25 seeds each. The major and minor axes of each seed in different samples were measured using NIS-Element Br. 2.10 with images from a Nikon Digital Sight DS-U1 digital camera, mounted on a Carl Zeiss stereo microscope.

The determination of SFM of *P. americana* was conducted on four lots containing 50 seeds, for each of the three stages of maturity and for each of the three sites considered. Since the collections contained fewer than 200 seeds from fully immature fruits, measurements were carried out for all available seeds (ranging from a minimum of 18 to a maximum of 22 seeds).

Seed dry mass determination was conducted on seeds used for fresh mass measurements. Seeds were placed in an oven at 103°C for 17 h to remove water and then allowed to cool to room temperature for 15 min

(Bacchetta et al., 2006), before any dry mass measurements. All measurements were carried out using a Gilbertini precision balance with a precision of 0.1 mg.

In accordance with Roberts (1973), the percentage of water within the seed, expressed as MC, allows discrimination between orthodox and recalcitrant seeds (Chin et al., 1989). Orthodox seeds achieve an internal MC lower or equal to 15% (Roberts, 1973). The moisture percentage of a seed can be determined experimentally using various methodologies (International Seed Testing Associations, ISTA, 2005). For the calculation of the internal moisture of seeds, the formula proposed by ISTA was used (2005, Equation (1)).

$$\begin{aligned} \text{Water Content (mc, \%)} \\ = [(\text{fresh weight} - \text{dry weight}) / \text{fresh weight}] \\ \times 100. \end{aligned} \quad (1)$$

2.3 | Effect of different temperature and light regimes on seed germination

The purpose of the germination tests was to determine the germination potential of one or more seed lots by subjecting seeds to different germination temperatures and controlled dark–light cycles, following the protocols of Bacchetta et al. (2006) and ISTA (International Seed Testing Association, 2005). The analyses were conducted exclusively on seeds from ripe fruits.

For both experiments (i) and (ii), we evaluated the germination process of seeds from each sampling site at different temperature ranges, that is, 6°C–15°C (cool temperature), 10°C–20°C (moderate temperature), 15°C–25°C (warm temperature), combined with two different light conditions, that is, 12-h dark (at the lower temperature) and 12-h light (at the higher temperature) and 24-h dark. These experiments (i, ii) were conducted using four Petri dishes (25 seeds per dish) for each thermal regime. We placed the Petri dishes inside three germination cabinets (each germination cabinet is set to a different temperature among those mentioned earlier).

The germination cabinets were adjusted so that the 12 h of light coincided with the highest temperature, mirroring natural conditions. Seeds from the 24-h dark cycle were covered with a double layer of aluminum foil to produce a no-light condition. Light treatments were performed by incubating Petri dishes with cool white-fluorescent tubes, providing a photon flux density of 80 μmol (photon $\text{m}^{-2} \text{s}^{-1}$). Seeds for each replicate were placed on filter paper and moistened with distilled water. Distilled water (1 mL) was added every 3 days to maintain seed moisture. Germination was assessed daily for each seed in each Petri dish for 60 days.

Seeds were considered germinated when they produced a visible radicle with a length greater than 1 mm (ISTA, 2005). The dishes placed in the dark were opened every day from the aluminum foil covering for up to 1 min, and the number of germinated seeds was counted. The capsules were then covered with a double layer of new aluminum foil.

In our experimental design, temperature was considered a qualitative variable with three levels, and light treatment was considered a qualitative variable with two levels.

2.4 | Data analysis

We estimated Poisson generalized linear models (GLMs) with logarithm as link function to investigate any potential variations in the number of seeds per berry and the number of mature, immature, and fully immature fruits depending on the sampling sites. For overdispersed counts we estimated a quasi-Poisson GLM.

The difference in MC (%) among the three sites was investigated by estimating a beta regression model. This model is suitable for a response variable within (0,1) interval since proportions, generally, exhibit strong skewness, and thus inference based on the assumption of normality is often incorrect (Ferrari & Cribari-Neto, 2004). For this reason, all the percentages of the MC were divided by 100 to obtain values between 0 and 1. The link function, used in beta regression models, is the logit.

To compare the minor and major axes of the seeds of the three different sites, based on the maturity stage, we estimated a linear model.

Germination rates were evaluated using nonlinear models with logistic curves. Logistic models are used for germination assay analysis, as they provide a good fit to data derived from germination (Ritz et al., 2015). The analysis was performed using log-logistic curves (Equation (2)). In the logistic curves, the response variable is the percentage of germinated seeds over time.

$$Y_t(\%) = d / (1 + \exp[b(\log(t) - \log(e))]). \quad (2)$$

In Equation (2), Y_t represents the percentage of germinated seeds at time t . The theoretical lower limit was set to 0. From this model, we estimated three parameters: “ b ,” “ d ,” and “ e .” The parameter “ b ” corresponds to the slope of the logistic model at the inflection point. The parameter “ d ” corresponds to the upper asymptote and represents the maximum of the germination process. The parameter “ e ,” here, represents the T50, that is, days needed to reach a percentage of germinated seeds equal

to the half of d (Ritz et al., 2015). The data analysis was carried out with the R software (version 4.2.0).

3 | RESULTS

3.1 | Seed morphometric traits

The number of mature and immature fruits did not differ among the sampling sites ($p > 0.05$), as indicated by the estimated GLMs, which showed no difference in the numbers of fully immature, immature, and ripe fruits across racemes from collection sites (Figure 2, Table S1). The estimated GLMs indicated no variation in the number of seeds per fruit among the three different sites ($p > 0.05$), demonstrating that the fruits sampled at these sites did not differ in seed quantity (Figure 2d, Table S1). The estimated linear model did not show axis differences for seeds taken from ripe fruits among the three sites ($p > 0.05$). Differences in axis sizes ($p < 0.05$), however, were found among the three sites for seeds taken from immature and fully immature fruits, but such differences are likely not biologically significant (Figure 3, Table S2).

The beta regression model was estimated to analyze the differences of MC among sites. In the model output, BG represents the corner point of the qualitative variable “Site.” The ZAG and OS levels are not statistically different from the BG level ($p > 0.05$). There was no evidence that collection site influenced the MC (%) of seeds (Figure 4, Table S3).

3.2 | Effect of different temperature and light regimes on seed germination

The logistic model output without cold stratification (i) for warm temperature level and dark level showed partially overlapping confidence intervals for the three sampling sites.

For the moderate temperature level, the nonlinear models did not show a trend, as germination was concentrated in the last week of the experiment, except for ZAG (Figure 5a). The limited number of seeds germinated in BG and OS resulted in inconclusive estimates, as the model could not accurately estimate growth under the cool temperature level. The outputs of the minimum temperature level showed no meaningful estimates, as seeds germinated to very low percentages (<30%) (Figure 5a).

Model results showed that, at the warm temperature level, seeds had increased germination, speed, and anticipated T50 in each sampling site (Figure 5).

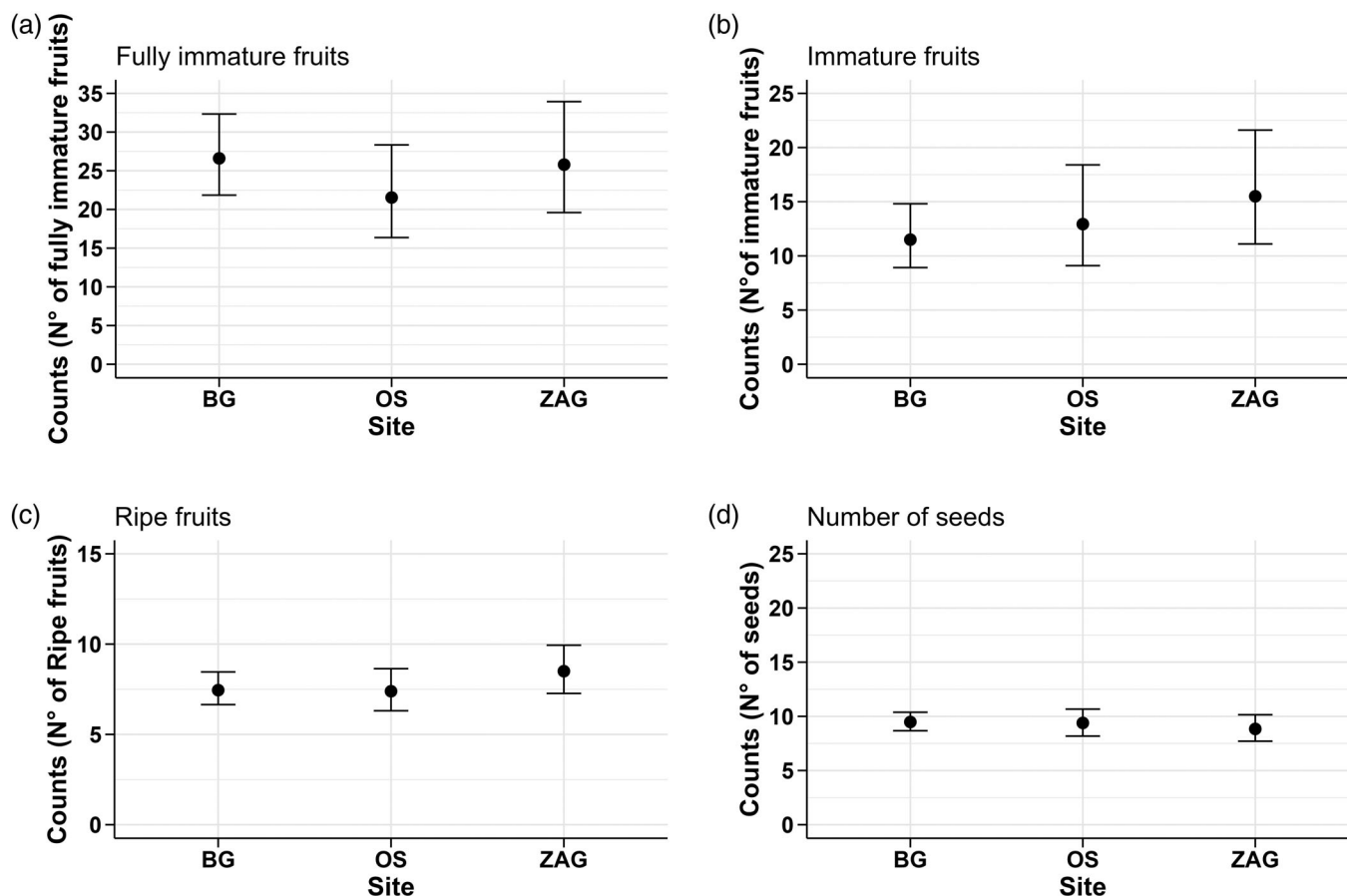


FIGURE 2 Results of the estimated quasi-Poisson generalized linear models (GLMs). Dots represent the model estimates, and lines represent the 2.5% (lower bar) and 97.5% (upper bar) confidence intervals. There were no differences in the number of ripe, immature, and fully immature fruits and seeds per berry among the three sampling sites. (a) Fully immature fruits; (b) immature fruits; (c) ripe fruits; (d) number of seeds per berry. BG, Botanical Garden of Rome; OS, Ostia; ZAG, Zagarolo.

When cold stratification was applied to seeds (ii), the output of the models showed completely different results compared with non-pretreated seeds (Figure 5b).

At warm temperature and for both light levels, seeds from the BG and OS showed partially overlapping confidence intervals (Figure 5b). Sites from lower elevations (BG and OS) had faster germination rates and an earlier T50 than seeds from ZAG when exposed to the warm temperature level and cold stratification. Warm temperature accelerated seed germination and increased the percentage of germinated seeds (Figure 5b). The ZAG site had seeds with a lower percentage of germination than OS and BG for the same parameters (warm temperature at both light levels). Moreover, this site showed a lower T50 and germination speed than the other sites (Figure 5b).

Moderate temperature and both light parameters always show differences among the three sites. The sites at lower elevations (BG and OS) showed seeds with faster germination rates and earlier T50 than seeds from ZAG, located at the highest elevation. However, seed exposure

to light level increases the germination rate and anticipates T50, stimulating germination.

At the cool temperature level, the site with the most germinating seeds, both in light and dark conditions, was located at the highest elevations (ZAG).

4 | DISCUSSION

In this study, the seeds' MC (%), morphometry, and germination capability among different populations of pokeweed (*P. americana*) were assessed. The preliminary determination of seed recalcitrance or orthodoxy showed that the MC of seeds was below 15%. These results are consistent with previous classifications of the species' seed as orthodox, improving the chances to produce a seed bank in the soil for a prolonged period, as other allochthonous species (Gioria et al., 2019; Presotto et al., 2020). Moreover, seeds from the three sites did not show biologically significant differences in internal seed moisture, highlighting the same invasive potentiality.

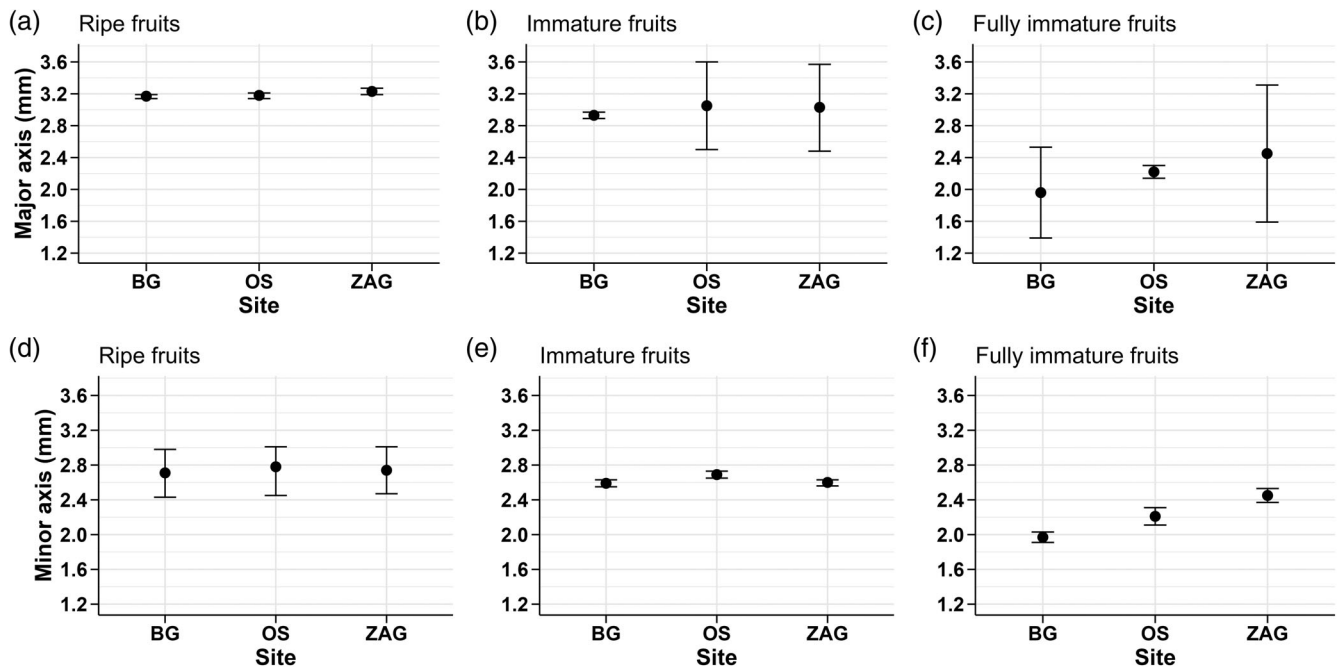
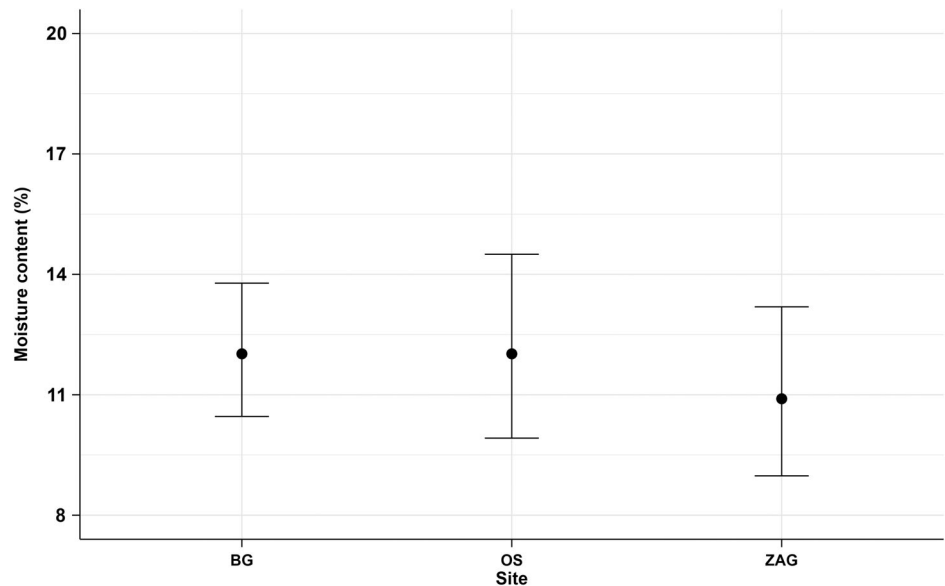


FIGURE 3 Results of the estimated linear models. (a–c) Major axis length among sampling sites. (d–f) Minor axis length among sampling sites. Dots represent the model estimates, and lines represent the 2.5% (lower bar) and 97.5% (upper bar) confidence intervals. Seeds from immature and fully immature fruits show differences between the three stations in both the major axis (b, c) and minor axis (e, f). BG, Botanical Garden of Rome; OS, Ostia; ZAG, Zagarolo.

FIGURE 4 Results of the estimated beta regression model. Dots represent the model estimates, and lines represent the 2.5% (lower bar) and 97.5% (upper bar) confidence intervals. BG, Botanical Garden of Rome; OS, Ostia; ZAG, Zagarolo.



One of the characteristics that allows a species to invade an area is the abundance of seeds produced by the introduced species (Widrechner et al., 2004). The results of the number of seeds per fruit and fruits per maturity stage showed no differences among sites. *Phytolacca americana* produces a high number of seeds, and this could be one of the features that make it an invasive plant, in addition to its high adaptability to different environments (Grotkopp et al., 2002). Consequently,

ecological factors do not appear to limit fruit and seed production in these areas.

Morphometric analyses are a crucial tool for discerning diverse characteristics of examined seeds, with seed size widely recognized as a key indicator of seed quality (Harms et al., 2000; Leishman et al., 2000). Seed quality encompasses a range of characteristics (biological, physiological, and agronomic) that seeds must possess to demonstrate viability and germinability (Harms

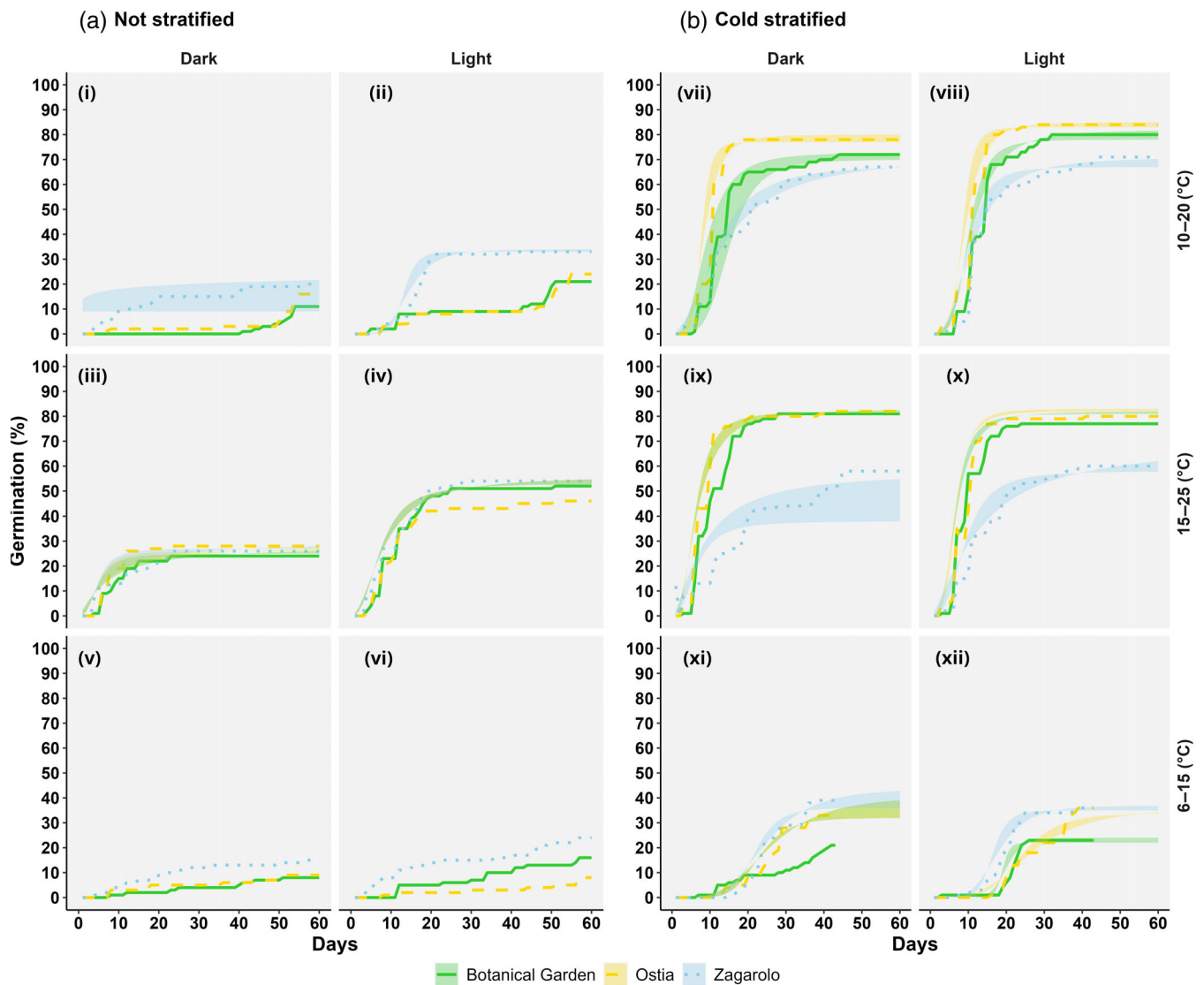


FIGURE 5 Percentages and trends of germinated seeds (left y-axis) for each treatment ([a]: no pretreatment/not stratified; [b]: cold stratification) over 60 days (x-axis) for the three stations (green = Botanical Garden; yellow = Ostia; light blue = Zagarolo) at different temperatures (rows) and different light regimes (columns). The semitransparent areas (same color as the stations) show the confidence intervals of the estimated logistic models, while the dashed and dotted lines represent the germination data. Panel (a) shows the results for non-pretreated seeds, whereas panel (b) shows results for pretreated seeds: germination of seeds in dark (i) and light (ii) conditions at a temperature of 10°C–20°C; germination of seeds in dark (iii) and light (iv) conditions at a temperature of 15°C–25°C; germination of seeds in dark (v) and light (vi) conditions at a temperature of 6°C–15°C; germination of previously cold-stratified seeds in dark (vii) and light (viii) conditions at a temperature of 10°C–20°C; germination of previously cold-stratified seeds in dark (ix) and light (x) conditions at a temperature of 15°C–25°C; and germination of previously cold-stratified seeds in dark (xi) and light (xii) conditions at a temperature of 6°C–15°C. The three stations without cold stratification (a) and exposed to minimum temperatures (panel [a]: v and vi) do not show an estimated model because germinations were concentrated in the last days of the experiment. The Botanical Garden station with pretreatment (b) and at minimum temperatures (panel [b]: xi and xii) has no estimated model because the trend deviates from a sigmoid. Some lines do not show confidence intervals, as the model did not estimate any results due to very few germinations or germinations concentrated in the last week of the experiment.

et al., 2000; Leishman et al., 2000). Seed size is influenced by the environmental conditions in which seeds are produced (Mamo et al., 2006). Therefore, differences in environmental conditions among the three sites do not appear to have any impact on seed size. These two similar results for the morphometric analysis, regarding fruit

number and seed dimensions, could be an indicator of the species' adaptation versatility to biotic and abiotic ecological factors.

The germination capability of a plant and the characteristics of seeds are useful to understand and interpret the mechanisms and dynamics of invasiveness of an

allochthonous species (Pyšek et al., 2008). In fact, invasive species exhibit a rapid growth rate (Grotkopp et al., 2002; Widrlechner et al., 2004). The abundance of seeds produced by plants and the rate of germination are key determinants in the initial invasion phase of a plant (Grotkopp et al., 2002; Widrlechner et al., 2004). The results of the germination test with and without pretreatment indicate greater germination of the three populations after stratification, under light and at maximum alternating temperatures, compared with the corresponding experimental sets not subjected to such stratification. From an ecological perspective, the cold stratification seems necessary to release dormancy of the seeds, enabling the germination in the correct season with the right levels of humidity and temperature, preventing germination in unfavorable environmental conditions. In addition, the percentage of germinating seeds increased with higher temperature (Schütz & Rave, 1999). Light probably does not have a considerable effect on the germination of *P. americana* at average and minimum temperature as this plant is weakly sciaphilous (Steinmetz, 1963). The ability of *P. americana* to germinate under both light and dark conditions allows it to colonize a range of environments, including forest edges. The reduced role of light in seed germination at maximum temperatures can be explained by the fact that the seeds exposed to alternating daily temperatures possess low light requirements, whereas seed germination is more controlled by temperature conditions (Catara et al., 2016; Koutsovoulou et al., 2014; Pepe et al., 2020; Thompson & Grime, 1983; Vázquez-Yanez & Orozco-Segovia, 1994).

Temperature appears to significantly influence seed behavior. In fact, warmer temperatures promote seed germination, intensify germination speed, and reduce T50 days. Higher temperatures in cities, influenced by urban heat islands, could make them more susceptible to invasion by *P. americana*. This species has demonstrated increased germination rates under higher temperatures, as evidenced by the results (McKinney, 2006). It is not a coincidence that this species often appears on the checklists of European urban floras (Casanelles-Abella et al., 2021; Zavyalova, 2008); furthermore, it has even increased in other areas and cities (Fratarcangeli et al., 2022).

Collection sites, on the other hand, appear to influence seed germination. In fact, the seeds collected in the three sites, located at different elevations, show differences in germination. Without cold stratification, differences are detectable only at moderate temperatures, both in light and dark conditions. In fact, seeds taken in ZAG (337 m a.s.l.) have a higher germination. This could be because seeds gathered from the site

from the highest elevation had already been exposed to a longer period of cold weather at the time of sampling, resembling a form of “cold stratification.” This period of exposure to cold could have partially released seed dormancy. Alternatively, studies have suggested that seeds from colder climates often exhibit decreased dormancy, requiring shorter dormancy-breaking periods compared with seeds from warmer climates (Baskin & Baskin, 2022). Cold stratification amplifies the differences between the three sites. It is likely that seeds of OS and BG are adapted to warmer environments, with optimum germination occurring at higher temperatures. The seeds from the ZAG site, at moderate temperature, always show a lower germination percentage and a higher T50 than the other two sites. A possible explanation could be that the individuals from BG and OS are more adapted to warm and moderate temperatures than the ZAG ones. Likely, lowland individuals are less adapted to cool temperatures. Consequently, seeds collected from the ZAG site can germinate more at cool temperatures than the other two. Cool temperatures, therefore, limit the sites at low elevations (5 m and 50 m a.s.l.) more than the site at high elevations. To prevent germination in autumn, most seeds probably rely on a combination of environmental and endogenous factors (low temperatures, hard integument, and autotoxicity; Bewley & Black, 1994). The inhibition of germination by lower temperatures is an established adaptation in several species that mature seeds in the fall season (Bewley & Black, 1994). In addition, variability among plants may be due to factors such as differences in phenotypic plasticity, resource availability, and/or plant age (Armesto et al., 1983).

Variations in germination rates may have a genetic origin or be caused by the environmental conditions (day length, temperature, light quality, water availability, and elevation) in which the seed matured (Loha et al., 2006) and can be greatly influenced by the position of the seed on the fruit and/or infructescence (Gutterman, 2000). The discordant behavior of *P. americana* seeds at different sites may be a response to the great phenotypic plasticity of this plant. The OS and BG sites do not differ much from an altitudinal point of view, and, in fact, their germination capabilities are much more similar compared with the ZAG collection. Elevation seems to possess an effect on seed germination ability in *P. americana*. This likely explains the colonization success in different environments of this species. However, genetic differences among *P. americana* populations should also be explored in the future.

The results on *P. americana* highlight important invasiveness characteristics: It has high seed production regardless of elevation and has seeds that do not require

light to germinate. The ability to germinate in the absence of light and the possession of orthodox seeds allow *Phytolacca americana* to persist in the soil for prolonged times without germinating and, consequently, to survive harsh conditions not suitable for establishment.

In general, *P. americana* seems adapted to warmer temperatures at lower elevations and cooler temperatures at higher elevations, with all populations demonstrating increased germination in response to cold stratification. Knowing the mechanism of invasion, the extent of germination, and the altitudes at which it spreads optimally, is important for planning strategies to control invasive allochthonous species. These results, in a scenario of climate change, show that the invasiveness of *Phytolacca americana* may increase in the future.

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REFERENCES

- Aislabie, L., & Cefas, L. (2020). Risk assessment template developed under the “Study on Invasive Alien Species—Development of risk assessments to tackle priority species and enhance prevention” Contract No 07.0202/2018/788519/ETU/ENV. D. 21.
- Armesto, J. J., Cheplick, G. P., & McDonnell, M. J. (1983). Observations on the reproductive biology of *Phytolacca americana* (Phytolaccaceae). *Bulletin of the Torrey Botanical Club*, 110, 380–383. <https://doi.org/10.2307/2996194>
- Bacchetta, G., Belletti, P., Brullo, S., Cagelli, L., Carasso, V., Casas, J. L., & Virevaire, M. (2006). *Manuale per la raccolta, studio, conservazione e gestione ex situ del germoplasma* (Vol. 37, pp. 1–244). APAT.
- Bagnouls, F., & Gaussen, H. (1953). Dry periods and vegetation. *Comptes rendus hebdomadaires des seances de l'Academie des sciences*, 236(10), 1075–1077.
- Balogh, L., & Juhasz, M. (2008). American and Chinese pokeweed (*Phytolacca americana*, *Phytolacca esculenta*). In Z. Botta-Dukát & L. Balogh (Eds.), *The most important invasive plants in Hungary* (pp. 35–46).
- Baskin, C. C., & Baskin, J. M. (2022). Cold stratification in winter is more than enough for seed dormancy-break of summer annuals in eastern North America: Implications for climate change. *Seed Science Research*, 32(2), 63–69. <https://doi.org/10.1017/S0960258522000125>
- Bewley, J. D., & Black, M. (1994). Seeds. In *Seeds* (pp. 1–33). Springer.
- Campana, C., Gauvin, S., & Ponge, J. F. (2002). Influence of ground cover on earthworm communities in an unmanaged beech forest: Linear gradient studies. *European Journal of Soil Biology*, 38(2), 213–224.
- Casanelles-Abella, J., Frey, D., Müller, S., Aleixo, C., Ortí, M. A., Deguines, N., Hallikma, T., Laanisto, L., Niinemets, Ü., Pinho, P., Samson, R., Villarroya-Villalba, L., & Moretti, M. (2021). A dataset of the flowering plants (Angiospermae) in urban green areas in five European cities. *Data in Brief*, 37, 107243. <https://doi.org/10.1016/j.dib.2021.107243>
- Catara, S., Cristaudo, A., Gualtieri, A., Galesi, R., Impelluso, C., & Onofri, A. (2016). Threshold temperatures for seed germination in nine species of Verbascum (Scrophulariaceae). *Seed Science Research*, 26(1), 30–46. <https://doi.org/10.1017/S0960258515000343>
- Chin, H. F., Krishnapillay, B., & Stanwood, P. C. (1989). Seed moisture: Recalcitrant vs. orthodox seeds. *Seed Moisture*, 14, 15–22. <https://doi.org/10.2135/cssaspecpub14.c2>
- Daru, B. H., Davies, T. J., Willis, C. G., Meineke, E. K., Ronk, A., Zobel, M., Pärtel, M., Antonelli, A., & Davis, C. (2021). Widespread homogenization of plant communities in the Anthropocene. *Nature Communications*, 12(1), 6983. <https://doi.org/10.1038/s41467-021-27186-8>
- de Lima, C. M. R., Borghetti, F., & de Sousa, M. V. (1997). Temperature and germination of the Leguminosae *Enterolobium contortisiliquum*. *Revista Brasileira de Fisiologia Vegetal*, 9(2), 97–102.
- Díaz-Segura, O., Golubov, J., Mandujano, M. C., & Zavala-Hurtado, J. A. (2020). Reproductive characteristics that favor invasiveness in *Leonotis nepetifolia* (L.) R. Br. *Plant Species Biology*, 35(4), 270–282.
- Dickson, T. L., Hopwood, J. L., & Wilsey, B. J. (2012). Do priority effects benefit invasive plants more than native plants? An experiment with six grassland species. *Biological Invasions*, 14, 2617–2624.
- Dullinger, S., Essl, F., Rabitsch, W., Erb, K. H., Gingrich, S., Haberl, H., & Hulme, P. E. (2013). Europe's other debt crisis caused by the long legacy of future extinctions. *Proceedings of the National Academy of Sciences*, 110(18), 7342–7347. <https://doi.org/10.1073/pnas.1216303110>
- Dumas, Y. (2011). American grape (*Phytolacca americana*): An invasive alien species. *RenDez-Vous Techniques*, 33/34, 47–57.
- Engelhardt, M. J., & Anderson, R. C. (2011). Phenological niche separation from native species increases reproductive success of an invasive species: *Alliaria petiolata* (Brassicaceae)—garlic mustard1. *The Journal of the Torrey Botanical Society*, 138(4), 418–433.
- Ferrari, S., & Cribari-Neto, F. (2004). Beta regression for modelling rates and proportions. *Journal of Applied Statistics*, 31(7), 799–815. <https://doi.org/10.1080/0266476042000214501>
- Fratarcangeli, C., Fanelli, G., Testolin, R., Buffi, F., & Travaglini, A. (2022). Floristic changes of vascular flora in the city of Rome through grid-cell census over 23 years. *Urban Ecosystems*, 25, 1–14.
- Gioria, M., Le Roux, J. J., Hirsch, H., Moravcová, L., & Pyšek, P. (2019). Characteristics of the soil seed bank of invasive and non-invasive plants in their native and alien distribution range. *Biological Invasions*, 21, 2313–2332.
- Gioria, M., & Osborne, B. A. (2014). Resource competition in plant invasions: Emerging patterns and research needs. *Frontiers in Plant Science*, 5, 501.
- Gioria, M., & Pyšek, P. (2017). Early bird catches the worm: Germination as a critical step in plant invasion. *Biological Invasions*, 19, 1055–1080.

- Godoy, O., Castro-Díez, P., Valladares, F., & Costa-Tenorio, M. (2009). Different flowering phenology of alien invasive species in Spain: Evidence for the use of an empty temporal niche? *Plant Biology*, 11(6), 803–811.
- Grotkopp, E., Rejmánek, M., & Rost, T. L. (2002). Toward a causal explanation of plant invasiveness: Seedling growth and life-history strategies of 29 pine (*Pinus*) species. *The American Naturalist*, 159(4), 396–419.
- Gutterman, Y. (2000). Environmental factors and survival strategies of annual plant species in the Negev Desert, Israel. *Plant Species Biology*, 15(2), 113–125.
- Harms, K. E., Wright, S. J., Calderón, O., Hernandez, A., & Herre, E. A. (2000). Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, 404(6777), 493–495.
- ISTA. (2005). *International rules for seed testing*. International Seed Testing Association.
- Keller, R. P., Geist, J., Jeschke, J. M., & Kühn, I. (2011). Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe*, 23(1), 1–17.
- Koutsovoulou, K., Daws, M. I., & Thanos, C. A. (2014). Campanulaceae: A family with small seeds that require light for germination. *Annals of Botany*, 113(1), 135–143. <https://doi.org/10.1093/aob/mct250>
- Kowarik, I. (2011). Novel urban ecosystems, biodiversity, and conservation. *Environmental Pollution*, 159(8–9), 1974–1983. <https://doi.org/10.1016/j.envpol.2011.02.022>
- Lambdon, P. W., Pyšek, P., Basnou, C., Hejda, M., Arianoutsou, M., Essl, F., ... Zikos, A. (2008). Alien flora of Europe: Species diversity, temporal trends, geographical patterns and research needs. *Preslia*, 80(2), 101–149.
- Lazzaro, L., Bolpagni, R., Buffa, G., Gentili, R., Lonati, M., Stinca, A., Acosta, A. T. R., Adorni, M., Aleffi, M., Allegranza, M., Angiolini, C., Assini, S., Bagella, S., Bonari, G., Bovio, M., Bracco, F., Brundu, G., Caccianiga, M., Carnevali, L., ... Lastrucci, L. (2020). Impact of invasive alien plants on native plant communities and Natura 2000 habitats: State of the art, gap analysis and perspectives in Italy. *Journal of Environmental Management*, 274, 111140. <https://doi.org/10.1016/j.jenvman.2020.111140>
- Leishman, M. R., Wright, I. J., Moles, A. T., & Westoby, M. (2000). The evolutionary ecology of seed size. *Seeds: The Ecology of Regeneration in Plant Communities*, 2, 31–57.
- Loha, A., Tigabu, M., Teketay, D., Lundkvist, K., & Fries, A. (2006). Provenance variation in seed morphometric traits, germination, and seedling growth of *Cordia africana* Lam. *New Forests*, 32(1), 71–86. <https://doi.org/10.1007/s11056-005-3872-2>
- Mamo, N., Mihretu, M., Fekadu, M., Tigabu, M., & Teketay, D. (2006). Variation in seed and germination characteristics among *Juniperus procera* populations in Ethiopia. *Forest Ecology and Management*, 225(1–3), 320–327. <https://doi.org/10.1016/j.foreco.2006.01.026>
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, 127(3), 247–260. <https://doi.org/10.1016/j.biocon.2005.09.005>
- Min, B. M. (2014). Distribution of *Phytolacca americana* in a coastal sand dune. *Journal of Ecology and Environment*, 37(2), 81–90.
- Moravcova, L., Pyšek, P., Pergl, J., Perglova, I., & Jarošík, V. (2006). Seasonal pattern of germination and seed longevity in the invasive species *Heracleum mantegazzianum*. *Preslia*, 78(3), 287–301.
- Orrock, J. L., & Damschen, E. I. (2005). Corridors cause differential seed predation. *Ecological Applications*, 15(3), 793–798.
- Orwig, D. A., & Foster, D. R. (1998). Forest response to the introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society*, 125, 60–73.
- Pepe, M., Gratani, L., Fabrini, G., & Varone, L. (2020). Seed germination traits of *Ailanthus altissima*, *Phytolacca americana* and *Robinia pseudoacacia* in response to different thermal and light requirements. *Plant Species Biology*, 35(4), 300–314. <https://doi.org/10.1111/1442-1984.12286>
- Pergl, J., Sádlo, J., Petrušek, A., Laštůvka, Z., Musil, J., Perglová, I., Šanda, R., Šefrová, H., Šíma, J., Vohralík, V., & Pyšek, P. (2016). Black, Grey and watch lists of alien species in The Czech Republic based on environmental impacts and management strategy. *NeoBiota*, 28, 1–37.
- Pignatti, S., Guarino, R., & La Rosa, M. (2017). *Flora d'italia* (Vol. 1). Bologna: Edagricole.
- Presotto, A., Hernández, F., Casquero, M., Vercellino, R., Pandolfo, C., Poverene, M., & Cantamutto, M. (2020). Seed bank dynamics of an invasive alien species, *Helianthus annuus* L. *Journal of Plant Ecology*, 13(3), 313–322.
- Pyšek, P., & Richardson, D. M. (2007). Traits associated with invasiveness in alien plants: Where do we stand? *Biological Invasions*, 193, 97–125.
- Pyšek, P., Richardson, D. M., Pergl, J., Jarošík, V., Sixtová, Z., & Weber, E. (2008). Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution*, 23(5), 237–244.
- Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *PLoS One*, 10(12), e0146021. <https://doi.org/10.1371/journal.pone.0146021>
- Roberts, E. H. (1973). Predicting the storage life of seeds. In *Proceedings*.
- Schirmel, J. (2020). Differential effects of American pokeweed (*Phytolacca americana*) invasion on ground-dwelling forest arthropods in southwest Germany. *Biological Invasions*, 22(4), 1289–1298.
- Schlaepfer, D. R., Glaettli, M., Fischer, M., & van Kleunen, M. (2010). A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *The New Phytologist*, 185, 1087–1099. <https://doi.org/10.1111/j.1469-8137.2009.03114.x>
- Schütz, W., & Rave, G. (1999). The effect of cold stratification and light on the seed germination of temperate sedges (*Carex*) from various habitats and implications for regenerative strategies. *Plant Ecology*, 144(2), 215–230.
- Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., Causton, C. E., Celesti-Gradow, L., ... Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences*, 115(10), E2264–E2273. <https://doi.org/10.1073/pnas.1719429115>
- Steinmetz, E. F. (1963). *Phytolacca americana*. *Quarterly Journal of Crude Drug Research*, 3(4), 478–492.
- Thompson, K., & Grime, J. P. (1983). A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology*, 20, 141–156.

- van Kleunen, M., Dawson, W., Essl, F., Pergl, J., Winter, M., Weber, E., Kreft, H., Weigelt, P., Kartesz, J., Nishino, M., Antonova, L. A., Barcelona, J. F., Cabezas, F. J., Cárdenas, D., Cárdenas-Toro, J., Castaño, N., Chacón, E., Chatelain, C., Ebel, A. L., ... Pyšek, P. (2015). Global exchange and accumulation of non-native plants. *Nature*, 525(7567), 100–103. <https://doi.org/10.1038/nature14910>
- Van Kleunen, M., & Johnson, S. D. (2007). South African Iridaceae with rapid and profuse seedling emergence are more likely to become naturalized in other regions. *Journal of Ecology*, 95, 674–681. <https://doi.org/10.1111/j.1365-2745.2007.01250.x>
- Vázquez-Yanez, C., & Orozco-Segovia, A. (1994). Signals for seeds to sense and respond to gaps. In M. N. Cardwell & R. W. Pearcy (Eds.), *Exploiting of environmental heterogeneity by plants: Ecophysiological processes above and belowground*, Academic Press.
- Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., & Hulme, P. E. (2010). DAISIE partners: How well do we understand the impacts of alien species on ecosystem services? A pan-European cross-taxa assessment. *Frontiers in Ecology and the Environment*, 8, 135–144. <https://doi.org/10.1890/080083>
- Vilà, M., & Ibáñez, I. (2011). Plant invasions in the landscape. *Landscape Ecology*, 26(4), 461–472.
- Widrlechner, M. P., Thompson, J. R., Iles, J. K., & Dixon, P. M. (2004). Models for predicting the risk of naturalization of nonnative woody plants in Iowa. *Journal of Environmental Horticulture*, 22(1), 23–31.
- Zavyalova, L. V. (2008). Alien fraction of Chernihiv urban flora: Analysis and checklist. *Biodiversity Research and Conservation*, 11(12), 17–26.
- Zeng, J.-J., Zhou, B., & Wang, N. (2021). Comparing the reproductive biological characteristics of the alien invasive *Coreopsis lanceolata* to those of the non-invasive alien congener *Coreopsis tinctoria*. *Plant Species Biology*, 36, 379–389. <https://doi.org/10.1111/1442-1984.12323>

SUPPORTING INFORMATION

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