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RESEARCH ARTICLE

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Germination niche breadth of invasive *Iris pseudacorus* (L.) suggests continued recruitment from seeds with global warming

Morgane B. Gillard[1] | **Jesús M. Castillo**[2] | **Mohsen B. Mesgaran**[3] | **Caryn J. Futrell**[1] | **Brenda J. Grewell**[1,3]

[1]USDA-Agricultural Research Service, Invasive Species and Pollinator Health Research Unit, Department of Plant Sciences MS-4, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA

[2]Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla 41080, Spain

[3]Department of Plant Sciences MS-4, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA

Correspondence

Brenda J. Grewell, Department of Plant Sciences MS-4, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA.
Email: bjgrewell@ucdavis.edu

Abstract

Premise: Understanding recruitment processes of invasive species is central to conservation and management strategies. *Iris pseudacorus*, an emergent macrophyte, has established invasive populations across a broad global range, and reduces biodiversity in wetland ecosystems. Climate change is altering germination cues, yet studies on the invasion of wetland macrophytes often ignore germination ecology despite its importance to their establishment and spread.

Methods: We explored germination of seeds from invasive *I. pseudacorus* populations in California in response to seed coat presence or absence, and several environmental factors. Using experimental results in a thermal time model, we derived germination temperature thresholds.

Results: Germination of *I. pseudacorus* seeds did not require cold or warm stratification, and was not affected by seed coat presence or absence. Germination occurred in the dark, although germinability was two- to threefold times greater under light. At constant temperature, thermal time model estimates included 18.3 ± 1.8°C base germination temperature (T_b); 28.2 ± 0.5°C optimal temperature (T_o); and 41.0 ± 1.7°C ceiling temperature (T_c). Seeds exposed to 36.0°C achieved over 10% germination, and embryos of ungerminated seeds presented 76% viability. Overall, germinability remained relatively low at constant temperatures (≤25%) but was close to 90% under alternating daily temperatures.

Conclusions: Exposure to diurnally fluctuating temperatures is essential for this species to achieve high germination rates. Our study reveals that *I. pseudacorus* has a broad germination niche supporting its establishment in a relatively wide range of environments, including at high temperatures more frequent with climate change.

KEYWORDS

climate change, germination ecology, global warming, invasive species, plant invasions, seed coat, seed dormancy, thermal time models

Ecosystem susceptibility to biological invasions and their ecological effects can increase when the functional traits, such as reproductive and dispersal traits, of invasive species support their capacity to colonize new niches (Hellmann et al., 2008; Drenovsky et al., 2012; Moravcová et al., 2015). Vegetative reproduction is commonly associated with perennial plants in aquatic environments, although plant life strategies often include multiple reproductive modes (Barrett, 2015). Therefore, the seed life stage and variation in seed ecological traits in response to environmental drivers are often ignored regarding plant invasions in wetland and riparian habitats, even though long-distance waterborne dispersal and colonization by seeds often underlies the spread of macrophytes in these environments (Boedeltje et al., 2004; Catford and Jansson, 2014).

Seed germination is a fundamental stage in the life cycle of plants, which can be a key to the spread of invasive plants (Gioria and Pyšek, 2017). Trait-based focus on germination ecology is needed to improve understanding of ecological adaptations of seed germination traits (Poschlod, 2020). This approach can inform the potential for success of a seed-dispersed invasive species in novel environments and thereby refine predictions of future invasions in the context of environmental change (Pepe et al., 2020). By recognizing the need to address changing environmental conditions in up-to-date invasive species risk assessments and management plans, managers have called for increased scientific support to better understand range-expanding invasive plants to make informed decisions (Beaury et al., 2020).

The wetland species *Iris pseudacorus* (L.) (yellow flag iris, Iridaceae), native to Europe, western Asia, and northern Africa, was introduced as an ornamental aquatic plant worldwide (Gervazoni et al., 2020). In many ecozones, *I. pseudacorus* has escaped cultivation and has been naturalized, becoming invasive in wetlands and marshes of South America, North America, South Africa, eastern Asia, Australia, and New Zealand where it is displacing native plant species (Mopper et al., 2016; Hayasaka et al., 2018; Minuti et al., 2022). Seeds of *I. pseudacorus* disperse via hydrochory; a large majority of seeds can float for months (Coops and Vandervelde, 1995; van den Broek et al., 2005). Analysis of molecular markers highlighted the reliance of this species on sexual rather than clonal reproduction for both invasive and native populations (Lamote et al., 2002; Gaskin et al., 2016). The high risk of *I. pseudacorus* colonization and establishment from seeds into novel habitats highlights the increasing need for an investigation of seed germination ecology of invasive populations.

Knowledge of conditions that alleviate dormancy is fundamental, yet it remains unclear if seeds of *I. pseudacorus* require a stratification period or seed coat damage to break their dormancy. Baskin and Baskin (2014) reported that *I. pseudacorus* has morphophysiological dormancy that requires cold stratification and does not require scarification to germinate. However, Crocker (1906) and Suzuki and Yamagata (1980) achieved germination in *I. pseudacorus* seeds only after removing the cap from the seeds or by damaging the seed coat. Coops and Vandervelde (1995) did not mention the need for such operation prior to germination but reported using a cold stratification treatment of two months. Similarly, the American Iris Society reported that germinating *Iris* seeds requires several months of cold temperatures followed by a warming period (Waters, 2017). Conversely, Guppy (1912) reported that seeds of *I. pseudacorus* in England do not present dormancy and germinate rapidly. Our field observations align with those by Guppy (1912); during seed collections at multiple population sites in hot, early-fall conditions in California, we have observed vivipary, with a small fraction of germinated seeds within capsules while still on parent (Gillard et al., 2021). These observations suggest that some seeds lack dormancy.

Temperature and moisture are among the most important abiotic factors for seed germination, with a great influence on germination timing (Donohue, 2005) that shapes community composition (Jiménez-Alfaro et al., 2016). In a previous study, we found *I. pseudacorus* seeds quickly reached 50% germination fractions at ~10 days in freshwater flooded conditions compared to ~12 days in moist conditions, and aqueous salinity drove differences in germination fractions independent of water levels (Gillard et al., 2021). Most of the information about the effect of temperature on germination of *I. pseudacorus* has been reported by Sutherland (1990) who stated no germination below 15°C and the highest germination fraction at 30°C, without providing detailed methods or detailed results. Thus, the cardinal temperatures for germination of *I. pseudacorus* seeds remain unclear. Based on experimental results, threshold models allow for the prediction of seed germination in response to environmental factors (Bradford, 2002), and are increasingly used to improve weed management and crop protection (Batlla and Benech-Arnold, 2005; Boddy et al., 2012). Such models developed for agronomic purposes can also increase understanding of invasion ecology in natural systems (Gioria and Pyšek, 2017). They can be applied to predict the emergence in the field under a range of environmental conditions and could thus be used to evaluate and refine timing of efforts for optimal management of *I. pseudacorus*.

Other environmental factors, such as light, may promote or inhibit germination (Batlla and Benech-Arnold, 2014). The light requirements to initiate germination of *I. pseudacorus* are not well defined because Deno (1993) reported that *I. pseudacorus* seeds do not germinate in the dark, while Rosbakh et al. (2020) described a decrease of germination in dark conditions.

In summary, information about the germination ecology of *I. pseudacorus* is scarce, often contradictory, and draws primarily from anecdotal observations or from reports lacking methodological details. Unfortunately, much of this unvetted information is included in widely distributed invasion risk assessments (e.g., U.S. Fish and Wildlife Service, 2019; GBIF, 2021), used as management prioritization tools to prevent invasions or control their spread. Global climate change is altering environmental cues that drive germination, population dynamics, the distribution of plant species (Walck et al., 2011), and the role of germination in the invasion process (Gioria and Pyšek, 2017). The limited information about the germination of *I. pseudacorus* is largely from studies in freshwater wetland populations in its native European range. Functional traits of invasive intertidal populations of *I. pseudacorus* being influenced by climate change are likely different from historic observations of wild native populations in Europe. Increased research on seed ecology in under-studied ecosystems is needed to comprehend and mitigate plant responses to changing environments (Walck et al., 2011). There have been little data on critical germination thresholds for invasive plant species in nonagricultural systems (Dürr

et al., 2015). Thus, empirical data from experimental studies are needed to validate functional aspects of seed ecology and provide ecologically-based recommendations for updating risk assessments and management of *I. pseudacorus* invasions. Through a series of experiments, we investigated the germination ecology of invasive *I. pseudacorus* intertidal populations. Our specific objectives were to (1) determine stratification or scarification requirements for germination, (2) investigate the impact of light on germination, and (3) explore the temperature requirements of the tested populations and derive important germination thresholds. Our results should contribute to improved understanding of the seed recruitment capacity of *I. pseudacorus* under a range of environmental conditions relevant to preventing and mitigating of the spread of invasion in estuaries under climate change.

MATERIALS AND METHODS

Study sites

In northern California, the recent spread of *I. pseudacorus* from the Sacramento–San Joaquin Delta to multiple sites in the sensitive tidal wetlands of Suisun Marsh and the Carquinez Strait of the San Francisco Estuary represents a concern considering the invasiveness of the species and the vulnerability of these ecosystems. In this study, we investigated the germination characteristics of seeds sourced from five populations of invasive *I. pseudacorus* (San Joaquin River at Buckley Cove [BC], Three Mile Slough at Brannon Island [BI], San Joaquin River at Antioch [AN], Montezuma Slough at Grizzly Island [MS], Glen Cove shoreline at Vallejo-Carquinez Strait [CS]) separated by 35–40 km in intertidal habitats along the species' current estuarine distribution in the Sacramento–San Joaquin River Delta–San Francisco Estuary (Appendix S1).

Seed collection

Mature capsules (fruits) of *I. pseudacorus* were collected in August–September 2017 from the five invasive populations described above, before natural release of seeds by loculicidal dehiscence. The collection effort was distributed among all the extant patches within each site, with an average of 132 capsules (63 ± 23 seeds per capsule) collected per population. Capsules were stored in plastic bags at 4°C for up to two days after collection. They were then air dried, and seeds were stored in paper bags in dry conditions at room temperature until they were used for experiments performed between May and December 2018.

From our experience with seeds of *I. pseudacorus* through prior trials, we observed that seeds from this species have high imbibition capacity. Therefore, we included a soaking period of one week (for four experiments) or two weeks (for one experiment) in deionized (DI) water at the beginning of each of the following experiments to ensure sufficient seed hydration.

Experimental designs

Of the five populations for which seeds were collected, all five were used only in one of the experiments to provide strong data to the complex models applied; three of the five populations, evenly covering the known estuarine distribution, were used in two of the experiments. Finally, two single populations selected randomly were used in two smaller trials. In all germination experiments, we used only those seeds that appeared to have fully developed embryos. Germination was monitored daily at first, every other day once the germination peak had passed, and then every three days as the rate of germination slowed. For the alternating temperature experiment, for which germination occurred at a faster pace, we continued monitoring every other day after the peak. A seed was considered germinated once the radicle protruded the testa and was visible. Germinated seeds were removed from the dishes, and DI water was added to the dishes as needed to keep the filter paper saturated.

Stratification experiment

Using populations BC, AN, and CS (BC and CS being at the extreme ends of the species current estuarine distribution, and AN being intermediate; Appendix S1), for each population we sampled a total of 300 seeds that were mixed, separated in 12 lots of 25 seeds, and weighed. Seed surfaces were sterilized by immersion in a sodium hypochlorite solution (5%) for 1 min and rinsed with running tap water, with a final rinsing with deionized (DI) water. Six lots (replicates) of seeds per population were soaked for 16 days in DI water either at 5°C (cold stratification) or at 25°C and 15°C at 12 h each, both under warm stratification, in the dark. (Hereafter, the differences in photoperiod, such as this one, with 12 h light and 12 h dark cycles, are denoted as 25/15°C.) After this stratification treatment, seed lots were transferred into a Petri dish (90 mm diameter) and placed on moist sterile filter paper (Whatman Grade 1, Cytiva Global Life Sciences Solutions LLC, Marlborough, Massachusetts, USA) placed above a layer of 6 mm diameter beads (Darice, Inc. Stongsville, Ohio, USA), and watered using DI water. All Petri dishes were randomly arranged in an environmental chamber (Percival I-22VL, Percival Scientific, Perry, Iowa, USA) set at 20°C day and 12°C night (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 12-h light/12-h dark photoperiod). Germination was monitored for 59 days.

Seed coat experiment

Five seeds were extracted from each of 40 capsules taken randomly from population MS and mixed. The 200 seeds sampled were randomly separated into eight lots of

25 seeds, and seeds were soaked for one week in Petri dishes filled with DI water. Then, the seed coat of 100 seeds (half of the lots) was removed without damaging the endosperm, by carefully incising the coat with a razor blade. All seeds were then placed on a single layer of moist filter paper above a layer of 6 mm diameter beads (Darice, Inc., Stongsville, Ohio, USA) in a Petri dish (90 mm diameter). Thus, for each treatment (with and without seed coat), there were four replicates of 25 seeds. The Petri dishes were arranged randomly in a greenhouse located at the U.S Department of Agriculture, Agricultural Research Service (USDA-ARS) Aquatic Weed Research Facility at the University of California, Davis. Light conditions in the greenhouse were approximately 280 $\mu mol \cdot m^{-2} \cdot s^{-1}$, photoperiod 12-h light/12-h dark, and air temperature during the experiment was 25.5 ± 5.7°C. We terminated the experiment after 71 days when no germination had been observed for 10 consecutive days.

Light experiment

Ten seeds were taken from 45 capsules that had been collected from three different patches (15 capsules per patch) from AN, BC, and CS populations. Thus, a total of 450 seeds were mixed, separated in 18 lots of 25 seeds per population, and weighed. Seeds were soaked for seven days in DI water in the dark at 22°C in vials thoroughly wrapped in aluminum foil to block light exposure. The seed lots were then split in three batches of six replicates per population, assigned to one of three light treatments: "light," "darkness + green light," and "total darkness." The investigation of germination in continuous dark conditions usually includes the use of a green safelight to monitor germination because the wavelength of this light does not trigger germination for most species, although there are exceptions (Luna et al., 2004). Each lot of seeds was placed in a Petri dish (90 mm diameter) on a moist filter paper (Whatman Grade 1, Cytiva Global Life Sciences Solutions LLC, Marlborough, Massachusetts, USA) placed above a layer of 6 mm diameter beads (Darice, Inc. Stongsville, Ohio, USA) and watered using DI water. All Petri dishes were randomly arranged in one of three environmental chambers (Percival Scientific I-22VL, Perry, Iowa, USA) assigned to one of the light treatments, and exposed daily to 20°C and 12°C by 12-h windows. Seeds of the "light" treatment were placed on Petri dishes under artificial light and were then exposed to 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a photoperiod 12/12-hrs, the daylight hours corresponding to the highest temperature, i.e., 20°C. Seeds of the "darkness + green light" treatment were placed on Petri dishes over 20 min in a dark room using a green light (520–560 nm), and then placed in continuous darkness. The Petri dishes of the "total darkness" treatment were placed in Petri dishes in the dark in a dark room, sealed with Parafilm M (Amcor Flexibles North America, Inc., Neenah, Wisconsin, USA) to prevent water loss, wrapped in aluminum foil, and seeds were not exposed to light or green light because the environmental chamber was sealed and remained unopened throughout the experiment. Therefore, germination was only monitored over time for the "light" and "darkness + greenlight" treatments. For the "darkness + green light" treatment, the germination measurements and addition of water were performed in a dark room under green light. The experiment was run for 52 days. At the end of the experiment, germinated seeds of the "total darkness" treatment were counted (Appendix S2).

Constant temperature experiments

Using all five populations (AN, BC, BI, CS, MS), we selected 20–30 seeds from 10 capsules from six random *Iris* patches, or in the case of population CS, from 20 capsules from the available three patches. For each population, eight replicates of 25 seeds were placed in Petri dishes at eight constant temperatures (12, 16, 20, 24, 28, 32, 36, 40°C), in environmental chambers (Percival I-22VL, Percival Scientific, Perry, Iowa, USA) with a 12/12-h light-dark photoperiod (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Seeds were soaked in DI water in these conditions for seven days and were then placed on moist filter paper above a layer of 6 mm diameter beads (Darice, Inc., Stongsville, Ohio, USA). Germination monitoring took place from the beginning of soaking until we observed no germination for 10 consecutive days (except at 32°C and 36°C, where the experiment was stopped after six consecutive days without germination). Depending on temperature, the experiments lasted between 21 and 90 days (Appendix S3).

Alternating temperature experiments

We selected 20 seeds from 10 capsules from each of six *Iris* patches for population AN. Seeds were split into lots of 25 in Petri dishes, which were randomly arranged in environmental chambers (Percival PGC-105, Percival Scientific, Perry, Iowa, USA) at 25/15°C or 35/25°C (day/night) with a 12/12 photoperiod (≈30 $\mu mol \cdot m^{-2} \cdot s^{-1}$). There were eight replicates of 25 seeds per treatment. Seeds were soaked in DI water for seven days, and then placed on a moist filter paper imbibed with DI water, above a layer of 6 mm diameter beads (Darice, Inc., Stongsville, Ohio, USA). Germination was monitored for 55 days from the beginning of seed soaking.

Embryo viability

For stratification, seed coat, light, and alternating temperature experiments, the embryo viability of all ungerminated seeds was tested with tetrazolium (2,3,5-triphenyl-2H-tetrazolium chloride) solution at 0.1% at the end of the experiments. Seeds were cut in half to bisect the embryo and then submerged in the tetrazolium solution for at least 48 h

at 4°C; viable embryos presented a pink or red color. For the constant temperature experiments, given the large number of ungerminated seeds, embryo viability was only tested for a subsample of half of the ungerminated seeds for each of the five highest temperatures (24, 28, 32, 36, 40°C). The initial viability of a subset of seeds was tested following seed collection, using seeds from one to two capsules per extant patches for each population, with a total of 55 capsules tested and 31 ± 5 seeds per capsule (Appendix S4).

Germination indices and statistical analyses

All statistical analyses were performed using R 4.0.0. software (R Core Team, 2020), and plots were generated using the R package ggplot2 (Wickham, 2016). Three indices were determined to describe germination patterns: maximum germination (as time, *t*, approaches infinity), germination lag time, and time to reach 50% germination (T50) when applicable.

Maximum germination and germination lag time were determined by fitting a Weibull model with a lag phase parameter to cumulative germination data, and T50 was determined by fitting a log-logistic model to cumulative data, using the 'drc' package (Ritz et al., 2015). Differences among treatments, or treatments and populations, were established by comparing the overlapping of 95% confidence intervals from the predicted values of the models, except for constant temperatures, which was a continuous variable.

For the stratification, light, and constant temperature experiments, we compared estimated embryo viability between populations and the environmental factor tested (either stratification, light, or temperature treatment) using a generalized linear model (GLM) fitted with a quasibinomial distribution and logit link function. For the light experiment, given that only final germination percentages were available for the "total darkness treatment" and not germination over time, maximum germination was only estimated for the "light" and "darkness + green light" treatments. We also estimated germinability for the three treatments using a GLM fitted with a quasibinomial distribution and logit link function. Differences among factors were established by comparing the overlapping of 95% confidence intervals from the predicted values of the GLMs. A two-way analysis of variance (ANOVA) was performed on individual seed weight to test the effect of population, the tested abiotic factor, and their interaction using the package 'car'. Data homoscedasticity and normality of residuals were checked using Levene's and Shapiro's tests, respectively. Data were square-root transformed if necessary to meet ANOVA assumptions. Tukey's HSD test was applied when *p*-values were significant (<0.05) to determine differences among population, treatment, or their interaction using the package 'agricolae' (de Mendiburu, 2013).

For the seed coat experiment and the alternating temperature experiment, Student's t-tests were performed on embryo viability and individual seed weight to explore differences between tested conditions. The normality of the data was checked, and Wilcoxon test was performed when the assumption of data normality was not met.

Thermal time model

Using results from the constant temperature experiment, we fitted a thermal time model to cumulative germination data. First, we calculated germination rates for seven of the eight temperatures tested (12°C was excluded because of the many final germination percentages of zero among the replicates) for six germination fractions (0.025, 0.05, 0.075, 0.1, 0.125, 0.15). Then, to estimate the base temperature, optimal temperature, and ceiling temperature, we fitted a beta function (nonlinear regression model) to the relation between germination rate (rapidity) and constant temperature as

$$GR = \frac{1}{t_g} = G_m\left(\left(\frac{T - T_b}{T_o - T_c} \times \frac{T_c - T}{T_c - T_o}\right)^{\frac{T_c - T_o}{T_o - T_b}}\right)^a,$$

where GR is germination rate, i.e., reciprocal of time to a given germination fraction, t_g; T is the germination temperature; T_b is the base temperature; T_o is the optimal temperature; T_c is the ceiling temperature; a is a shaping parameter; and G_m is the highest germination rate that occurs at optimal temperature, T_o.

RESULTS

Stratification experiment

There was no effect of the stratification treatment on maximum germination (Figure 1A). Overall, the maximum germination of population BC was 47.9 ± 2.7%, 1.8–2.6-fold greater than that of populations CS (26.3 ± 2.6%) and AN (17.9 ± 2.2%), respectively, and that of CS was 1.4-fold greater than that of AN (Figure 1A). There was no effect of population or stratification treatment on the germination lag (Figure 1B) of approximately six days, or on the ~93% embryo viability of ungerminated seeds (Appendix S5). The individual seed weight of population AN was the highest (0.067 ± 0.002), that of population BC was intermediate (0.062 ± 0.001 g), and that of population CS was the lowest (0.057 ± 0.001 g) (Appendices S5, S6).

Seed coat experiment

The absence of the seed coat slightly increased maximum germination, with the germination percentage 6% greater

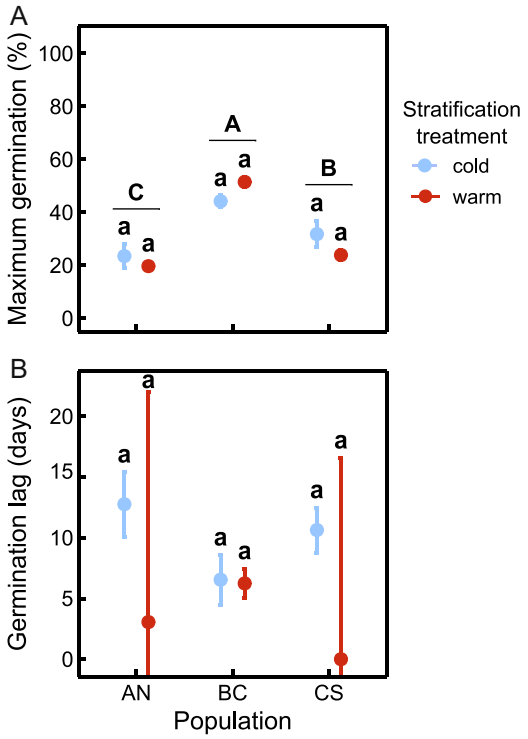

FIGURE 1 Predicted means for maximum germination and germination lag (±95% confidence interval) for seeds of *Iris pseudacorus* from three invasive populations subjected to two temperature stratification treatments. Similar small letters indicate there were no significant differences among treatments. Error bars not visible are smaller than the points. Populations: AN, Antioch; BC, Buckley Cove; CS, Carquinez Strait.

than with seed coat presence (92.7 ± 1.5 vs. 87.2 ± 2.1%) (Figure 2A). Germination lag was about eight days, independent of the presence or absence of the seed coat (Figure 2B). Finally, the embryo viability of ungerminated seeds was similar (~19%) between the two treatments (Appendix S7; Wilcoxon test: p-value = 1.00).

Light experiment

Maximum germination was 1.4-fold greater for population AN (55.5 ± 3.9 vs. 40.8 ± 2.4%) and 2.1-fold greater for population CS (35.9 ± 3.2 vs. 16.7 ± 3.0%) when seeds were exposed to light compared to when they were exposed to darkness and monitored with green light (Figure 3A). The predicted maximum germination of population AN (48.1 ± 1.8%) was overall 2.3-fold and 1.8-fold than that of population BC (21.0 ± 1.9%) and CS (27.2 ± 2.0%), respectively; population CS was 1.3-fold greater than that of population BC (Figure 3A). Germinability was two-fold greater for population AN (54.7 ± 6.9 vs. 30.7 ± 7.5%) and three-fold greater for population CS (37.3 ± 7.5 vs. 13.3 ± 3.9%) when seeds had been exposed to light compared to when they had been permanently exposed to dark (Figure 3B). However, germinability was comparable for population BC between the light and dark treatments (18.2 ± 3.6 vs. 24.0 ± 11.4%, Figure 3B). For all three

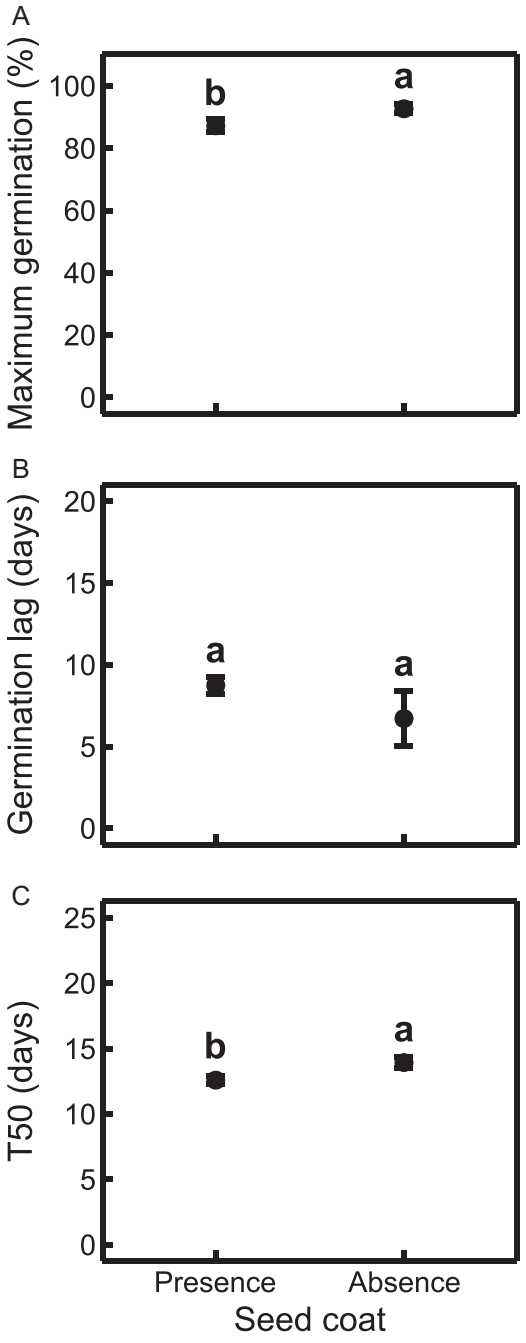

FIGURE 2 Estimated means for maximum germination, germination lag, and time to 50% germination (T50) (±95% confidence interval) for seeds of *Iris pseudacorus* from the Montezuma Slough population (MS) with or without seed coat. Different letters indicate significant differences between seed coat treatments. Error bars not visible are smaller than the points.

populations, there was no difference in germinability for seeds monitored using green light while exposed to darkness compared to those that were uniquely in darkness without green light monitoring throughout the experiment (Figure 3B). Mean germination lag ranged from 4.1–9.2 days but was not different among populations nor between light treatments (Figure 3C). The individual seed weight of population AN was

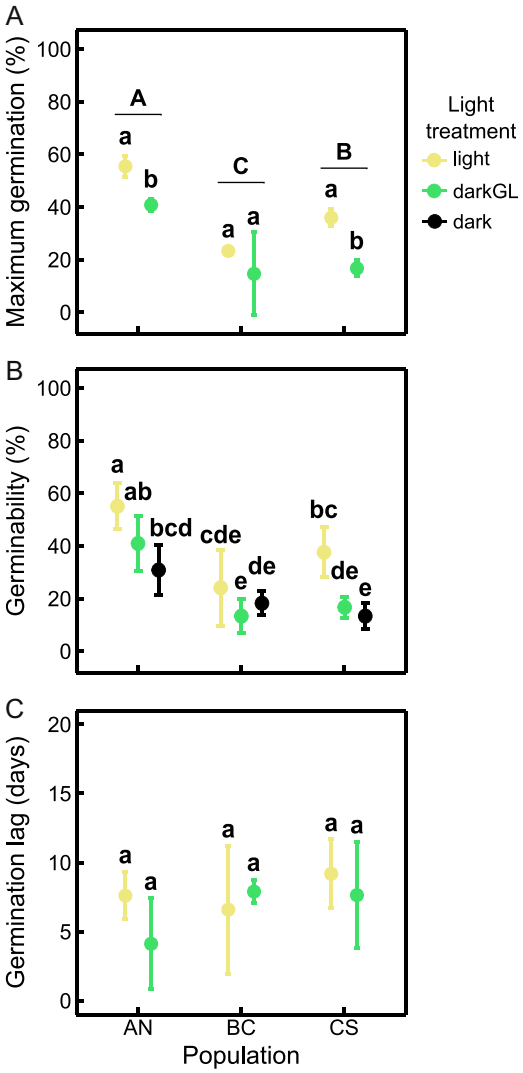

FIGURE 3 Estimated means for maximum germination, germinability, and germination lag (±95% confidence interval) for seeds of *Iris pseudacorus* from three invasive populations subjected to three light treatments. Different capital letters indicate differences among populations. Different small letters indicate significant differences among treatments. No letters indicate there were no differences. Error bars not visible are smaller than the points. Populations: AN, Antioch; BC, Buckley Cove; CS, Carquinez Strait.

the highest (0.072 ± 0.002 g), that of population BC was intermediate (0.065 ± 0.001 g), with population CS being the lowest (0.056 ± 0.002 g) (Appendices S6, S8).

Constant temperature experiments and thermal time model

The thermal time model applied estimated that base temperature (T_b) of *I. pseudacorus* was 18.3 ± 1.8°C, optimal temperature (T_o) was 28.2 ± 0.5°C, and ceiling temperature (T_c) was 41.0 ± 1.7°C (Figure 4), based on germination percentiles of 0.025, 0.05, 0.075, 0.1, 0.125, and 0.15 (for which the model was computing). In the

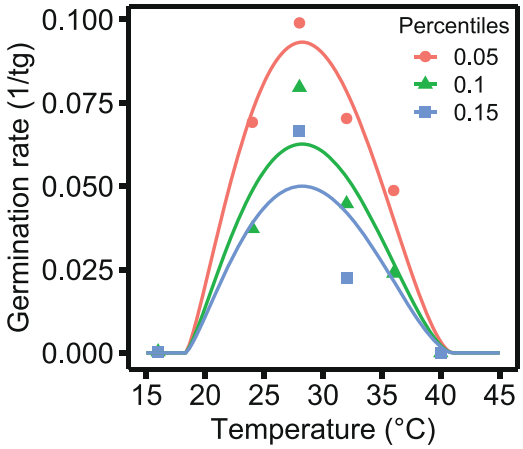

FIGURE 4 Fit of the beta function to germination rates (lines) and germination rate values (points) for percentiles 0.05, 0.1, and 0.15.

series of constant temperature experiments, seeds exposed to 28°C presented the highest maximum germination (24.9 ± 0.3%), followed by seeds exposed to 24, 32, and 36°C (12.1 ± 0.7, 16.2 ± 0.3 and 13.1 ± 0.5%, respectively; Figure 5A). Seeds exposed to 20°C had maximum germination of 5.7%, and seeds exposed to 12, 16, and 40°C all presented germinability <3%. Germination lag was different depending on temperature; seeds exposed to 28, 32, and 36°C germinated after about 6 to 7 days, and those at 24°C germinated after about nine days (Figure 5B). Estimated germination lag for seeds exposed to 16°C and 20°C was 9 to 11 days, lag was 21 days for those incubated at 12°C, and germination was almost immediate for seeds at 40°C. Ungerminated seeds, for which embryo viability was measured, showed 76–88% viability after exposure to the constant temperatures (Figure 5C).

Alternating temperature experiments

Independent of the temperature regime applied, maximum germination was 89% (Figure 6A). However, the time to germination lag was 1.6-fold times lower at 35/25°C than at 25/15°C, occurring after about 10.5 days at 25/15°C and after 6.5 days at 32/25°C (Figure 6B). Seeds at 25/15°C reached 50% of germination 1.4-fold sooner than those at 35/25°C (20 days vs. 14 days; Figure 6C). The embryo viability of ungerminated seeds was not different between the two temperature treatments (Appendix S9; Wilcoxon test: p-value = 0.24).

DISCUSSION

Our results characterized *I. pseudacorus* as an invasive species with the capacity for germination within a range of changing environmental conditions. Thus, we observed no enhancement of maximum germination due to cold or

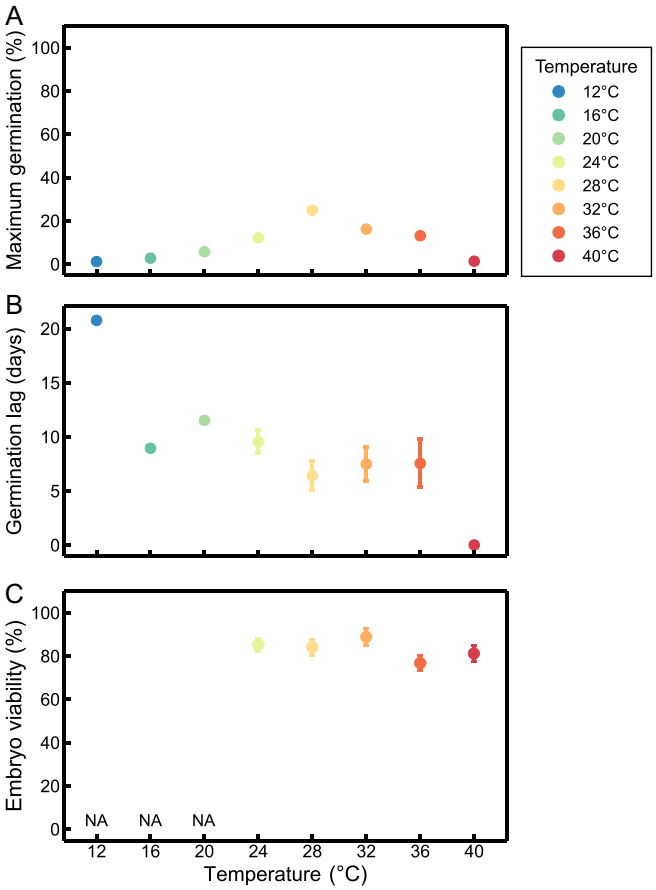

FIGURE 5 Estimated means for maximum germination, germination lag, and embryo viability (±95% confidence interval) for seeds of *Iris pseudacorus* from five invasive populations subjected to eight constant temperature stratification treatments. NA = Not Applicable. Error bars not visible are smaller than the points (except for temperatures 12, 16, 20, and 40°C in maximum germination and germination lag, for which standard error could not be computed by the model and returned NAs).

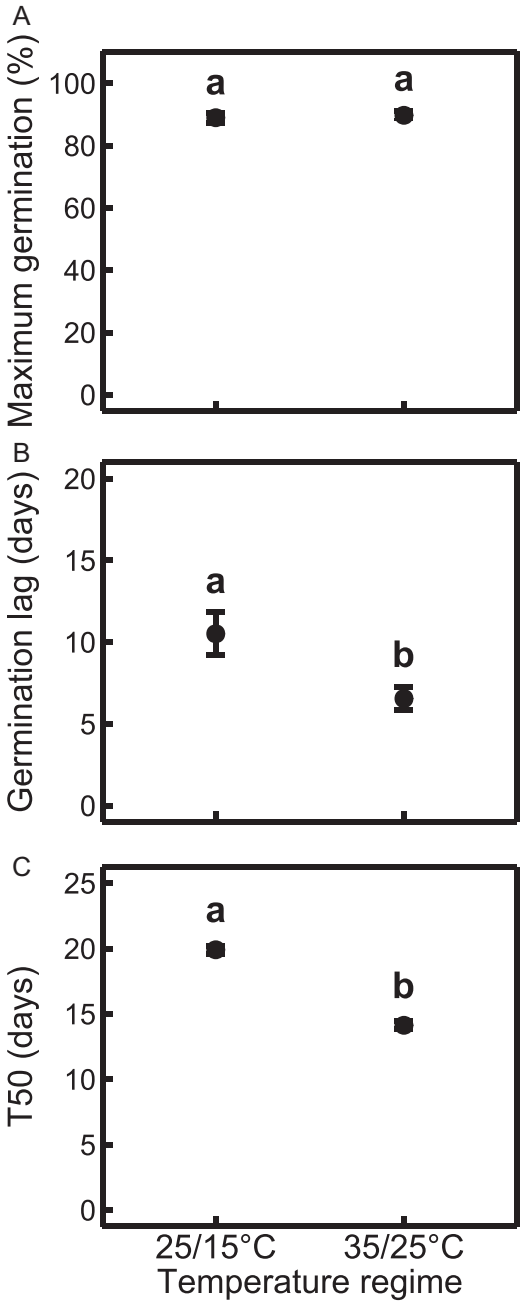

FIGURE 6 Predicted means for maximum germination, germination lag, and time to reach 50% germination (T50) (±95% confidence interval) for seeds of *Iris pseudacorus* from the Antioch population (AN) exposed to two alternating temperature regimes. Different small letters indicate significant differences between temperature treatments.

warm stratification treatments, and no effect of seed coat removal. Germinability was greater under light treatment, but also occurred in the dark. At constant temperatures, seeds of *I. pseudacorus* did not reach high germination percentages (≤25%), but we found they were able to germinate at high temperatures up to 36°C. Exposure to alternating temperatures led to germinability of approximately 90%.

A seven-day stratification treatment in cold or warm water did not result in significant differences in maximum germination or germination lag between the two conditions. The absence of a stratification requirement for most of *I. pseudacorus* seeds indicates the lack of a generalized primary physiological dormancy that would allow invasive seeds to germinate year-around. However, a small fraction (<10%) of the seeds from the studied populations could have either been quiescent or had a physiological dormancy mechanism because we never achieved 100% germination in our experiments, and most of the embryos from ungerminated seeds were viable. In addition, the investigated populations of I. *pseudacorus* did not present physical dormancy because the presence or absence of seed coat barely influenced seed germinability and velocity. In addition, we did not observe underdeveloped embryos, which does not corroborate with the potentiality of morphological dormancy (Baskin and Baskin, 2004).

Our results demonstrate that *I. pseudacorus* can germinate in total darkness, although for two out of three populations tested, light promoted germinability by two- to

threefold compared to germination in darkness. Our results contrast with that of Deno (1993) who concluded *I. pseudacorus* could not germinate in the dark. However, our results are consistent with that of Rosbakh et al. (2020), who observed fourfold greater germination fractions when seeds from native *I. pseudacorus* populations were exposed to light compared to darkness. The seeds tested in our experiment did not present sensitivity to green light. Therefore, the use of green light is appropriate to monitor germination of *I. pseudacorus* in darkness. In view of our results, a fraction of seeds could germinate if buried under a layer of sediment and/or turbid water that would prevent light to reach the seeds. Overall, large seeds are less dependent on light than small ones (Milberg et al., 2000). Nonetheless, despite its relatively large seeds compared to other wetland species (Soons et al., 2008; Kleyheeg et al., 2018), *I. pseudacorus* is sensitive to light, while the germination of other wetland species tends to not be affected by a reduction of light level (Fraser et al., 2014). In this sense, germination under buried conditions is frequent among macrophyte species colonizing tidal marshes where seed burial is common due to highly dynamic sedimentation processes (Abbas et al., 2020). However, the greater light requirement of *I. pseudacorus* compared to other wetland species could increase its persistence in seed banks (Jankowska-Blaszczuk and Daws, 2007) and promote its germination when floating on water and on shallow waters where seedling establishment would be favored (Nilsson et al., 2010).

Using the results from our series of experiments at eight constant temperatures ranging from 12–40°C, we developed a thermal time model to derive important germination thresholds, with estimated base temperature of 18.3 ± 1.8°C, optimal temperature of 28.2 ± 0.5°C, and ceiling temperature of 41.0 ± 1.7°C for the tested populations of *I. pseudacorus*. In addition, we observed significant germinability at a very high temperature (13% at 36°C), and 76% of ungerminated seeds were still viable after 90 days in moist conditions at 36°C. Similarly, 81% of ungerminated seeds were still viable after 21 days in moist conditions at 40°C, although very few seeds had germinated. The high germination fraction obtained for seeds exposed to an alternating temperature regime of 35/25°C confirmed that tested seeds of invasive *I. pseudacorus* are tolerant of relatively high temperatures. Therefore, in a context of global warming, it seems unlikely that increases in temperature will prevent germination or significantly decrease viability of *I. pseudacorus*. Furthermore, the low seed dormancy of tested populations in combination with their tolerance to high temperatures may favor a shift in the timing of germination to late summer and early fall germination in the year of seed production, including viviparous germination, which has already been observed in the field (Gillard et al., 2021). Early germination has long been considered a trait underlying the invasiveness of plant species (Pyšek and Richardson, 2008). Invasive species tend to have broader germination cues in their nonnative range than in their home range (Gioria and Pyšek, 2017), and early germination can be a preadaptation to novel habitats that facilitate exploitation of vacant niches where competition can be avoided (Gioria et al., 2018). This trait may allow seedlings of *I. pseudacorus* to establish before winter, potentially offering them a competitive advantage over other plant species that germinate and resprout in spring (Pywell et al., 2003).

The germination threshold values indicate that seedling emergence of *I. pseudacorus* from the tested populations is expected to occur from spring to fall when temperatures reach at least 18.3°C, and do not exceed 41.0°C, with the maximum germination occurring at 28.2°C. Weed control strategies of the species could include monitoring of seedling emergence in the temperature window favorable to seed germination, with regular eradication of the seedlings during the growing season. In addition, new knowledge obtained from our work can be applied to improve noxious weed risk assessments, refine predictions of future invasions, and support development of ecologically-based management practices with focus on early detection networks and rapid response management to prevent further spread of *I. pseudacorus* before seeds are dispersed. This could consist in a strenuous manual flower cutting during flowering season, when the plants are the most visible and detectable in the landscape.

We observed <3% germinability below 20°C and above 36°C, and overall, *I. pseudacorus* presented relatively low germinability at constant temperature (≤25%). The two alternating temperature regimes applied revealed that fluctuating temperatures are necessary for germination of a good fraction of seeds of invasive *I. pseudacorus*, suggesting that this seed fraction required alternating temperatures to break their primary dormancy. Our observations of naturalized *I. pseudacorus* in California are consistent with those from Rosbakh et al. (2020) who observed the germination fraction of a native European population of *I. pseudacorus* to be fivefold lower at a constant temperature (19% germination at 22°C) compared to germination under alternating temperature (100% germination at 22/14°C). We noted that a fraction of the seeds did not require alternating temperature to germinate, depending on the temperature applied. Despite the relatively low germination fraction observed at constant temperatures, we were able to use a thermal time model to derive important temperature thresholds for this species, which is a positive outcome of the study because the use of alternating temperatures to estimate base temperatures is still in its infancy (Masin et al., 2017).

We performed germination trials on up to five *I. pseudacorus* populations in three of our experiments. There was no difference in germinability among the five populations tested in the constant temperature trials. However, the three populations tested in the stratification experiment and the light experiment, which were also present in the constant temperature trials, presented interpopulation differences. Seeds from population AN in the brackish reach of the estuary had the highest seed mass

and presented the highest germinability in the light experiment, while seeds sourced from the most upstream freshwater tidal population BC had the highest germinability in the stratification experiment. In the light experiment, time to first germination was lower for AN than for CS, with the lowest seed mass of the three tested populations. The observed population effects are unrelated to seed mass but could potentially be due to genotypic differences.

CONCLUSIONS

Our results have important implications for predicting conditions under which *I. pseudacorus* could become invasive in the future, and they highlight a germination niche breadth that will likely support its continued invasiveness where environmental conditions are changing. *I. pseudacorus* seeds do not require cold or warm stratification for germination. Germinability is not affected by the presence or absence of the seed coat, and seeds can germinate in the dark although germination is enhanced by light. Germination occurs mostly under alternating temperatures, but also at constant temperatures, including high temperatures expected with continued global warming. Furthermore, this broad capacity for germination can support shifts in timing of germination that may promote increased recruitment and influence distributional range of invasions. Finally, our results provide a functional perspective of seed ecology and germination of *I. pseudacorus* across a range of environmental conditions, providing information about the threat this species poses in response to climate change.

AUTHOR CONTRIBUTIONS

M.B.G., B.J.G., and J.M.C. conceived the ideas and selected study populations. B.J.G., C.J.F., and J.M.C. collected seeds. M.B.G., B.J.G., J.M.C., and M.B.M. designed the experiments. M.B.G. and C.J.F. implemented experiments, monitored germination, and performed embryo viability tests. M.B.G. and M.B.M. developed and analyzed the thermal time model, and M.B.G. performed data analyses. All authors contributed to interpretation of results. M.B.G. led the manuscript writing with substantial contributions from B.J.G. and J.M.C. All authors reviewed and commented on the final draft.

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DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.25338/B8WH0Q.

ORCID

Brenda J. Grewell http://orcid.org/0000-0001-6768-3836

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Table and figure showing location of population study sites where seeds of *Iris pseudacorus* were collected.

Appendix S2. Photograph showing examples of germinated seeds of *Iris pseudacorus* observed after exposure to total darkness for 52 days.

Appendix S3. Table summary providing details of the different experiments.

Appendix S4. Initial embryo viability following collection for five populations of invasive *Iris pseudacorus*.

Appendix S5. Graph of stratification experiment results showing fitted cumulative germination data and predicted means for embryo viability (± 95% confidence interval) for seeds of three populations of *Iris pseudacorus* exposed to two stratification treatments, and mean individual seed weight (± 95% CI) within the lots of seeds used in each condition.

Appendix S6. Results of two-way analysis of variation (ANOVA) tests performed on individual seed weight of three to five populations of *Iris pseudacorus* in response to stratification, light, and constant temperature treatments.

Appendix S7. Fitted cumulative germination data and mean embryo viability (± 95% confidence interval) for seeds of one population of *Iris pseudacorus* with presence or absence of seed coat, and mean individual seed weight (± 95% confidence interval) within the lots of seeds used in each condition.

Appendix S8. Fitted cumulative germination data and predicted mean embryo viability (± 95% confidence interval) for seeds of three populations of *Iris pseudacorus* exposed to three light treatments, and mean individual seed weight (± 95% confidence interval) within the lots of seeds used in each condition.

Appendix S9. Fitted cumulative germination data and mean embryo viability (± 95% confidence interval) for seeds of one population of *Iris pseudacorus* at two temperature regimes, and mean individual seed weight (± 95% confidence interval) within the lots of seeds used in each condition.

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