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The seeds of invasion: enhanced germination in invasive European populations of black locust (Robinia pseudoacacia L.) compared to native American populations

X. P. Bouteiller[1], F. Moret[1], R. Ségura[1], M. Klisz[2], A. Martinik[3], A. Monty[4], J. Pino[5], M. van Loo[6], T. Wojda[2], A. J. Porté[1] & S. Mariette[1]

1 BIOGECO, INRAE, Univ. Bordeaux, Cestas, France
2 Department of Silviculture and Genetics, Forest Research Institute, Raszyn, Poland
3 Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic
4 Gembloux Agro-Bio Tech, Biodiversity and Landscape Unit, University of Liège, Gembloux, Belgium
5 Centre for Ecological Research and Forestry Applications (CREAF), Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain
6 Department of Forest Growth, Silviculture and Genetics, Research Centre for Forests (BFW), Vienna, Austria

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Correspondence
X. P. Bouteiller, BIOGECO, INRAE, Univ. Bordeaux, Cestas, France.
E-mail: bouteiller.xavier@gmail.com

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ABSTRACT

- Local adaptation and the evolution of phenotypic plasticity may facilitate biological invasions. Both processes can enhance germination and seedling recruitment, which are crucial life-history traits for plants. The rate, timing and speed of germination have recently been documented as playing a major role during the invasion process. Black locust (*Robinia pseudoacacia* L.) is a North American tree, which has spread widely throughout Europe. A recent study demonstrated that a few populations are the source of European black locust. Thus, invasive populations can be compared to native ones in order to identify genetic-based phenotypic differentiation and the role of phenotypic plasticity can thereby be assessed.
- A quantitative genetics experiment was performed to evaluate 13 juvenile traits of both native and invasive black locust populations (3000 seeds, 20 populations) subjected to three different thermal treatments (18 °C, 22 °C and 31 °C).
- The results revealed European populations to have a higher germination rate than the native American populations (88% *versus* 60%), and even when genetic distance between populations was considered. Moreover, this trait showed lower plasticity to temperature in the invasive range than in the native one. Conversely, other studied traits showed high plasticity to temperature, but they responded in a similar way to temperature increase: the warmer the temperature, the higher the growth rate or germination traits values.
- The demonstrated genetic differentiation between native and invasive populations testifies to a shift between ranges for the maximum germination percentage. This pattern could be due to human-mediated introduction of black locust.

INTRODUCTION

Analysing the roles that local adaptation and phenotypic plasticity play in biological invasions is central to gaining a better understanding of invasion dynamics (Hulme 2008; Engel *et al.* 2011). Indeed, if native and new ranges have contrasting environmental conditions, selection is expected to favour traits advantageous for survival and reproduction in the new range. Such selection may lead to genetic differentiation between the two ranges, and eventually to local adaptation in the new range. Several studies have documented that recent evolution occurring in the new range may have facilitated biological invasions (Lee 2002; Blair & Wolfe 2004; Maron *et al.* 2004; Barrett *et al.* 2008; Keller & Taylor 2008; Colautti & Barrett 2013; Li *et al.* 2014). For instance, invasive populations of *Lythrum salicaria* were found to exhibit genetically-based differentiation for a fitness-related trait, which was attributed to the rapid evolution of the species in its new range in response to selection pressures, leading to local adaptation (Barrett *et al.* 2008; Colautti & Barrett 2013). A recent meta-analysis revealed that the local adaptation of invasive plant species to a new range is common, with at least the same proportion of adaptation as native species (Oduor *et al.* 2016).

Phenotypic plasticity (*i.e.* the ability of a genotype to express different phenotypes depending on the environmental conditions; Pigliucci 2006; Ghalambor *et al.* 2007) is another way for plants to deal with environmental differences between the native and the new ranges. Therefore, by comparing native and invasive populations, it is possible to determine whether plasticity has evolved from one range to another (Richards *et al.*

2006; Monty *et al.* 2013; Oduor *et al.* 2016). Contrasting results have been found regarding the magnitude of phenotypic plasticity in invasive species; for example, several growth traits of invasive populations of the trees *Melaleuca quinquenervia* in Florida and *Acer negundo* in Europe were found to have significantly higher plasticity than those of native populations (Kaufman & Smouse 2001; Lamarque *et al.* 2014), whereas no differences in plasticity between these two ranges were found for populations of the shrub *Clidemia hirta* (DeWalt *et al.* 2004). Thus, the role of phenotypic plasticity in biological invasions is still unclear and more empirical studies are needed.

Of the traits that directly affect the fitness of plant populations and can lead to local adaptation (Donohue *et al.* 2010), germination and seedling establishment are the most crucial in plant life-history stages (Eriksson & Ehrlén 2008; Walck *et al.* 2011). For instance, for the model plant *Arabidopsis thaliana*, the timing of seed dispersal has been demonstrated to be under selection and directly linked to the timing of germination (Donohue *et al.* 2005). Juvenile traits related to the germination process of plants appear to be under strong selection pressure; they can therefore be considered as key traits for the study of local adaptation (Postma & Ågren 2016).

Moreover, differences in the timing, speed and rate of germination have been recently documented in various studies on invasive species, in which populations from both the native and invaded ranges were compared (Blair & Wolfe 2004; Erfmeier & Bruelheide 2005; Beckmann *et al.* 2011; Hirsch *et al.* 2012, 2017; Leiblein-Wild *et al.* 2014; Gioria & Pyšek 2016). The results point to the major role of germination and juvenile development during the invasion process. Germination earlier in the season, larger seed production, higher germination percentage and/or a large tolerance to broad environmental conditions at the juvenile stage all contribute to plant invasiveness (Monty *et al.* 2013; Gioria & Pyšek 2016). To date, only a few studies have been carried out on life-history traits and the functional traits of juveniles by comparing populations from the native and new ranges. Some of these studies found that invasive populations often have higher growth rates and biomass production than native populations; for example, *Sapium sebiferum* (Zou et al. 2007), *Acer negundo* seedlings (Lamarque *et al.* 2013) and *Senecio* plants (Caño *et al.* 2008). This may be a result of stronger carbon acquisition traits, such as Rubisco activity, chlorophyll content or PSII yield (Caño *et al.* 2008; Hyldgaard & Brix 2012). Contrasting responses have also been observed, depending on the species; for example, seedlings of invasive populations of the shrub *Rhododendron ponticum* exhibited earlier phenology in response to temperature than native populations (Erfmeier & Bruelheide 2005). Conversely, no differences in plasticity to temperature in terms of germination and biomass production were found between the invasive and native populations of *Ulmus pumilla,* despite the invasive populations always outperforming the native ones in terms of germination (Hirsch *et al.* 2012, 2016). Moreover, significant differences have been observed between various germination-related traits in native and invasive populations, depending on the species: invasive populations of the herbs *Hypericum perforatum* and *Achillea millefolium* were found to have higher germination percentages than native populations; while no differences were found for this trait between native and invasive populations of *Hieracium pilosella* (Beckmann *et al.* 2011). However, although sometimes contradictory, these outcomes suggest that enhanced germination-related traits within invasive populations likely contribute to species invasiveness.

Temperature seems to be a key abiotic variable to consider when evaluating the role of local adaptation and plasticity in invasion success. Indeed, temperature is known to be one of the most important abiotic factors shaping tree species distribution on a global scale (Woodward 1987; De Frenne *et al.* 2010), in particular by influencing fitness (Prentice *et al.* 1992; Iverson & Prasad 1998). The selection pressures acting on invasive species during the invasion process may be related to changes in temperature in the new range (Broennimann *et al.* 2007). Moreover, germination and seedling development strongly respond to temperature, which affects the initiation and ending of dormancy, leaf and fructification phenology, seedling development, survival and growth (Walck *et al.* 2011).

This study focuses on the black locust, *Robinia pseudoacacia* L. (Fabaceae). This tree species, which is native to North America, is considered as invasive worldwide (eight out of 14 defined regions; Richardson & Rejmánek 2011). It naturally occurs in the eastern United States, over two disjoint geographic areas (Little, 1971): the largest native area corresponds to the Appalachian Mountains and partially encompasses several states; the smallest area is located further west in the Ozark Mountains. The black locust was introduced to Europe at the beginning of the 17th century and is now recognized as one of the 100 worst invasive species (DAISIE 2006, 2009). A broad population genetics study has demonstrated that only individuals from a few populations from the Appalachian region were introduced to Europe (Bouteiller *et al.* 2019) and a moderate genetic bottleneck associated with a loss of allelic richness in the introduced range was detected using single nucleotide polymorphism (SNP) markers. Studying the phenotypic variation in germination and juvenile traits in invasive black locust populations from Belgium and France revealed a very high percentage germination, with no sign of inter-population genetic differentiation. Furthermore, all the studied traits showed high plasticity to temperature, with a higher percentage germination when seedlings were grown at 22 °C compared to 18 °C (Bouteiller *et al.* 2018). In the present study, germination-related traits were investigated by extending the sampling area to both the native and invasive ranges. The following questions were posed: (i) is there genetic differentiation in terms of germination and juvenile traits between ranges or between populations; and (ii) do invasive populations have higher plasticity to temperature than native populations?

MATERIAL AND METHODS

Sampling

Twenty populations of *R. pseudoacacia* were sampled in both the native (11 populations) and European invasive (9 populations) ranges. Sampling was conducted between the spring and autumn of 2016 following a common protocol (Fig. 1, Appendix S1). Naturally regenerated populations were sampled, and recently cultivated plantations were avoided. In North America, populations were sampled throughout the species' native

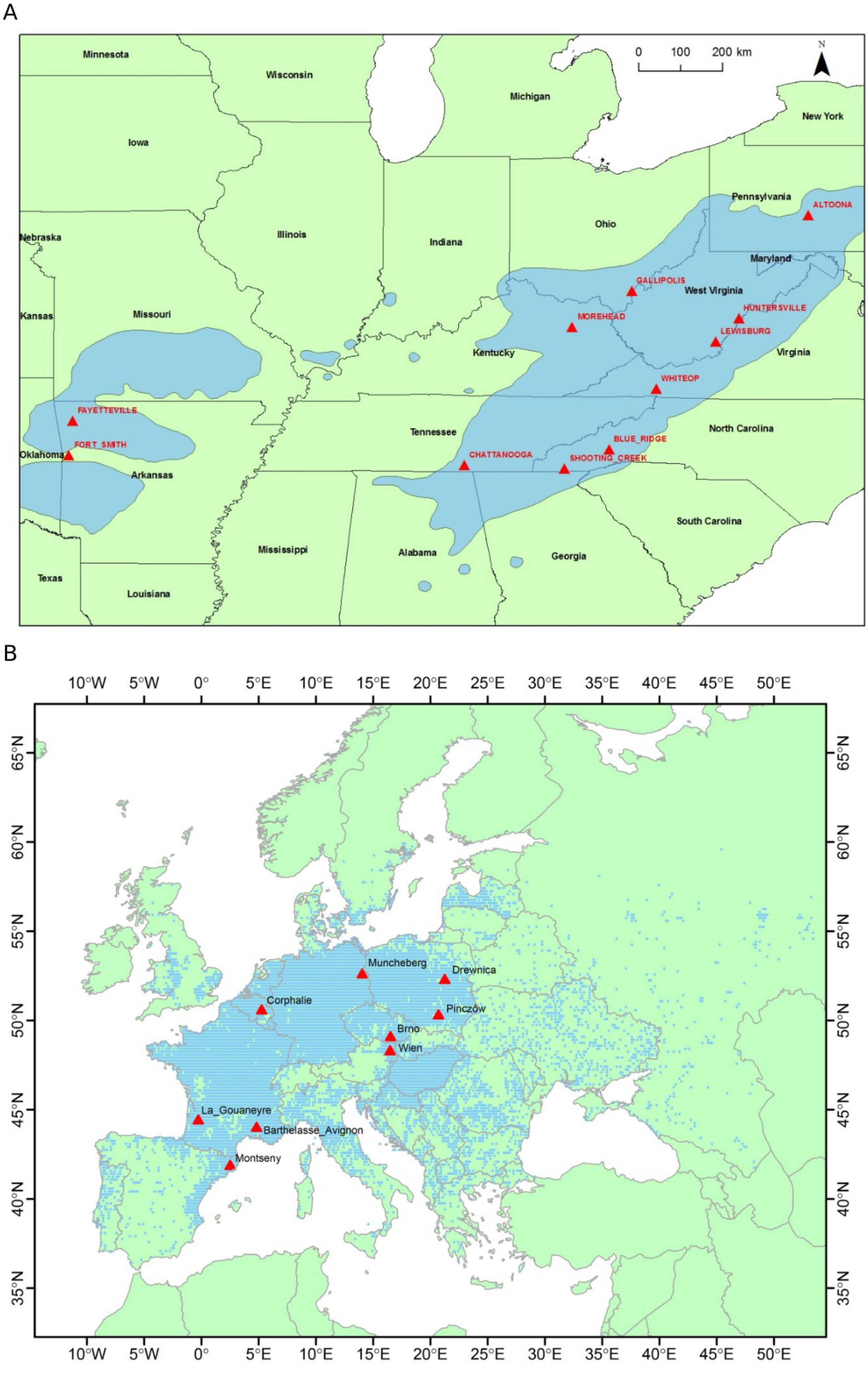


Fig. 1. Maps of sampled populations in (A) the USA and (B) Europe. The sampled populations are indicated with red triangles. Blue shading represents the native distribution of black locust in USA (Little, 1971) and the distribution of black locust in Europe (Puchałka *et al*. 2021).

range: from the Ozark Mountains to the Appalachian Mountains. In Europe, populations were sampled throughout the European introduced range: *i.e.* from Spain to Poland (Puchałka *et al.* 2021). In each population, ten to 100 pods were collected from ten trees using a tree pruner. Since the species is able to spread by root-suckering (Cierjacks *et al.* 2013), a minimum distance of 20 m was kept between adjacent sampled trees in order to minimize the risk of collecting the same genotype. GPS coordinates of the population or each sampled tree were recorded using a portable GPS (GPSMAP62; Garmin, Olathe, KS, USA).

Based on a previous study which demonstrated that black locust was introduced to Europe from just a small area of its native range (Bouteiller *et al.* 2019), the Altoona and Lewisburg American native populations were considered to be the most related. The criterion of selection was based on the location within this area and the SNP inferred genetic structure membership (see Bouteiller *et al.* 2019 for more details).

Seeds were extracted manually from the pods, placed into a paper tea filter and stored in a cold room at 0–5 °C (Forest Research 2015; Kew Royal Botanic Garden 2015). Only well-conserved seeds (*i.e.* seeds without mould stains or damaged tegument, or those which did not appear to be empty) were retained, while those considered unhealthy were discarded. In order to estimate the average seed weight for each tree, the seeds of each maternal tree were counted and weighed to an accuracy of 0.1 mg (Practum 224-1S; Sartorius, Goettingen, Germany).

Seed germination

A controlled experiment was set up using three climate chambers (Micro-Clima Series Arabidopsis Chamber MCA1600; Snijders Labs, Tilburg, the Netherlands). The set environmental conditions for each chamber were the same (except for air temperature): 12/10 h day/night photoperiod with progressive day/night transitions of 1 h, 60 ± 5% relative air humidity, and 185 ± 45 µmol m^2 s^{-1} photosynthetic photon flux density (LiCoR Li190; LiCor, Lincoln, NE, USA). The set CO_2 concentration was equal to the ambient atmospheric concentration. Each of the three chambers had a different day/night air temperature: 18 °C/16 °C, 22 °C/20 °C and 31 °C/29 °C. The 18 °C and 22 °C day temperatures were chosen based on the mean maximum temperature in May in the northern and southern parts of the invasive area, roughly corresponding to the period of seed germination. It is also worth noting that the same temperatures were used as in Bouteiller *et al.* (2018), so that comparisons could be made. The third day temperature of 31 °C was chosen to mimic a heatwave.

Five seed families were sown (*i.e.* per maternal tree) in the same tray, using ten families per population (9 European populations and 11 American populations; see Sampling) to obtain 1000 seeds per chamber and 3000 seeds in total. Within each climate chamber, the families were randomly spread. Prior to sowing, the seeds were mechanically scarified using an automated sand blasting technique (Bouteiller *et al.* 2017) to ensure controlled breaking of dormancy. Seeds were sown in plastic trays (QuickPot 35RW; HerkuPlast Kubern, Ering, Germany), each of which was filled with 25 g substrate (Substrate 307; Peltracom, Gent, Belgium), then a further 6 g after sowing to cover the seeds (Bonner & Karrfalt 2008). Watering (50 ml) was carried out every 2 days in order to supply a non-limiting resource. After 31 days, the seedlings were fertilized with liquid fertilizer (NPK 7:5:6; Florendi Jardin SAS, Dinard, France), which was renewed twice every 10 days.

The germination rate of the seeds from both ranges whose cotyledons had broken were compared *a posteriori*. A total of 10.7% and 10.0% of European and American seed cotyledons were damaged, respectively. The similarity of these two values strongly indicates that the sand blasting had had an similar impact on the seeds from both ranges.

Life-history traits

Germination and seedling phenological phases were monitored daily for each individual over 372 Growing Degree Days (GDD). The GDD were calculated by multiplying the day temperature in the climate chamber (in °C) by the number of days that the seeds were in the climate chamber; the base temperature was considered equal to 0 since no reference value was found for black locust with physical seed dormancy that is not temperature-related (Jastrzębowski *et al.* 2017). Five phenological phases were defined as: 0 = no sign of germination, 1 = emergence, 2 = straight stem, 3 = open cotyledons, and 4 = first leaf (see Bouteiller *et al.* (2017) for details and photographs of the phenological phases). The phenological survey was stopped after 12, 17 and 21 days in the 31, 22 and 18 °C climate chambers, respectively. For each phenological phase and individual, the minimum number of days required to reach the respective phase was noted. When data for a phase were missing, the number of days was calculated as the average of the number of days required to reach the previous phase to the missing phase and the phase following this. When an individual died after germination, it was recorded as a missing value.

Germination was also monitored as a Boolean trait (germinated/not germinated). Strictly speaking, this trait corresponded to the potential percentage germination, as all the black locust seeds had been previously scarified to remove their physical dormancy; for simplicity, the percentage germination will be used hereafter.

Functional traits

At the end of the phenological survey (372 GDD), seedling total height was measured from the root collar to the apical bud using a ruler (Height 1, cm ~1 mm). The seedlings were then potted: three to five plants per pot in order to avoid competition. After 1450 GDD, corresponding to 49, 66 and 82 days in the 31 °C, 22 °C and 18 °C climate chambers, respectively, the total height (Height 2, cm ~1 mm), root collar diameter (Diameter, mm ~0.01 mm) and effective quantum yield of photosystem II (PSII yield) for each seedling were measured using a ruler, an electronic caliper and a portable chlorophyll fluorometer system (PAM 2100; Walz, Effeltrich, Germany), respectively.

The PSII yield was measured on the terminal leaflet of the youngest fully developed compound leaf and calculated according to the equation $(F_m' - F_t)/F_m'$, where F_m' is maximum fluorescence yield after a pulse of saturating light when the sample is already illuminated, and F_t is measured fluorescence yield at a given time. Fluorescence yield provides information about the proportion of photons captured by PSII; the proportion of photons is used in photochemistry, being a main determinant of net primary productivity and related to temperature (Genty *et al.* 1989; Santiso *et al.* 2015).

Finally, seedlings were separated into leaves and stem, then oven dried at 65 °C (Universal oven; Memmert, Swabach, Germany) for a minimum of 1 week until a constant dry weight was reached. Leaves (Foliar weight) and stem (Stem weight) were weighed to an accuracy of 0.1 mg (Entris 224-1S; Sartorius, Goettingen, Germany) and Total weight was the sum of these two values. Growth Rates (GR) in height (Height GR 1 and 2, cm day^{-1}), diameter (diameter GR, mm day^{-1}) and leaf weight (Foliar weight GR, mg day^{-1}), stem weight (Stem weight GR, mg day^{-1}) and total weight (Total weight GR, mg day^{-1}) were calculated by dividing the trait value by the number of days spent in each climate chamber.

Data analysis

Trait–environment regressions

To analyse the relationship between traits and the environmental conditions of the sites from which the populations were sampled, the mean annual temperatures of the 1970–2000 period were extracted from WorldClim version 2.2 (Fick & Hijmans 2017) using ARcGIS (ESRI, 2018) with a 30-s resolution raster (http://worldclim.org/version2). A linear model was used to fit a regression between the average trait of each population against mean annual temperature of the population (R version 3.3.1; R Core Team, 2016). The R^2 values for statistically significant linear regressions ($P < 0.05$) were extracted and visualized in the form of a heatmap (package ggplot2 v2.2.1).

Statistical model

To estimate trait differentiation between ranges, populations and treatments for each measured trait, a Bayesian mixed model (hereafter, the global model) was fitted using the mother tree's average seed weight as a covariate, and thus as a proxy of a potential maternal effect (Bouteiller *et al.* 2018).

The complete between-chambers model was defined as:

$$\underbrace{Y_h}_{traitvalue} = b_0 + \underbrace{b_{1,i[h]}}_{temp} + \underbrace{b_{2,j[h]}}_{range} + \underbrace{B_{3,k[h]}}_{population} + \underbrace{B_{4,l[h]}}_{tree} + \underbrace{B_{5,i[h]k[h]}}_{temp\times pop} + \underbrace{c\left(x|l[h] - \grave{x}\right)}_{maternal\,effect} + \varepsilon_h \tag{1}$$

For h = 1...3000.

With the residual as:

$$\varepsilon_h N\left(0, \sigma^2\right) \tag{2}$$

Lowercase letters indicate fixed effects and uppercase letters indicate random effects: $i \in 1, 2, 3 =$ the 18, 22 and 31 °C chamber conditions, respectively; $j \in 1, 2 =$ the two ranges, Europe and the USA; $k \in [|1, 9|]$ = the 9 European populations; $k \in [|1, 11|]$ = the 11 US populations; $l \in [|1, 10|]$ = the 10 trees per population; x_l = seed weight of the tree (family level) and x = mean seed weight of all families.

To compare differences in seed weight between ranges, analyses were conducted using the same model, but without the temperature effect (nor the interaction) as the seeds were sampled in the field, and without the tree effect as there was only one averaged value per tree.

Lastly, a similar model (hereafter the related model) was fitted using only the European populations and the related American populations, in order to test if there are still any differences between the aforementioned populations.

Codes provided by O'Hara *et al.* (2005) and Kruschke (2015) were adapted to our design using uninformative inverse gamma conjugate priors $\Gamma(10^{-4}, 10^{-4})$ for random effects and normal priors $N(0, 10^6)$ for fixed effects (O'Hara & Merilä 2005; Kruschke 2015). The code used for implementing the Bayesian mixed model is provided on the GitHub platform https://github.com/xbouteiller/BayesMix, and more details can be found in Bouteiller *et al.* (2018); in particular of how it was tested and compared to frequentist methods. In addition, a Bernoulli distribution with a logit link function was used in equation (2) to analyse germination data as binary data (0 non-germinated/1 germinated). The code used for implementing this model is also provided on the GitHub platform https://github.com/xbouteiller/BayesMixBinary.

All the Bayesian models were computed using JAGS 3.4.0 (Plummer 2005), the R2jags package of R (Su & Yajima 2012) and R version 3.2.2 (R Development Core Team 2013). For each trait, 100,000 iterations were run with a burn-in of 75,000 iterations and a thinning interval of 50, using four chains. Autocorrelation and convergence were assessed using native JAGS functions and the Rhat convergence criterion was less than 1.01, as recommended by Kruschke (2015).

Plasticity index

The plasticity of each trait in response to the experimental conditions was evaluated using the Relative Distance Plasticity Index (RDPI; Valladares *et al.* 2006; Godoy *et al.* 2012). RDPI is an index ranging from 0 to 1, which is defined as the absolute phenotypic distance between families across various environments and is divided by the sum of the phenotype values. Thus, for a given family j, it was calculated using the formula:

$$RDPI = \sum_{\substack{i,\, i' = 1 \\ i \neq i'}}^{n} \frac{\left|X_{ij} - X_{i'j}\right|}{\left(X_{ij} + X_{i'j}\right)} \times \frac{1}{n} \tag{3}$$

where *i* and *i'* refer to two of the *n* levels of the environmental treatment (here, *n* is defined as 1 to 3 for each of the growth chamber temperature conditions), and *j* refers to a given family (*i.e.* a mother tree). Thus, X_{ij} is the phenotype value for a given trait for family *j* in environment *i* (*i.e.* number of pairwise environments; 3 in this study). Thus, one value of RDPI is obtained per family. RDPI values were calculated using R, with a function made available on the GitHub repository: https://github.com/xbouteiller/Plasticity. The differentiation in plasticity between ranges and populations was tested using a similar Bayesian mixed model as shown in equation (1), but without the temperature effect and tree level effect. [Data will be accessible on Open Science Framework repository after acceptance.

RESULTS

Differences in life-history traits between invasive and native populations of black locust

Only two out of the 13 measured traits showed differences between the two ranges: the maximum percentage germination and early emergence (number of days to reach stage 1; *i.e.* cotyledon emergence). In all three temperature conditions, the maximum percentage germination was higher in the European range than in the native ones (Fig. 2, Table 1, Appendix S2). This trait ranged from 84% to 90% for invasive populations, depending on the growth chamber temperature, whereas it ranged from 55% to 84% for native populations. The number of days to reach stage 1 was also significantly less for European populations than for native populations (Table 1; 9.1/5.1/3.0 days *versus* 10.0/6.0/3.7 days in 18/22/31 °C temperature conditions), indicating faster germination in the European range.

Moreover, the impact of genetic relatedness between populations was tested on these results in two ways. (i) The matrix of pairwise genetic distances between populations was incorporated into the model to account for population relatedness; while the differences between ranges in terms of number of days to reach stage one were no longer significant, they were significant in terms of maximum percentage germination when taking into account molecular similarity (Appendix S3). (ii) The same analyses were repeated on only the native populations that had been identified as the potential origins of black locust in the USA (*i.e.* 9 invasive populations and 2 American populations); as in (i), the differences in the number of days to reach stage 1 were no longer significant between ranges, but the differences in the maximum percentage germination between ranges were significant (Appendix S4).

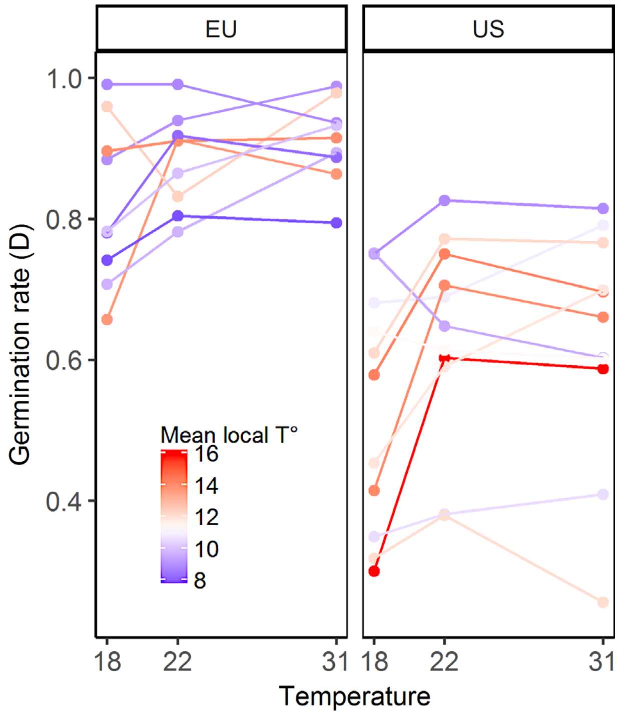


Fig. 2. Percentage germination of black locust populations in response to growth temperature (18, 22 and 31 °C). EU: European populations, US: American populations. Each line represents one population (values averaged over all individuals), colour indicates mean annual temperature at the sampling location (from coldest in blue to warmest in red).

Lastly, it is worth noting that seed weight was significantly different between ranges (Appendix S5), being lower in the American families (17.13 ± 4.30 mg, from 4.32 mg to 25.51 mg) than in the European ones (21.89 ± 4.47 mg, from 14.26 mg to 36.85 mg). There were also significant differences between populations within ranges; however, the American population with the heaviest seeds (Altoona: 19.89 mg) showed seed weights equivalent to the lightest seeds observed in the European populations (Brno: 19.86 mg, Drewnica: 17.45 mg, Montseny: 19.73 mg).

Most of the traits exhibited a similar level of plasticity between ranges (Table 1, Appendix S6). However, differences in plasticity were observed between ranges for two traits: maximum percentage germination and stem weight increment; RDPI was significantly higher for populations of the native American range (Table 1; germination rate RDPI: EU = 0.090, US = 0.218; stem weight RDPI: EU = 0.318, US = 0.376).

Functional trait response to temperature treatments

Overall, a strong response to temperature was observed (Fig. 3, Appendices S2, S7): individuals generally performed better in one or both of the warmest temperature conditions (22 °C and 31 °C) than in the coldest (18 °C). Germination responded strongly to temperature, and the highest maximum percentage germination was obtained under both the 22 and 31 °C experimental conditions (Fig. 2, Table 1; 0.84/0.90/0.90 (EU) and 055/0.64/0.60 (US) in 18/22/31 °C temperature conditions). For all the other traits, a significant temperature effect was observed (Fig. 3, Appendices S2, S7) but with slight variations in the optimal temperature, as illustrated in Fig. 3. In general, most trait values increased continuously with temperature: *e.g.* in terms of height, growth rate doubled with each temperature change, from 18 to 22 °C and 31 °C (Height GR 0.072 cm day^{-1} at 18 °C, 0.141 cm day^{-1} at 22 °C and 0.303 cm day^{-1} at 31 °C over both ranges). For some traits, a maximum was reached at 22 °C, with a further plateau at 31 °C, such as for total weight GR. Conversely, significantly higher values for PSII yield were observed in the coldest experimental conditions (0.133/0.107/0.109 at 18/22/31 °C over both ranges).

Moreover, European and American populations showed similar responses to temperature (Fig. 3, Appendices S2, S7): in terms of height, the same positive response was observed for growth rate (Height GR 2; 0.07/0.13/0.30 cm day^{-1} (EU) and 0.07/0.15/0.31 cm day^{-1} (US) in 18/22/31 °C experimental conditions) and in weight increment (Total weight GR; 1.60/3.91/5.39 mg day^{-1} (EU) and 1.67/4.62/5.37 mg day^{-1} (US) in 18/22/31 °C experimental conditions) whatever the range. A negative response was observed for populations of both ranges for leaf physiology assessed as PSII yield (0.14/0.11/0.11 (EU) and 0.12/0.11/0.11 (US) in 18/22/31 °C experimental conditions) or phenological development (time to reach stage 3 EU: 13.84/8.90/5.89 days; US: 14.03/9.44/6.15 days in 18/22/31 °C temperature conditions).

Table 1. Trait values (mean ± SD) within-range and within-temperature growth conditions (18, 22 and 31 °C).

		T18		T22		T31		RDPI	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Germination percentage	EU	**0.84**	**0.36**	**0.90**	**0.31**	**0.90**	**0.31**	**0.090**	**0.097**
	US	**0.55**	**0.50**	**0.64**	**0.48**	**0.60**	**0.49**	**0.218**	**0.229**
Stage 1	EU	**9.11**	**2.74**	**5.08**	**1.57**	**3.05**	**1.11**	0.346	0.069
	US	**10.01**	**3.27**	**6.01**	**2.23**	**3.68**	**1.40**	0.332	0.082
Stage 2	EU	11.12	2.46	6.76	1.60	4.28	1.02	0.303	0.043
	US	11.89	2.77	7.72	2.25	4.82	1.18	0.299	0.054
Stage 3	EU	13.84	2.55	8.90	1.88	5.89	1.03	0.274	0.044
	US	14.03	2.45	9.44	2.07	6.15	1.12	0.273	0.050
Stage 4	EU	20.47	0.82	13.56	1.54	7.82	0.93	0.315	0.022
	US	20.47	0.84	14.09	1.76	8.10	1.06	0.304	0.024
Height GR 1	EU	0.157	0.044	0.232	0.054	0.380	0.087	0.287	0.086
	US	0.152	0.045	0.225	0.063	0.362	0.098	0.294	0.088
Height GR 2	EU	0.070	0.039	0.131	0.050	0.301	0.103	0.439	0.072
	US	0.074	0.037	0.150	0.065	0.306	0.102	0.442	0.096
Diameter GR	EU	0.028	0.007	0.033	0.011	0.027	0.009	0.136	0.068
	US	0.025	0.006	0.033	0.012	0.027	0.009	0.147	0.064
No. of Leaves	EU	4.65	1.73	7.56	2.08	11.21	2.16	0.292	0.092
	US	4.72	1.93	7.69	2.03	11.01	2.36	0.292	0.108
Foliar weight GR	EU	0.89	0.86	2.57	1.93	4.00	2.55	0.480	0.147
	US	1.09	1.16	3.11	2.35	4.02	2.67	0.466	0.163
Stem weight GR	EU	0.71	0.53	1.33	1.19	1.39	1.24	**0.318**	**0.141**
	US	0.58	0.38	1.51	1.31	1.35	1.20	**0.376**	**0.127**
Total weight GR	EU	1.60	1.24	3.91	3.02	5.39	3.73	0.410	0.140
	US	1.67	1.48	4.62	3.45	5.37	3.80	0.428	0.143
PSII yield	EU	0.140	0.044	0.109	0.039	0.110	0.039	0.173	0.083
	US	0.125	0.046	0.105	0.039	0.107	0.033	0.164	0.073

Bold indicates traits showing significant differences between ranges. RDPI values (mean ± SD)

Relationships between temperature, life history, functional traits and local environmental conditions

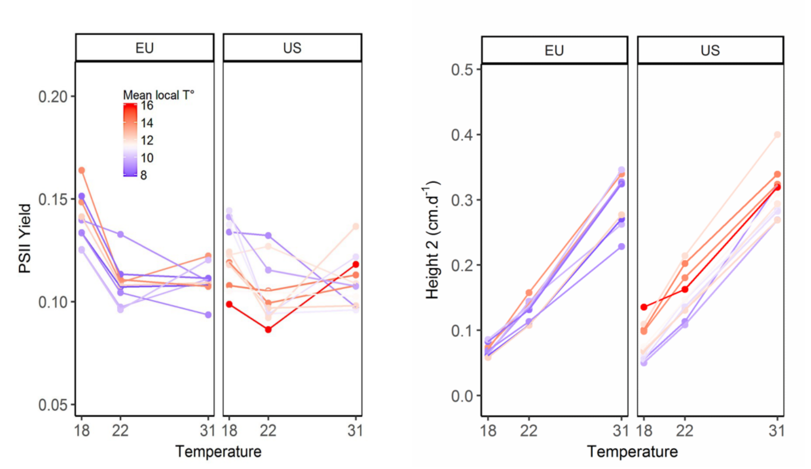
Regardless of the trait examined, the relationship with temperature at the sampling location was not significant in the invasive range. However, significant relationships between traits and temperature conditions at the sampling site were detected within the native American range (Fig. 4). These relationships were detected for almost all life history and functional traits in the 18 °C climate chamber, whereas at 22 and 31 °C, only the phenology of seedling emergence (stages 1–4) was significantly related to temperature in the sampling location. Thus, the phenological traits (*i.e.* germination stages) were always positively associated with mean annual temperature at the sampling site, irrespective of the growing conditions of the native populations.

DISCUSSION

A genetically based increase in maximum percentage germination, combined with increased germination speed (*i.e.* time to reach stage 1) was observed in the invasive range in comparison with the native range of *R. pseudoacacia*. An increase in percentage germination is generally not the rule in invasion, and several studies have not been able to demonstrate it (Erfmeier & Bruelheide 2005; Hodgins & Rieseberg 2011; Hirsch *et al.* 2012), whereas a faster germination process has been more frequently observed within the invasive range (Gioria & Pyšek 2016).

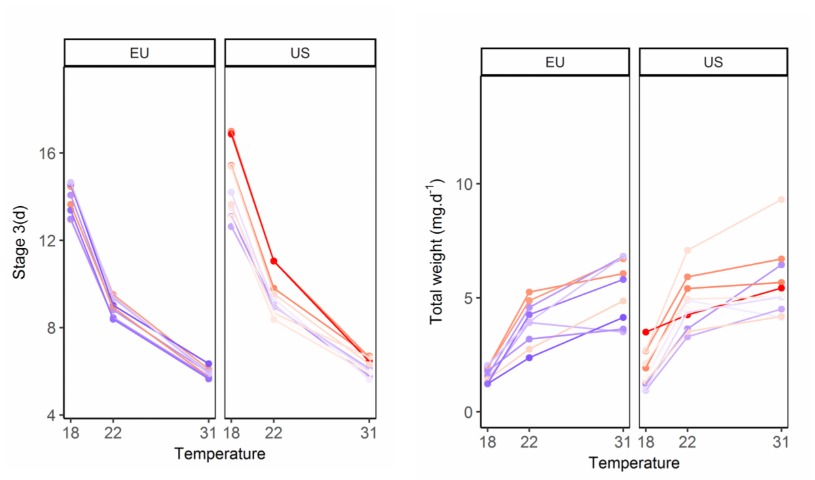
Furthermore, the reduced plasticity to temperature of European populations resulted in a remarkably stable high percentage germination, regardless of the treatment (*i.e.* temperature), whereas plasticity to temperature was high for all other traits for both ranges. The role of plasticity in invasion success remains unclear. High phenotypic plasticity was found to be involved in the invasive success of *Acer pseudoplatanus* in New Zealand (Shouman *et al.* 2017), whereas the analysis of germination response to a temperature increase (24/27 °C) for invasive populations of two *Ludwigia* species resulted in opposite patterns: for one of the species, percentage germination and speed increased with temperature, while for the other, percentage germination was high and showed no response to temperature (Gillard *et al.* 2017), as observed in the present study.

Overall, the increase in temperature was beneficial to black locust development, even when simulating a heatwave. Similarly, resilience to extreme temperature was found in invasive shrubs compared to native shrubs in Australia (French *et al.* 2017). Consequently, as long as water is available, the increase in temperature induced by climate change is likely to favour the development of black locust in Europe. Further studies are needed to more thoroughly investigate the extent to which such a major increase in percentage germination among European populations is key to the success and dynamics of black



A. PSII yield

B. Height 2 GR



C. Stage 3

D. Total weight GR

Fig. 3. Reaction norms to experimental growth conditions (18, 22, 31 °C) for four of the measured traits in black locust. (A) PSII yield; (B): Height 2 GR; (C): Stage 3; (D): Total weight GR. Each line represents one population (values averaged over all individuals), colour indicates mean annual temperature at sampling location (from coldest in blue to warmest in red).

locust in Europe, as well as to assess its resistance to drought and evaluate the impact of climate change on its invasiveness.

The results of this study clearly point to significant phenotypic differentiation among populations for all traits within both ranges. Since the plants were cultivated under the same experimental conditions we can conclude that these differentiations had a genetic basis. When comparing Belgian and French populations of black locust, no significant differentiation was found between populations, since all the variability was observed at the tree level (Bouteiller *et al.* 2018). By enlarging the sampling range to the whole of Europe, it was possible to detect differentiation between populations from the invasive range. Differentiation between populations in the native range can be expected since local adaptation is commonly observed for plants in their natural range (Linhart & Grant 1996; Leimu & Fischer 2008), but differentiation between invasive populations is not so simple. For instance, while invasive populations of *Eschscholzia californica* exhibited significant genetic differentiation for several quantitative traits, more contrasting results were found for invasive populations of maple trees: *Acer negundo* exhibited genetic differentiation, but *Acer platanoides* exhibited none (Lamarque *et al.* 2014). Similarly, populations of the herbaceous *Phyla canescens* showed genetic differentiation in its native South American range; however, while it showed genetic differentiation in its Australian invasive ranges, there was no such differentiation in its European ranges (Xu *et al.* 2015). In the light of such divergent results for different species, further studies are needed to evaluate the role of evolution in biological invasions.

There are two possible explanations—which are not mutually exclusive—for the higher percentage germination in the invasive range of black locust populations: (i) during the introduction, only populations with the highest germination rate were introduced, and (ii) after the introduction, selection occurred in response to either the new environmental conditions or to human-mediated selection. However, maternal

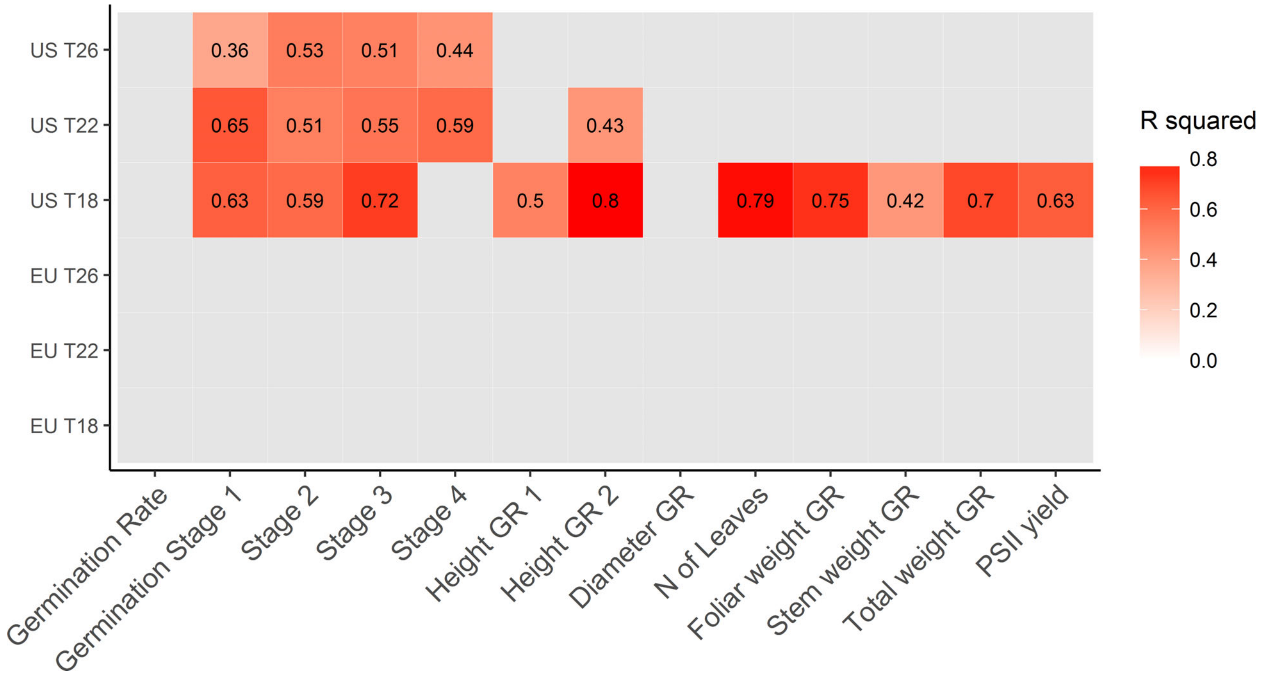


Fig. 4. Heatmap illustrating the R^2 regression between each phenotypic trait value averaged over all black locust populations within each range against mean annual temperature at sampled location of the population. Only R^2 for slopes that were significantly different from 0 were plotted ($P < 0.05$). Colour scale is relative to R^2 value, from highest R^2 in red to the null R^2 in white.

effects may also have contributed to this pattern. Despite having tried to reduce their impact on the analysis, it was hard to completely remove such effects, especially as black locust is a long-lived species.

A previous study demonstrated that black locust was introduced to Europe from a limited number of populations from the North Eastern Appalachian plateau (Bouteiller *et al.* 2019). In the present study, the two American populations identified as being the source of European black locust were Altoona and Lewisburg (mean percentage germination: 80% and 39%, respectively). This is partly consistent with explanation 1, since the percentage germination observed in Altoona is the highest of the native range populations, and since black locust was mainly introduced to Europe from seeds (Bouteiller *et al.* 2019). However, although this co-ancestry link likely contributed to the increase in germination rate observed in Europe, the percentage germination of the American populations (when considering all 11 populations or only the two genetically closest) was, on average, lower; there is thus still significant differentiation (range effect) between native and invasive populations.

It is therefore likely that some selection occurred after introduction (explanation 2). This can be tested *via* Qst-Fst comparisons, which can contribute to ascertaining the role of selection in the evolution of phenotypic traits (O'Hara & Merilä 2005; Leinonen *et al.* 2013). However, in the present study, such comparisons did not clearly indicate a departure from neutral evolution, because while mean Q_{ST} values were generally larger than F_{ST} values in both ranges, hardly any of the differences were significant (Appendix S8). Indeed, the Q_{ST} estimates had large confidence intervals, despite the use of a Bayesian approach (Bouteiller *et al.* 2018) to minimize bias in Q_{ST} estimation (O'Hara & Merilä 2005; Leinonen *et al.* 2013), seed weight as a covariate to account for maternal effects and the half-sib design to reduce dominance and epistatic effects (O'Hara & Merilä 2005; Whitlock 2008; Leinonen *et al.* 2013). Due to the sampling size and trait variability, these confidence intervals could not be reduced. Increasing Q_{ST} estimate precision would have required a much more intense experimental design, with a minimum of 20 populations per range (O'Hara & Merilä 2005; Goudet & Büchi 2006); therefore, a more thorough analysis of explanation 2, based on molecular markers, was not possible.

However, although natural selection can neither be demonstrated nor rejected, historical records testify to the role of human-mediated selection in the propagation of black locust throughout Europe (Nicolescu *et al.* 2020). After its first introductions from Pennsylvania during the 17th century, black locust seeds were used for further disseminating the species across its new range in Europe (Saint-Jean de Crève Coeur, 1786; François de Neufchateau, 1807; Cobbett, 1825). Hence, it is probable that humans unknowingly selected the best black locust seeds and propagated them throughout the new range—a process which, when repeated, may have resulted in mass selection of a more successful germination rate.

Finally, significant correlations between phenotypic values and the local environment (*i.e.* mean annual temperature of original position of the population) were only found in the native range, indicating that local adaptation is occurring in the native range but not in the invasive range. To obtain more rigorous results, an analysis of local adaptation in terms of germination would require a reciprocal sowing experiment using seeds collected from plants grown under the same conditions to remove potential maternal effects (Donohue *et al.* 2010). In the context of invasion ecology, Monty *et al.* (2009) performed such an experiment on *Senecio inaequidens* and no maternal effect was observed (Monty *et al.* 2009). However, this kind of experimental design would be difficult to undertake for a long-lived species whose first flowering period is only reached after several years (Nicolescu *et al.* 2020).

A recent meta-analysis highlighted that local adaptation was as common for invasive species in their new range as for native species (Oduor *et al.* 2016), but that study involved mostly herbaceous species (only five native and four invasive tree or shrub species out of 134 plant species). Time since introduction and number of generations that have occurred in the new range are likely crucial elements for the local adaptation of perennial woody species. For instance, *Lythrum salicaria* was introduced to America at the beginning of the 19th century, resulting in approximately 200 generations since the introduction, *i.e.* one generation per year. The minimum generation time for black locust being 6 years (Cierjacks *et al.* 2013; Nicolescu *et al.* 2020), it can be calculated that approximately 65 generations have occurred since the beginning of the 17th century. With optimal seed production starting at 15 years of age (Huntley, 1990), it is possible that the elapsed time since introduction is not long enough for local adaptation. Alternatively, human-assisted seed dispersal throughout the invasion range from a limited number of native populations might have homogenized phenotypic variation, resulting in the absence of local adaptation.

CONCLUSION

The present study demonstrated an increased percentage germination for invasive populations of black locust than for native populations. This result is likely linked to the introduction history of black locust, *i.e.* seed source and potential human-mediated selection during its active dissemination throughout Europe. This increased percentage germination may have contributed to the invasion success of the species beyond its plantation locations in Europe; however, further studies would be required to determine if such a shift in potential germination leads to increased invasiveness in the field.

The temperature plasticity of both native and invasive populations of black locust suggests that the current climate changes will favour the development of this species in the future. However, rising temperatures will lead to more and longer drought periods, which could reduce the survival and growth of black locust. The balance between the putative effect of temperature increase and precipitation modification should be further evaluated to reliably assess the evolution of invasiveness of black locust, as well as the risk to vulnerable ecosystems.

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SUPPORTING INFORMATION

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

Appendix S1. Information about the sampled populations. X and Y (longitude and latitude respectively) represent the GPS coordinates in the WGS84 system. Altitude and annual mean temperature were extracted from WorlClim database (Fick & Hijmans 2017).

Appendix S2. Results of the Bayesian analyses for all phenotypic traits using the global model presented in the main document. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2; (H) diameter GR, (I) Number of leaves; (J–L) leaf, stem and total weight GR, and M) PSII yield; with (a) temperature effects (b1), (b) range effects (b2), (c) population effects (b3), and (d) estimated population, tree and temperature × population interaction variance. Each dot represents the mean value of the estimated effect, and bars represent the 95 % credible interval over the mean value. Effects significantly different from 0 are drawn in red.

Appendix S3. Results of the Bayesian analyses for all phenotypic traits using the global model incorporating a matrix of pairwise genetic distance between populations. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2, (H) diameter GR, (I) Number of leaves, (J–L) leaf, stem and total weight GR, and (M) PSII yield; with (a) temperature effects (b1), (b) range effect (b2), (c) population effects (b3), and (d) estimated population, tree and temperature × population interaction variance. Each dot represents the mean value of the estimated effect, and bars represent the 95% credible interval over the mean value. Effects significantly different from 0 are indicated in red.

Appendix S4. Results of the Bayesian analyses for all phenotypic traits using the related model (9 invasive populations + 2 related native populations) presented in the main document. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2, (H) diameter GR; (I) Number of leaves, (J–L) leaf, stem and total weight GR, and (M) PSII yield; with (a) temperature effects (b1), (b) range effect (b2), (c) population effects (b3), and (d) estimated population, tree and temperature × population interaction variance. Each dot represents the mean value of the estimated effect, and bars represent the 95 % credible interval over the mean value. Effects significantly different from 0 are indicated in red.

Appendix S5. (A) Seed weight differentiation between ranges and populations; black dots represent mean population seed weight averaged over families associated with standard deviations (vertical black lines); red and blue dots represent mean family seed weight (European families and US families respectively); horizontal red lines indicate the mean seed weight per range. (B and C) Results of the seed weight analysis using the Bayesian model testing for range effects and population effects respectively; each dot represents the mean value of the estimate, and bars represent the Bayesian 95 % credible interval; effects significantly different from 0 are drawn in red; both the range and population effects are significant.

Appendix S6. Results of the Bayesian analyses for the RDPI. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2, (H) diameter GR, (I) Number of leaves, (J–L) leaf, stem and total weight GR, and (M) PSII yield; with (a) temperature effects (b1), (b) range effect (b2), (c) population effects (b3), and (d) estimated population, tree and temperature × population interaction variance. Each dot represents the mean value of the estimated effect, and bars represent the 95 % credible interval over the mean value. Effects significantly different from 0 are indicated in red.

Appendix S7. Reaction norms for all the studied traits. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2, (H) diameter GR; (I) Number of leaves, (J–L) leaf, stem and total weight GR, and (M) PSII yield. Each line represents one population (values averaged over all individuals); the color indicates the mean annual temperature at the sampling location (from the coldest in blue to the warmest in red).

Appendix S8. Differentiation indices calculated for all studied phenotypic traits (QST) and using SNP markers (FST). QST values (mean and 95 % confidence interval) were calculated on the 9 European populations (left) and 11 US populations (right) per trait and growing chamber (blue circle: 18 °C, pink circle: 22 °C, red circle: 31 °C). The dashed lines represent the FST 95 % confidence interval based on allelic variation in SNP's loci. QST estimations were calculated using Bayesian methods. (A) Germination rate, (B–E) phenological stages 1 to 4, (F–G) height Growth Rate (GR) 1 and 2, (H) diameter GR, (I), Number of leaves, (J–L) leaf, stem and total weight GR, and (M) PSII yield. GR: Growth Rate.

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