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RESEARCH ARTICLE

Allelopathic effects of native and invasive *Brassica nigra* do not support the novel-weapons hypothesis

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PREMISE: The novel-weapons hypothesis predicts that some plants are successful invaders because they release allelopathic compounds that are highly suppressive to naïve competitors in invaded ranges but are relatively ineffective against competitors in the native range. For its part, the evolution of enhanced weaponry hypothesis predicts that invasive populations may evolve increased expression of the allelopathic compounds. However, these predictions have rarely been tested empirically.

METHODS: Here, we made aqueous extracts of roots and shoots of invasive (North American) and native (European) *Brassica nigra* plants. Seeds of nine species from North America and nine species from Europe were exposed to these extracts. As control solutions, we used pure distilled water and distilled water with the osmotic potential adjusted with polyethylene glycol (PEG) to match that of root and shoot extracts of *B. nigra*.

RESULTS: The extracts had a strong negative effect on germination rates and seedling root lengths of target species compared to the water-control. Compared to the osmolality-adjusted controls, the extracts had a negative effect on seedling root length. We found no differences between the effects of *B. nigra* plant extracts from the invasive vs. native populations on germination rates and seedling root growth of target plant species. Responses were largely independent of whether the target plant species were from the invaded or native range of *B. nigra*.

CONCLUSIONS: The results show that *B. nigra* can interfere with other species through allelochemical interactions, but do not support predictions of the novel-weapons hypothesis and evolution of increased allelopathy.

KEY WORDS biogeographic differences; black mustard; Brassicaceae; competition; invasion biology; novel-weapons hypothesis; osmolality; post-introduction evolution.

Identification of the mechanisms that underlie the invasiveness of exotic plant species is a major goal in ecology (Sakai et al., 2001; van Kleunen et al., 2018). Several non-mutually exclusive hypotheses including the novel-weapons hypothesis (Callaway et al., 2004), enemy-release hypothesis (Keane and Crawley, 2002), and shifting-defense hypothesis (Doorduin and Vrieling, 2011) have been proposed to explain plant invasiveness. The novel-weapons hypothesis predicts that some plants are successful invaders because they release allelopathic compounds that are highly suppressive to naïve competitors in the non-native range but are relatively ineffective against competitors in the native range because of co-adaptation (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004). Plants may release allelopathic compounds through leaf leachates, root exudates, volatiles, and decaying litter (Callaway and Ridenour, 2004; Callaway et al., 2005; Inderjit et al., 2011). Indeed, there is mounting empirical evidence in support of the novel-weapons hypothesis (e.g., Callaway and Aschehoug, 2000; Ridenour and Callaway 2001; Prati and Bossdorf, 2004; Abhilasha et al., 2008; Gómez-Aparicio and Canham, 2008; Thorpe et al., 2009; Inderjit et al., 2011a; Becerra et al., 2018; Irimia et al., 2019), suggesting that allelopathic effects could indeed play a crucial role in facilitating the ability of invasive plants to displace their neighbors in the new range. Moreover, if allelopathic effects confer an advantage against naïve competitors as suggested in the novel-weapons hypothesis, then genotypes that produce high concentrations of allelochemicals might be selected for in the introduced range (Callaway and Ridenour, 2004). However, little is known about potential biogeographical differences in allelopathic effects of invaders (Gruntman et al., 2016; Becerra et al., 2018; Irimia et al., 2019).

The enemy-release hypothesis predicts that plants that are introduced to new ranges leave behind their specialized herbivores and are therefore released from harmful herbivory pressure exerted by those specialist herbivores (Keane and Crawley, 2002). In support of the enemy-release hypothesis, several empirical studies have found that invasive plants often interact with fewer herbivores (Liu and Stiling, 2006) and pathogens (van Kleunen and Fischer, 2009) in the invaded range than in the native range. Invasive plants, however, might mainly be released from specialist enemies, and not from generalists (Liu and Stiling, 2006; Strauss et al., 2009). Based on this, the shifting-defense hypothesis predicts that invasive-range populations will evolve higher concentrations of physiologically less costly qualitative defense compounds (e.g., glucosinolates and pyrrolizidine alkaloids) that will deter most of the generalist herbivores that occur in the invaded range (Müller-Schärer et al., 2004; Doorduin and Vrieling, 2011). The numerous studies that reported significantly higher concentrations of qualitative defense compounds in invasive populations than in conspecific native-range populations (e.g., Lewis et al., 2006; Caño et al., 2009; Doorduin and Vrieling, 2011; Oduor et al., 2011; Zhang et al., 2018) support the predictions of the shifting-defense hypothesis. Anti-herbivore compounds can serve other functions including allelopathy (Lankau and Kliebenstein, 2009). Therefore, invasive genotypes that express higher concentrations of defense compounds may have stronger allelopathic effects than conspecific native-range genotypes.

Only a few studies have tested whether plants of invasive populations have stronger allelopathic effects than conspecifics from the native range, with mixed results. For example, leaf extracts of *Impatiens glandulifera* from the invaded range had stronger allelopathic effects on *Urtica dioica* than extracts of *I. glandulifera* from the native range (Gruntman et al., 2016). In contrast, no evidence was found that leaf leachates of invasive populations of *Centaurea solstitialis* generally had stronger allelopathic effects than those of native populations of *C. solstitialis* on *Lactuca sativa* (Irimia et al., 2019). In fact, allelopathic effects of *C. solstitialis* plants from parts of its invaded range in California and Australia were significantly weaker than those of *C. solstitialis* plants in the native regions of Spain and Turkey (Irimia et al., 2019). Moreover, extracts from invasive populations in Chile and Argentina had inhibitory effects similar to those of extracts from native populations in Spain and Turkey (Irimia et al., 2019). The limited number of studies pre-empt drawing a general conclusion as to whether invasive plants can evolve increased allelopathy in their novel range.

Bioassays are a powerful direct way to study allelopathic interactions. Such assays involve the preparation of aqueous extracts of shoot and root material and observing the influence of these extracts on germination and early seedling growth of target species (Inderjit and Nilsen, 2003). Although it is often assumed that the response of seeds or seedlings to plant extracts is due entirely to allelochemical action, such responses can also be due to changes in osmolality (Inderjit and Nilsen, 2003). Therefore, it is important to include controls for the changes in osmolality. However, such controls have rarely been included; hence, the relative importance of allelochemical action and changes in osmotic potential in driving changes in germination and growth of target plant remains largely unclear (Loydi et al., 2015).

Brassica nigra (L.) W.D.J.Koch (Brassicaceae), an annual herb native to Europe, North Africa, and Asia, was introduced to North America ca. 200 years ago (Bell and Muller, 1973; Feeny and Rosenberry, 1982). Presently, *B. nigra* is invasive in certain regions of North America, where it can form thick monospecific stands (Oduor et al., 2015b). Invasive populations stemmed from multiple source populations in the native range (Oduor et al., 2015a). *Brassica nigra* deters herbivores with glucosinolates, a class of secondary compounds derived from several amino acids (Feeny and Rosenberry, 1982). Although over 120 different glucosinolate compounds have been identified (Hopkins et al., 2009), sinigrin (allyl-glucosinolate) represents 90–99% of the total glucosinolate concentration in *B. nigra* (Feeny and Rosenberry, 1982; Traw, 2002). Leaves of invasive populations produce significantly higher concentrations of sinigrin than leaves of the conspecific native-range populations (Oduor et al., 2011), potentially driven by escape from herbivory in the invaded range (Oduor et al., 2013). Sinigrin can mediate competitive interactions between *B. nigra* and heterospecific neighbors through allelopathy. High sinigrin-producing individuals of *B. nigra* suppressed heterospecific neighbors more than the low sinigrin-producers did (Lankau and Strauss, 2007; Lankau and Kliebenstein, 2009). However, it has not been tested whether these heterospecific suppressive effects of *B. nigra* are stronger if the competitor species are more naïve to the novel allelochemical weapons (as they would be in the invaded range).

Here, we tested whether aqueous extracts from invasive populations have a stronger effect on target species than extracts from native populations and whether the effects depend on the biogeographic range of the target species. Specifically, we tested whether below- and aboveground *B. nigra* extracts (from eight native and eight invasive populations) negatively affected germination and root growth of nine target species from the native European range and nine target species from the invaded North American range. We asked (1) whether the effect of invasive North American *B. nigra* was stronger than that of native European *B. nigra* and (2) whether target species from the invaded range were more strongly affected than target species from the native range. To test whether the effects of the extracts could solely be attributed to allelochemical action or involved additional changes in osmotic potential, we compared germination and root growth in the presence of extracts vs. a distilled water control and vs. two further controls with osmotic potentials adjusted, respectively, to those measured for below- and aboveground extracts.

MATERIALS AND METHODS

Study species and seed sources

For *B. nigra*, we used bulked seed samples collected from 10 maternal plants in each of eight invaded-range and eight native-range populations (Appendix S1). The plants were raised in a common greenhouse environment to eliminate potential maternal environmental effects. Because *B. nigra* is self-incompatible, we produced the seeds by supplying cross-pollen from multiple same-population donors. To test the potential allelopathic effects of *B. nigra*, we used 18 target species, of which nine are native to North America and another nine native to Europe (Table 1). The test species were selected on the basis that they co-occur with *B. nigra* across a wide range of habitats in both the invaded and native range (The Jepson Interchange, http://ucjeps.berkeley.edu/interchange/), so that our results can be generalized across the different habitats invaded by *B. nigra*.

TABLE 1. Information about the 18 target species that were used in the present study.

Native range	Species	Family	Life cycle	Growth form	Vegetative and sexual reproductive characteristics
Europe	*Artemisia vulgaris*	Asteraceae	Perennial	Herb	Clonal, NA
	Campanula rotundifolia	Campanulaceae	Perennial	Herb	Clonal, Self-incompatible
	Medicago lupulina	Fabaceae	Annual/Perennial	Herb	Nonclonal, Self-compatible
	Salvia pratensis	Lamiaceae	Perennial	Herb	Nonclonal, Self-compatible
	Alopecurus pratensis	Poaceae	Perennial	Grass	Clonal, Self-compatible
	Agrostis capillaris	Poaceae	Perennial	Grass	Clonal, Self-incompatible
	Bromus erectus	Poaceae	Annual	Grass	Nonclonal, Self-compatible
	Holcus lanatus	Poaceae	Perennial	Grass	Clonal, Self-incompatible
	Galium album	Rubiaceae	Annual	Herb	Nonclonal, NA
North America	*Lasthenia glabrata*	Asteraceae	Annual	Herb	Nonclonal, Self-incompatible
	Grindelia camporum	Asteraceae	Annual/Perennial	Herb	Nonclonal, Self-incompatible
	Lupinus succulentus	Fabaceae	Annual	Herb	Nonclonal, Self-compatible
	Trifolium wildenovii	Fabaceae	Annual	Herb	Nonclonal, Self-compatible
	Eschscholzia californica	Papaveraceae	Annual/Perennial	Herb	Nonclonal, Self-incompatible
	Bromus carinatus	Poaceae	Annual/Biennial	Grass	Nonclonal, Self-compatible
	Elymus glaucus	Poaceae	Perennial	Grass	Nonclonal, Self-compatible
	Stipa pulchra	Poaceae	Perennial	Grass	Clonal, Self-compatible
	Vulpia microstachys	Poaceae	Annual	Grass	Nonclonal, NA

Note:: NA = not available

Preparation of *B. nigra* extracts and controls

Between February and April 2015, to produce tissue to make extracts, we raised six plants for each of the 16 *B. nigra* populations in a greenhouse (24 ± 5°C, 16 h day: 8 h night, 50–70% relative humidity). Seeds of the 16 *B. nigra* populations were germinated in plastic plug-trays filled with a commercial potting soil (Profi-Classic CL-T, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany) in a phytochamber (12 h 21°C day/12 h 17°C night, 90% humidity). Two weeks after emergence, we transplanted seedlings to 2.5-L plastic, circular pots with the same potting soil as above. In each pot, we mixed the substrate with 10 g of a slow-release fertilizer (Osmocote Classic 14% N, 14% P_2O_5, 14% K_2O; Scotts, Geldermalsen, Netherlands). Watering was standardized by filling the plastic plates placed beneath each pot.

To make *B. nigra* extracts when plants were 3 months old (plants had started to flower at that point), we selected the youngest fully expanded leaves from each of the six individual plants for bulk sampling 300 g per population. From the same plants, we bulk-sampled 300 g of root tissues per population. We then ground the fresh tissue with a blender in 1.0 L of distilled water, thus creating 32 extracts (a shoot and a root extract from each of the 16 *B. nigra* populations) with a concentration of 300 g L^{-1}. These amounts of leaves and roots correspond to the average litter quantity and root debris of individual *B. nigra* plants (A. M. O. Oduor, personal observation), and the volume of water corresponds to a rough average amount of rainfall per 1 m^2 during wettest months in parts of the native and invaded range of *B. nigra* (World Weather Online, 2020). All extracts were made at the same time. The extracts were filtered three times through a fresh Whatman No. 1 filter paper. Immediately after filtering, we measured osmotic potential (Osm/kg H_2O) of the collected filtrates with a cryoscopic osmometer (Osmomat, model 030, Gonotec GmbH, Berlin, Germany) and stored samples at −80°C until use. We used pure distilled water as one of our controls. To allow interpreting whether any effects of the extracts are due to changes in allelopathic effects or osmotic potential, we also used two further solutions amended with polyethylene glycol-8000 (PEG-8000) (Sigma-Aldrich, Steinheim, Germany), adjusted to the mean osmolality of the *B. nigra* extracts, respectively, 0.092 Osm/kg H_2O for aboveground and 0.038 Osm/kg H_2O for belowground extracts. We used the osmotic agent PEG-8000, because it is biologically inert and cannot be absorbed into the seed (Michel and Kaufman, 1973; Paparella et al., 2015).

Experimental setup

To test the effect of *B. nigra* shoot and root extracts on seed germination and early seedling growth of the 18 target species, in May 2015, we placed 10 seeds per species in a circular formation at equal distances from each other in Petri dishes (diameter = 5 cm) lined with a sterilized Whatman filter paper (No. 1) moistened with 2.0 mL plant extracts or control liquids. Before sowing, the seeds were sterilized for 2 min in 1% v/v sodium hypochlorite solution and rinsed well with distilled water. We re-applied 1 mL of the liquids to the filter paper every 7 days until ending the experiment after 3 weeks. Corresponding to the 32 *B. nigra* extracts (above- and belowground for each of the 16 populations), the water control and the two osmolality-adjusted controls (above- and belowground), our experiment included 35 Petri dishes for each of the 18 target species. Each Petri dish was sealed with parafilm to keep moisture levels and concentrations of the extracts at approximately constant levels. The 630 Petri dishes were positioned randomly within trays that were kept in a phytochamber (12 h, 21°C day/12 h, 17°C night, 90% relative humidity). We recorded the proportion of seeds that germinated in each Petri dish on the 14th day from the start of the experiment and again on the 21st day. Because there were no new seedlings at that point, we stopped the experiment, and measured seedling root lengths.

Statistical analyses

All data were analyzed with generalized mixed models using the functions implemented in the package lme4 (Bates, 2011) in R v3. 6.2 (R Core Team, 2019). First, we tested whether germination rates differed between extracts and controls. For germination rate, models had a binomial error term. For average root length per Petri dish (excluding Petri dishes with no germination), models had a

Gaussian error term ($\log_e$-transformation was required to ensure homoscedasticity and normality of residuals). The model included the germination environment as a fixed factor with seven levels: water control; PEG control for aboveground extracts (0.038 Osm/kg); PEG control for belowground extracts (0.092 Osm/kg); above- and belowground extracts from invasive *B. nigra* populations; above- and belowground extracts from native *B. nigra* populations. Target species identity was included as a random effect. For post-hoc comparison of factor-level means, we defined a custom matrix to test whether: (1) the water control differed from the aboveground PEG control and the aboveground extracts; (2) the water control differed from the belowground PEG control and the belowground extracts; (3) the aboveground PEG control differed from the aboveground extracts; (4) the invasive aboveground extracts differed from native aboveground extracts; (5) the belowground PEG-control differed from the belowground extracts; (6) the invasive belowground extracts differed from native belowground extracts.

To test whether the target species differed in their responses depending on their biogeographic origin and the origin of the *B. nigra* plants used to make the extracts, we performed mixed model analyses for germination rate and root length (same transformations and error terms as above) for the subset of seeds that were exposed to *B. nigra* extracts (separately analyzing responses to above- and belowground extracts). The fixed part of the models included the range of *B. nigra* plants from which extracts were made (invasive or native), target species range (Europe or North America) and their interaction. Additionally, we included the osmolality of the extracts as a covariate. We included *B. nigra* population and target species identity as random effects. The function drop1 was used to assess the significance of the fixed effects in each model at $\alpha = 0.05$ by testing the significance of the change in deviance upon the removal of terms against a χ^2 distribution.

RESULTS

Effects of *B. nigra* extracts compared to controls

Both aboveground (AG) and belowground (BG) *B. nigra* extracts had a stronger negative effect on target species germination rate than the water-control (specific contrasts: AG vs. water control, $z = 11.5$, $p < 0.001$; BG vs. water control, $z = 4.5$, $p < 0.001$), but not the corresponding PEG osmolality controls (Fig. 1A; see test statistics in the figure). A similar pattern emerged for the root lengths (specific contrasts: AG vs. water control, $z = 13.1$, $p < 0.001$; BG vs. water control, $z = 7.7$, $p < 0.001$), but here the negative effect of the extracts was also significant compared to the PEG osmolality controls (Fig. 1B; see test statistics in figure).

Comparison of effect of extracts from native (European) vs. invasive (North American) *B. nigra* on European vs. North American target species

Within the subset of seeds treated with aboveground extracts, neither the range of origin of the *B. nigra* extracts, nor the range of the target species influenced germination rates or seedling root length

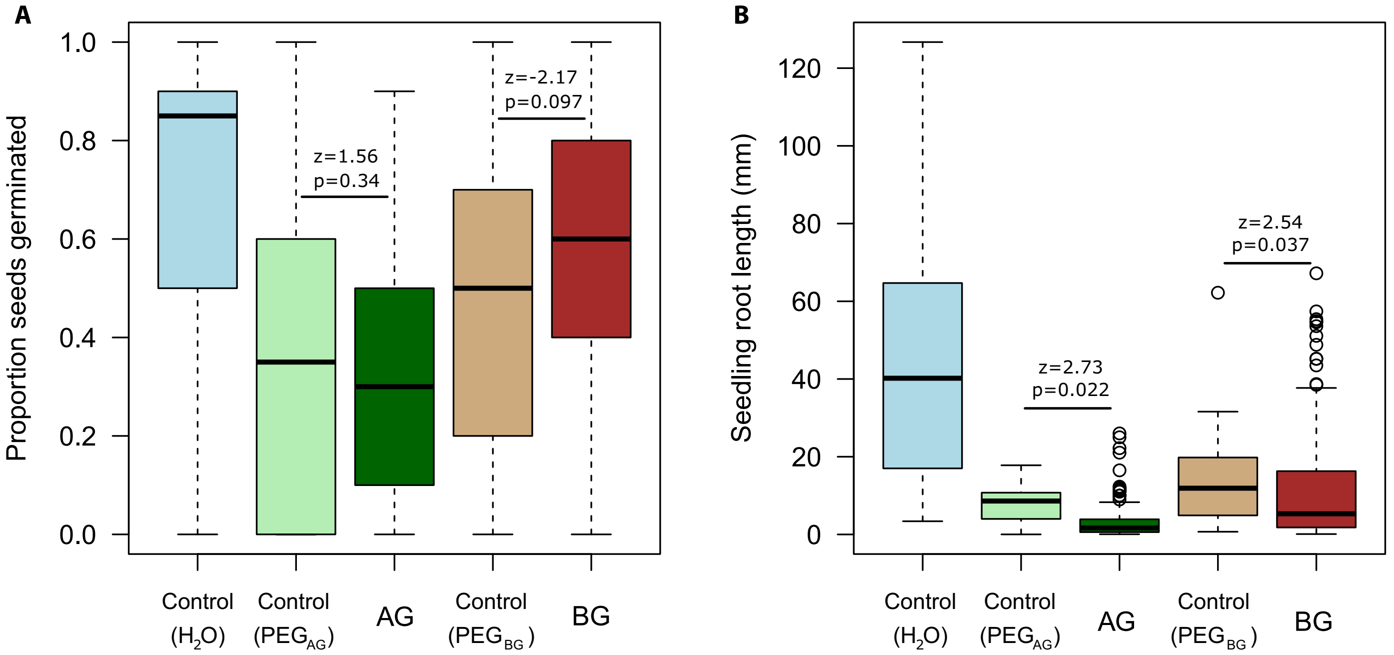

FIGURE 1. The effects of aboveground (AG) and belowground (BG) extracts of *Brassica nigra* plants relative to the controls on (A) germination and (B) seedling root length. Distilled water served as a general control (H_2O, blue) for both above- (green) and belowground (brown) extracts. To control for effects of changes in osmolality, we used a biologically inert polyethylene glycol (PEG-8000) dissolved in distilled water, respectively adjusted to match the mean osmolality of aboveground extracts (PEG_{AG}, light green) and to the mean osmolality of belowground extracts (PEG_{BG}, light brown). Boxes show the interquartile range around the median, whiskers extend to 1.5× the interquartile range (or to the minimum or maximum observed value within that). Data points beyond the whiskers are outliers (marked by circles). Test statistics are indicated for comparisons between extracts and their respective PEG controls.

(Table 2). The osmolality of the aboveground extracts also did not have a significant effect. Within the subset of seeds treated with belowground extracts, the range of origin of the *B. nigra* extracts had no effect on germination and root length (whether alone or in interaction with target species range). However, target species from North America tended to have higher germination rates and longer roots than European species (Fig. 2; marginally significant effects of target species range, Table 2). Additionally, the osmolality of the belowground extracts had a significant negative effect both on germination and on root length (Table 2).

DISCUSSION

The idea that invasive plants may not only possess novel weapons, but might also evolve increased expression of these weapons in the introduced range has seldom been tested (e.g., Callaway and Ridenour, 2004; Gruntman et al., 2016). Here, we tested both the potential allelopathic effects of *B. nigra* and whether the species has evolved greater allelopathy in the introduced range. We found that aqueous extracts of *B. nigra* plants from the invaded range had similar effects on germination rates and seedling root growth of target plant species as aqueous extracts of *B. nigra* plants from the native range. Target plant species from the invaded and native ranges of *B. nigra* responded similarly to the aqueous extracts. Our results mirror those of Irimia et al. (2019), who did not find significantly stronger allelopathic effects of invasive *C. solstitialis* relative to native *C. solstitialis* plants. While our results suggest that *B. nigra* can interfere with other species through allelochemical interactions, taken together, the results do not support predictions of the novel weapons hypothesis and evolution of increased allelopathy.

Both above- and belowground extracts of *B. nigra* plants had allelopathic effects on root growth, although the effects of aboveground extracts were relatively stronger (Fig. 1B). Plants within the Brassicaceae family can contain different concentrations of glucosinolates in the roots and shoots at different phenological stages (Agneta et al., 2014). For instance, shoots of flowering *B. nigra* plants had higher concentrations of total glucosinolate compounds than in the roots of the same individual plants (van Dam et al., 2009). Another study found that *B. nigra* can produce higher concentrations of glucosinolates in the roots than in the shoot during the pre-flowering stages (Bellostas et al., 2007). In the present study, we made above- and belowground extracts from *B. nigra* plants that were beginning to flower. Thus, although we did not measure glucosinolate contents of the extracts, it can be expected that the aboveground extracts should have had a higher concentration of glucosinolates.

We found that the negative effects of our extracts were mainly due to changes in osmolality, but also had an allelopathic component for root growth (Fig. 1B). This result contrasts with the apparent absence of inhibitory effects of *B. nigra* allelochemicals on germination of the test species (Fig. 1A), which is in line with other studies that found root growth to be more sensitive to allelopathic compounds than germination (e.g., Haugland and Brandsaeter, 1996; Chon et al., 2004; Perry et al., 2005). Possibly, the seed coat confers some degree of resistance to allelochemicals. Additive negative effects of increased osmolality and allelopathy, such as we found for root growth (Fig. 1B), have also been reported in the few other studies that included controls adjusted for changes in osmolality alongside distilled water controls (e.g., Wardle et al., 1992; Chon et al., 2004; Loydi et al., 2015). It remains to be tested whether the changes in osmotic potential in plant extracts or leachates also play a role in natural settings, but our findings underscore the importance of using proper controls to test the relative contribution of changes in osmotic potential and allelochemical action. In doing so, we could conclusively show that *B. nigra* allelochemically suppresses root growth of seedlings of target species with which it normally co-occurs. However, the specific chemicals that were responsible for the allelopathic effects of *B. nigra* were not identified.

Given their known role in allelopathic interactions (Turk and Tawaha, 2003; Rivera-Vega et al., 2015), glucosinolates could

TABLE 2. Results of linear mixed-effect models to test for differences in effects between extracts from invasive (North American) and native (European) *B. nigra* populations on germination and seedling root length of nine European and nine North American target species. Above- and belowground extracts were analyzed separately. Significant tests ($P < 0.05$) are highlighted in bold. Marginally significant tests ($0.05 < P < 0.10$) are italicized.

	Aboveground extracts				**Belowground extracts**			
	Germination (binomial, dispersion = 1.31)		**Root length[a] (Gaussian, log$_e$-transformed)**		**Germination (binomial, dispersion = 1.31)**		**Root length[a] (Gaussian, log$_e$-transformed)**	
	LRT (χ^2)	***P***	**LRT (χ^2)**	***P***	**LRT (χ^2)**	***P***	**LRT (χ^2)**	***P***
Fixed effects								
Osmolality (covariate)	0.002	0.964	0.15	0.700	**4.50**	**0.0338**	**5.43**	**0.0197**
B. nigra range	0.84	0.359	1.21	0.272	0.21	0.644	0.023	0.88
Target species range	0.23	0.628	0.51	0.476	*3.67*	*0.0553*	*2.82*	*0.093*
B. nigra range × target species range	1.56	0.211	0.74	0.389	0.0006	0.981	1.15	0.284
Random effects	SD explained		SD explained		SD explained		SD explained	
B. nigra population (nested within *B. nigra* range)	0.24		0.24		<0.01		0.31	
Target species identity (nested within target species range)	0.93		1.08		0.93		1.19	
Residuals	-		0.83		-		0.72	

[a] Log$_e$-transformed. Only for subset of plates with at least one germinated seed (n = 224 of 288 plates for aboveground extracts, n = 273 of 288 plates for belowground extracts)

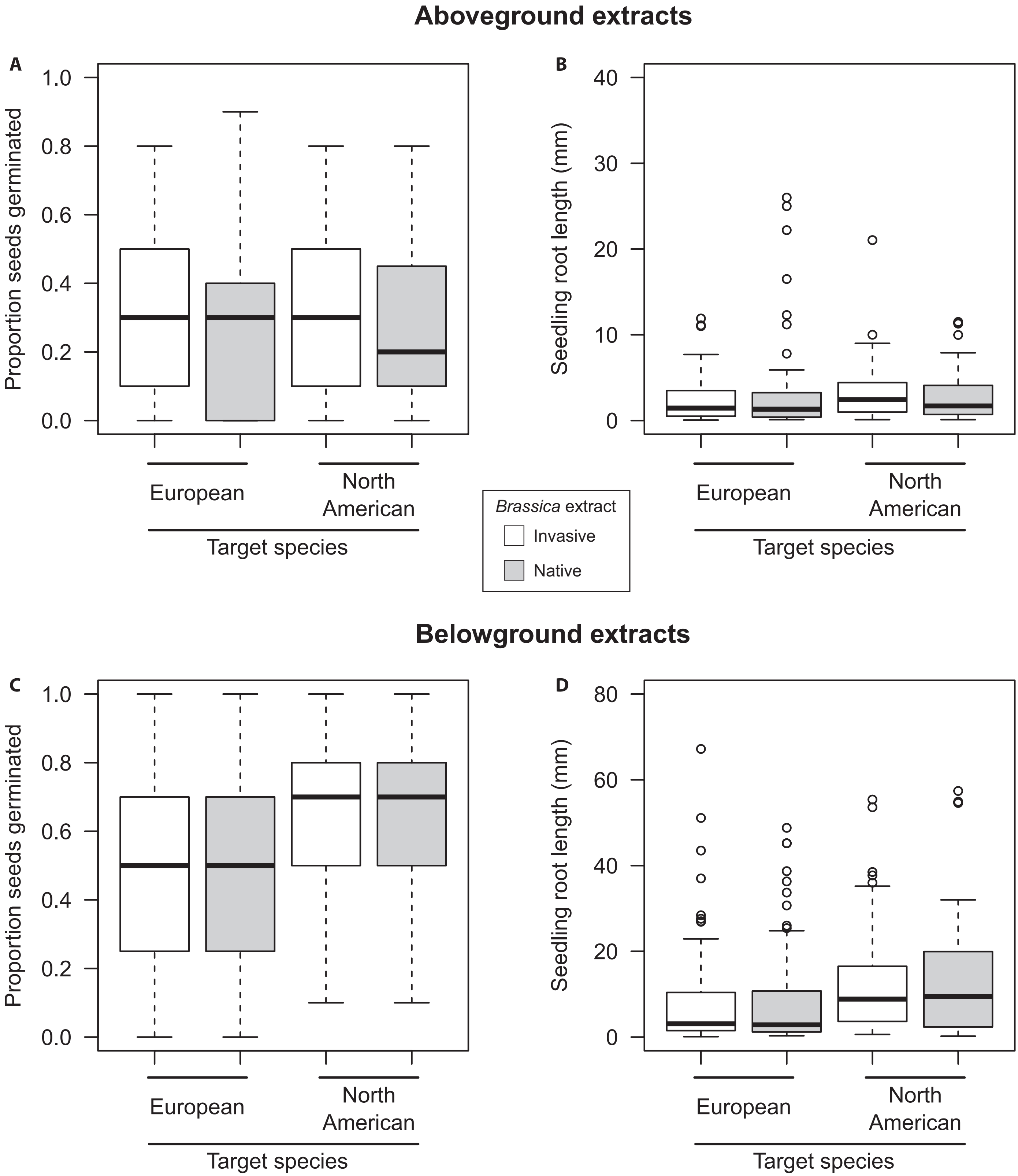

FIGURE 2. Comparison of effects of extracts from invasive and native *Brassica nigra* populations on germination (A, C) and seedling root length (B, D) of nine European and nine North American target species. Boxes show interquartile range around the median, whiskers extend to 1.5× the interquartile range (or to the minimum or maximum observed value within that). Data points beyond the whiskers are outliers (marked by circles).

underlie the allelopathic effects of *B. nigra* extracts. The most abundant glucosinolate in *B. nigra* is sinigrin, and invasive *B. nigra* had higher levels of sinigrin than native *B. nigra* when grown in the field (Oduor et al., 2011). Sinigrin is produced constitutively in tissues of Brassicaceae, but herbivory and mechanical damage on tissues can induce higher sinigrin production (Traw, 2002; Soler et al., 2005). In line with the involvement of sinigrin in competitive interactions, invasive *B. nigra* plants had stronger suppressive effects on competitors than native-range *B. nigra* plants in the presence of root and shoot herbivory on *B. nigra* (Oduor et al., 2017). Our current finding that invasive populations did not have stronger allelopathic effects than native populations may thus imply that constitutive production of sinigrin (our greenhouse-grown plants were not exposed to herbivory) is not inherently different between invasive and native *B. nigra* populations.

CONCLUSIONS

The present findings showed strong negative effects of *B. nigra* extracts on target species germination and seedling root growth. However, we did not find evidence of the evolution of enhanced allelopathic ability in invasive populations of *B. nigra*. Although the negative effect was mostly due to changes in osmolality, our data confirms that *B. nigra* can interfere with other species through allelochemical interactions. A better understanding of the allelopathic effect of *B. nigra* will require further studies in the field, identification of the allelochemicals involved, their mechanism of release into the soil, and their stability.

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AUTHOR CONTRIBUTIONS

A.M.O.O. conceived the research idea; A.M.O.O., M.v.K., and M.S. designed the research; A.M.O.O. performed the experiment; A.M.O.O., M.v.K., and M.S. performed statistical analyses; A.M.O.O., M.v.K., and M.S. wrote and revised the paper.

DATA AVAILABILITY

The data for this manuscript are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9cnp5hqfd (Oduor et al., 2020).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. *Brassica nigra* seed sources for the current experiment.

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