EICA FAILS AS AN EXPLANATION OF GROWTH AND DEFENCE EVOLUTION FOLLOWING MULTIPLE INTRODUCTIONS

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ABSTRACT

1. Rapid adaptation is aiding invasive populations in their competitive success. The evolution of increased competitive ability (EICA) hypothesis posits this enhanced performance results from escape from native enemies, yet its support is equivocal.
2. We here test EICA comprehensively by investigating adaptive divergence of various constitutive and inducible defence-related traits within the native North America and introduced European and Australian ranges, whilst controlling for divergence due to latitudinal trait clines, individual resource budgets and population differentiation using >11,000 SNPs.
3. We do not identify a general reduction in defence in concert with an increase in growth among the multiple introduced ranges as predicted by EICA. Yet, rapid, repeated clinal adaptation in defence-related traits was apparent despite distinct demographic histories. Divergence in energy budgets among ranges may explain some, but not all, defence-related trait divergence.
4. *Synthesis*: The rapid spread of invasive species is affected by a multitude of factors, likely including adaptation to climate and escape from natural enemies. Unravelling the mechanisms underlying invasives' success enhances understanding of eco-evolutionary theory and is essential to inform management strategies in the face of ongoing climate change.

KEYWORDS

EICA, latitudinal adaptation, growth-defence trade-offs, constitutive defence, inducible defence, invasive species, phenolic compounds, resource budgets

INTRODUCTION

Biological invasions are occurring at an accelerating pace due to the globalisation of anthropogenic activity (Ricciardi, 2007). Individuals colonizing new ranges likely face environments different from those previously experienced (Sax & Brown, 2000; Allendorf & Lundquist, 2003; Chown et al., 2014). Nonetheless, alien populations often display enhanced performance compared to their native counterparts (Blossey & Notzold, 1995; Thébaud & Simberloff, 2001; Parker et al., 2013), and this can be facilitated by rapid adaptation (Chown et al., 2014; Colautti & Lau, 2015; Dlugosch et al., 2015). The evolution of increased competitive ability (EICA) hypothesis posits that invasives' trait divergence results from release from natural enemies, allowing the allocation of defence resources to growth and reproduction (Blossey & Notzold, 1995). However, limited empirical support exists for the predicted evolutionary decrease in invasive plants' herbivory defence in concert with increased competitive ability (Bossdorf et al., 2005; Felker□Quinn et al., 2013) and EICA as yet falls short as a general hypothesis to explain invasion success.

The complex interplay between the evolutionary mechanisms shaping phenotypic divergence could confound inferences predicted by EICA. Distinct demographic processes, including founder effects, genetic drift and admixture, often characterize introduction and alone can lead to divergence between native and introduced populations (Lee, 2002; Facon et al., 2006; Prentis et al., 2008; Rius & Darling, 2014; Estoup et al., 2016). Dissection of the various evolutionary processes that can contribute to trait divergence is required to advance our understanding of rapid spread in invasive species. In addition, the repeatability of evolutionary patterns associated with introductions is unclear as the majority of studies on EICA focus on a single invaded range (e.g. Blossey & Notzold, 1995; Joshi & Vrieling, 2005; Hodgins

& Rieseberg, 2011; Uesugi & Kessler, 2016, but see Colomer‐Ventura et al., 2015). To reveal the general applicability of EICA, tests across multiple introduced ranges are essential.

Biotic and abiotic clines governing plant resistance within ranges (Endara & Coley, 2011; Moles et al., 2011a) can also obscure the adaptive underpinnings of trait divergence among ranges experienced during invasion. For instance, herbivore pressure in the native range is expected to increase towards lower latitudes and potentially drive clines in plant defence in some species (Moles et al., 2011a). This clinal pattern may be absent in the introduced range due to overall lack of herbivory, resulting in non-parallel defence gradients between ranges (e.g. Cronin et al., 2015; Allen et al., 2017). Moreover, high-resource environments support plant species with faster growth that are more vulnerable to herbivores (Coley et al., 1985; Zandt, 2007; Endara & Coley, 2011). Latitudinal clines in resource availability could thus lead to the evolution of high growth and reduced chemical defences at lower latitudes (Woods et al., 2012; Moreira et al., 2014), although this interspecific pattern may have limited application to intraspecific variation (Hahn & Maron, 2016, but see Woods et al., 2012). Taken together these patterns suggest that the evolutionary consequences of herbivore escape could change along latitudinal gradients (Blumenthal, 2006). Geographical clines therefore need to be considered in tests of adaptive divergence between ranges (Colautti et al., 2009).

Herbivore defence may also evolve indirectly if defensive traits are genetically correlated with other traits that are under strong selection. For example, the growth-defence trade-off hypothesis (Coley et al., 1985) suggests a negative genetic correlation between plant growth and defence-related traits, because allocation of limited resource to one trait necessarily reduces resource available for the other.

Nevertheless, empirical demonstrations of such trade-offs are rare (Uesugi et al., 2017), and may often be obscured by genetic variation in resource acquisition (Van Noordwijk & de Jong, 1986). High variation in resource acquisition among genotypes from across the species range, such as elevated resource returns of larger individuals (Van Noordwijk & de Jong, 1986) or individuals with high specific leaf area (SLA, Poorter & Remkes, 1990), is expected to mask the underlying trade-offs and result in a positive correlation between growth and defence (Agrawal, 2011; Züst & Agrawal, 2017). To test for the evolution of defence traits driven by allocation trade-offs, we thus need to assess how potential traits governing resource acquisition could impede detection of allocation trade-offs between growth and defence.

The frequency and level of attack could lead to various defence responses (Orrock et al., 2015; Bixenmann et al., 2016), expected to trade off due to their costs and redundancy (Koricheva et al., 2004; Agrawal et al., 2010). Predictable and strong attack should favour constitutive defence, whereas low, infrequent herbivory would render no, or an inducible response (Agrawal & Karban, 1999; Ito & Sakai, 2009). These responses have been shown to vary over latitudinal clines within ranges (Moreira et al., 2014 Rasmann & Agrawal, 2011). However, the studies exploring evolutionary shifts between native and introduced ranges showed mixed results (e.g. Cipollini et al., 2005; Eigenbrode et al., 2008). Various responses are predicted to result from a decrease in the intensity and frequency of herbivory following introduction (Maron & Vilà, 2001; Agrawal & Kotanen, 2003), including an increase in plasticity (Cipollini et al., 2005; Lande, 2015) or high variability in inducible response among populations (Eigenbrode et al., 2008). Testing such shifts in invasive species would provide insight into the evolution of induced/constitutive trait defence more generally.

Ambrosia artemisiifolia is a highly suitable system to study adaptive divergence in defence-related traits during invasion. This native North American weed has successfully established globally (Oswalt & Marshall, 2008), including recent introductions to Europe (~160 years ago Chauvel et al., 2006) and Australia (~80 years ago; Palmer & McFadyen, 2012; van Boheemen et al., 2017). Repeated clinal associations were found in *A. artemisiifolia* populations included in the current study, with declines in size and increase in SLA at higher latitudes (van Boheemen et al., 2018). At comparable latitudes, European plants were bigger and had lower SLA than natives, while Australian plants had higher SLA leaves (van Boheemen et al., 2018).

We test for adaptive trait divergence in 1) physical defence (trichome density), 2) chemical defence (phenolic compounds concentration and richness), and 3) inducibility of chemical defence among the native North American and introduced European and Australian ranges. Trichomes are found on the leaves and the stems of plants and deter herbivores (Kessler & Baldwin, 2002; Dalin et al., 2008; Tian et al., 2012). Phenolics are secondary metabolites that are often thought to confer resistance against herbivores (Bhattacharya et al., 2010; War et al., 2012; War et al., 2018). These compounds are also known to be inducible in response to herbivore damage, as well as simulated herbivory treatments including wounding and methyl jasmonate (MeJA) applications (e.g. Lee et al., 1997; Constabel & Ryan, 1998; Keinänen et al., 2001; Heredia & Cisneros-Zevallos, 2009). We accounted for non-adaptive genetic differences among populations potentially influencing trait variation using >11,000 double-digest genotype-by-sequencing SNPs. Moreover, we controlled for defence-related trait variation along latitudinal clines.

We predict reduced constitutive defence within the introduced ranges together with elevated inducible response due to lower certainty of attack (Cipollini et al., 2005) and a more plastic (inducible) response in recent colonisations (Lande, 2015). We expect non-parallel defence gradients between native and introduced ranges due to divergence of clines in herbivory (Moles et al., 2011b) and/or variable resource gradients (Blumenthal, 2006; Hahn & Maron, 2016). Finally, we explored whether defence-related trait divergence between ranges could be explained by divergence in growth and SLA (van Boheemen et al., 2018). By considering the complex interplay of the evolutionary mechanisms shaping defence divergence among multiple ranges, we effectively test adaptive evolutionary changes in herbivore defence.

METHODS

Study species

Ambrosia artemisiifolia is a highly invasive monoecious, self-incompatible annual (Brandes & Nitzsche, 2006), most commonly found in disturbed habitats (Bassett & Crompton, 1975; Lommen et al., 2017) and is expected to expand its range with ongoing climate change (Chapman et al., 2014). It is the leading cause of hay fever worldwide (Taramarcaz et al., 2005) and has a significant impact on crop yields (Kazinczi et al., 2008). Within Europe, admixture following multiple introductions from distinct native sources has been contributed to the success of these introduced populations, and genetic variation equals levels observed in North America (Chun et al., 2010; Gladieux et al., 2010; Gaudeul et al., 2011; van Boheemen et al., 2017). A subsequent single bottlenecked introduction from Europe has been determined to have sourced the Australian invasion, although the exact European source is unknown (van Boheemen et al., 2017).

Within the native range, around 450 herbivores have been associated with *Ambrosia* species, of which about 30% are specific to the *Ambrosia* genus (Gerber et al., 2011). Native specialist *Ophraella communa* is shown to exert high levels of damage (Throop, 2005). Up to 50 polyphagous insect species have been associated with *A. artemisiifolia* in Europe, yet most cause little damage (Gerber et al., 2011; Essl et al., 2015). *Ophraella communa* has been sighted in Southern Switzerland and Northern Italy since 2013 (Müller□Schärer et al., 2014), where it greatly affects *A. artemisiifolia* seedling survival and growth (Cardarelli et al., 2018). In Australia, generalists *Zygogramma bicolorata* (leaf-feeding) and *Epiblema strenuana* (stem-boring) are widespread and seemingly exert some control (Palmer & McFadyen, 2012).

Experimental set-up

To explore the divergence of constitutive quantitative defence traits between native and introduced ranges ("constitutive-defence experiment"), while accounting for divergence along latitudinal clines, we collected *Ambrosia artemisiifolia* seeds in 2013-2014 from broad geographical scales within the native North America and introduced Europe and Australia. We raised seedlings in a common garden (for detailed methods, see Supporting Information). Briefly, we stratified seeds for 6 weeks at 4°C (Willemsen, 1975). After a 2-week germination at 30°C with 12h light/dark cycle, we randomly transplanted into 100ml kwikpot trays with Debco mix, followed by a second transplant to 0.7L pots containing Debco and 1.5ml slow-release fertilizer (Osmocote Pro, eight to nine months) one month later. We top-watered all plants and artificially manipulated daylight following the light cycle at the median latitude for all populations (47.3°N). To explore constitutive defence, we selected a seedling from four maternal lines, originating from 28 North American, 32 European and 20 Australian locations (Supporting Information, Table S1).

A separate greenhouse experiment was conducted to test whether the inducibility of defence response varied among plant origins (hereafter, “induction experiment”). We used a subset of populations used in the constitutive experiment (10 North American, 17 European and 12 Australian locations, Table S1). For each population, we selected four maternal lines, and grew two seedlings per line as above. One seedling per mom was allocated to either the control or simulated herbivory treatment. We simulated herbivory by vertically cutting off half of the newest fully formed leaf (wounding) and subsequently spraying the whole plant with 1mM methyl jasmonate (MeJA) (Campos□Vargas & Saltveit, 2002; Heredia & Cisneros-Zevallos, 2009). Control plants were not wounded and were sprayed with distilled water.

Trait measurements

For the constitutive experiment, we recorded trichome density at the mid-point of each plant under a dissecting microscope (Olympus, SZ-PT) using a 1 cm x 0.3 cm stem area at the mid-point of each plant, nine weeks after the second transplant. Three weeks later, we scanned one young, fully expanded leaf from each plant and calculated leaf area using ImageJ and the R package LeafArea (Katabuchi, 2015). We dried leaves at 45 °C for seven days and an addition 12 hours prior to weighing and weighed to the closest milligram. We calculated specific leaf area (SLA) by dividing leaf area by dry leaf weight (mm^2/mg). We deconstructed plants for biomass measurements once the majority of seeds had ripened. We placed aboveground components in paper bags and dried these in ovens at 45 °C for at least 36 hours. Before dry weight biomass measures, we dried materials for an additional minimum of 24 hours to ensure the dry weight was constant at the time of measuring and it was not variable due to humidity in the air or incomplete drying. We weighed this shoot biomass to the closest 0.1 gram.

Leaf samples for phenolic analyses were collected four weeks after the second transplant by clipping approximately 200 mg of the newest fully expanded leaf, which was flash frozen in liquid nitrogen and stored in a -80 ˚C. In the induction experiment, we collected leaf samples 24 hours after the final treatment. Samples were extracted in 1 ml of 80% methanol using a Qiagen TissueLyser II for 30 seconds at 30 rps twice and centrifuged for 30 minutes at 2700 rpm. Phenolic samples from the constitutive-defence experiment were analysed using HPLC Agilent 1200 series (Agilent Technologies Australia, Mulgrave, VIC, Australia) equipped with C18 reverse-phase column (Waters, 5.0 µm, 250 mm x 4.6 mm; Alltech Australia, Baulkham Hills NSW, Australia). The elution system consisting of solvents (A) 0.25% H3PO4 in water (pH 2.2) and (B) acetonitrile was: 0–6 min, 0–12% of B; 6–10 min, 12-18% of B, and 10-30 min, 18-58% of B, with a flow rate of 1 mL/min and injection volume of 15 µL (Keinänen et al., 2001). Samples from the induction experiment were analysed with Agilent Infinity 1260 equipped with C18 reverse-phase column (Poroshell 120 EC-C18, 2.7 µm, 150 mm x 3.0 mm; Agilent Technologies Australia, Mulgrave, VIC, Australia). The elution method was modified from above and was: 0–2 min, 0–12% of B; 2–3.3 min, 12–18% of B, and 3.3–10 min, 18-58% of B, with a flow rate of 0.5 mL/min and injection volume of 5 µL. In both experiments, phenolic compound peaks were identified to their compound classes using UV spectra and relative abundance was quantified at 320 nm. To estimate phenolic compound richness, we counted the number of detectable peaks. Concentration of eight major phenolic peaks was estimated as area under each peak divided by sample fresh weight. Results could not be directly compared as the two experiments were performed in different greenhouses and samples from each experiment were run using different HPLC machines.

Statistical analyses

To test if constitutive defence differed among ranges (the constitutive experiment), we included range, latitude, their interaction and a latitude^2 effect as fixed factors in each model. We examined phenolic compound composition in a multivariate analysis of covariance (MANCOVA) by including the concentration of eight major phenolic compounds. We increased the power of this analysis by removing highly correlated peaks (Spearman's $\rho > 0.75$) (Scheiner, 2001), resulting in the inclusion of all eight peaks. We calculated Wilks' λ (multivariate F-value) to measure the strength of the associations. We then examined concentration of individual phenolic compounds, phenolic compound richness, total phenolic concentration, and trichome density in univariate mixed models. Here, we controlled for neutral population structure, possibly shaping trait variation between populations, by including q-values as obtained from STRUCTURE analysis performed on genetic data as a random effect. Within multi- and univariate analyses, we improved normality of the data by square-rooting or log-transforming traits where appropriate. We computed type III Wald F-values with Kenward-Roger degrees of freedom and step-wise removed non-significant effects, starting with the highest order interaction. For univariate models, we plotted the partial residuals of each response variable by ranges, thus accounting for latitudinal clines and neutral population genetic structure.

To explore the variation in inducibility among ranges (the induction experiment), we repeated the steps for the constitutive experiment, now including treatment and its interactions with range and latitude as fixed effects. For the MANCOVA, we excluded five peaks with $\rho < 0.75$. We retained treatment in these models, as this was the variable of interest. Here, a significant treatment effect would signify an inducible response, whereas a treatment x range interaction would imply this response differs between ranges. A treatment x latitude interaction would indicate different inducibility at different latitudes. To test if variation in induction differed between ranges (Eigenbrode et al., 2008), we compared the coefficient of variation (c_v) using the modified signed-likelihood ratio test for equality with 10^4 simulations in the cvequality package (Krishnamoorthy & Lee, 2014; Marwick & Krishnamoorth, 2018).

To examine associations between defence-related traits and plant growth and to assess if divergence in individual resource budgets could have resulted in range differences in defence-related trait investment, we tested responses of phenolic richness, phenolic concentrations or trichome density to shoot biomass or SLA. Each model included a defence-related trait as response, with shoot biomass or SLA, range and their interaction as predictors. We used individual trait values and included individual STRUCTURE q-values and sampling location as random factors. We explored significant range x defence interactions using a Holm p-value correction in the *phia* package (De Rosario-Martinez, 2013). In these models, a negative association between defence-related traits and shoot biomass would suggest a trade-off, while a positive one might indicate differences in resource acquisition. Range differences at similar values of shoot biomass or SLA would indicate defence-related trait divergence independent of genotypic differences in individual resource budgets.

To explore if constitutive and inducible defence trade off, we first calculated the induced level of total phenolics for each maternal line as the difference between damage and control treatments of the two half-sibs. This estimate of induction is thought to reduce correlations with control treatment estimates and thus the collinear associations (e.g. due to genotypic biases) will not mask the trade-off associations (Morris et al., 2006). We included population of origin and individual q-values as random factor in these models. A significant negative association between induced and constitutive levels of phenolic concentration and richness would indicate the presence of a trade-off. All statistical analyses were conducted in R v3.4.3 (R Core Team, 2017).

RESULTS

Constitutive defence trait divergence between ranges

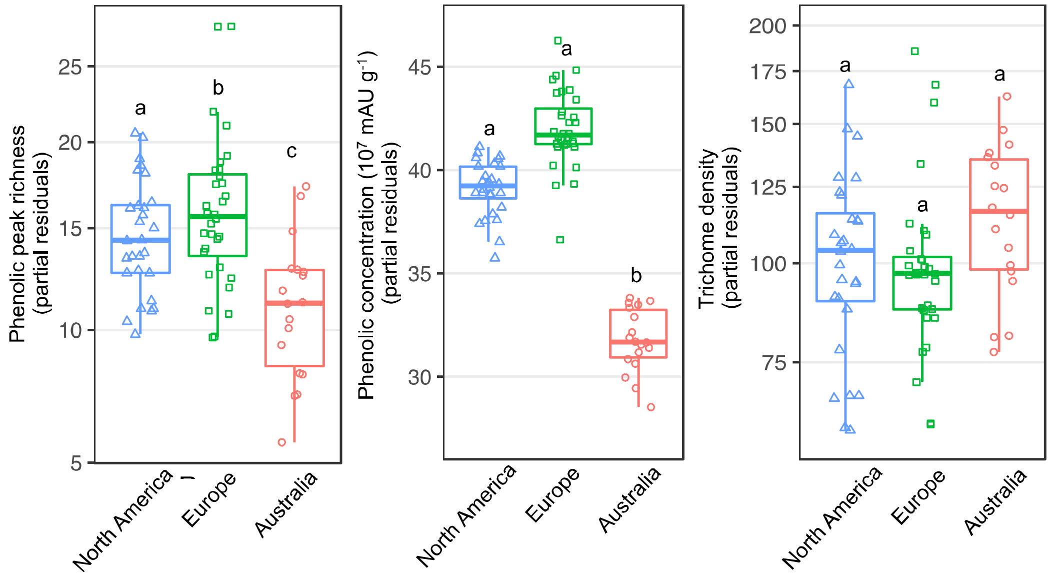
We found significant range divergence in constitutive phenolic composition (Table 1a), resulting from differences between the introduced Europe and the native North America ($F_{8,66}$=3.280, p=0.010, Wilks' λ=0.716; Table 1b). Phenolic peak richness differed among ranges (Table 1a): it was highest in the introduced European range (adjusted mean of 43 peaks) followed by the native North American (40 peaks) and introduced Australian ranges (33 peaks). Total phenolic concentration was similar between the native and European populations, but 28% lower in Australia (Table 1b, Fig. 1). Trichome density showed no differences between ranges (Table 1a, Fig. 1). The composition of individual phenolic compounds and peak richness depended on latitude, though no such effect was found for the total phenolic concentration or trichome density (Table 1a, Fig. 2). We did not observe range x latitude interactions for any of the defence-related traits (Table 1a), suggesting latitudinal clines, when present, did not differ between ranges.

Table 1. *Ambrosia artemisiifolia* defence-related trait responses (population means) to range, latitude, their interaction and latitude^2 in the constitutive experiment in multivariate (individual phenolic compounds) and univariate analyses (a), with dissection of significant range effects (p<0.05) in post-hoc tests (b). We reported Wald type III F (a) or χ^2 test values (b), Kenward-Roger degrees of freedom (subscript), significance (symbols) and Wilk's λ (multivariate analysis only). Models were step-wise reduced starting with the highest order non-significant interaction and univariate analyses included neutral population genetic structure as a random effect.

a.	Range	Latitude	Latitude^2	Range:Latitude
Individual phenolic compounds composition	$4.520_{16,132}$***, λ =0.417	$6.928_{8,66}$***, λ=0.544	$2.814_{8,66}$*, λ =0.746	$0.849_{16,128}$(ns), λ =0.817
Phenolic richness	$7.615_{2,58.48}$**	$7.79_{1,71.66}$**	$0.046_{1,53.51}$(ns)	$2.027_{2,69.64}$(ns)
Phenolic concentration	$8.601_{1,71.918}$***	$0.934_{1,73.244}$(ns)	$0.036_{1,72.973}$(ns)	$0.127_{1,70.054}$(ns)
Trichome density	$0.663_{2,71.183}$(ns)	$1.825_{1,66.941}$(ns)	$3.148_{1,74.991}$#	$0.121_{2,69.141}$(ns)

b.	North America - Europe	North America - Australia	Europe - Australia
Individual phenolic compounds composition	$3.280_{8,66}$**, λ =0.716	$1.580_{8,66}$(ns), λ=0.840	$1.994_{8,66}$(ns), λ =0.805
Phenolic richness	4.783_{1}*	12.725_{1}***	15.843_{1}***
Phenolic concentration	1.397_{1}(ns)	9.311_{1}**	17.321_{1}***

*ns: p>0.1; #: p<0.1, *: p<0.05; **: p<0.01; ***: p< 0.001*



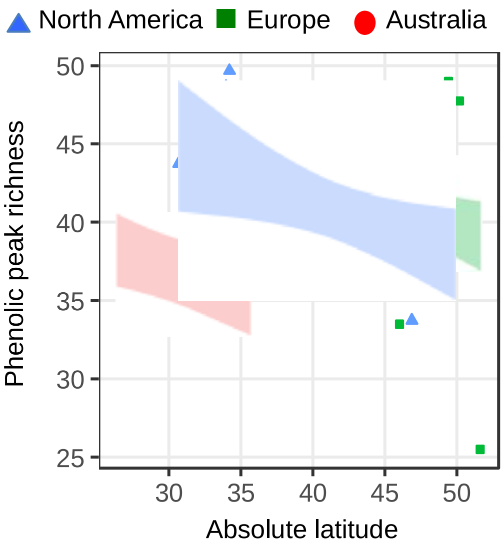


Fig. 2. Population mean response of phenolic peak richness to range (native North America, blue triangles; introduced Europe, green squares; introduced Australia, red circles) and latitude in *Ambrosia artemisiifolia,* with predicted latitudinal clines (+/- 95% confidence interval) corrected for neutral population structure.

Inducible defence trait divergence between ranges

We found a significant treatment effect on individual phenolic compound composition in the induction experiment ($F_{5,59}$=12.014, p<0.001, Wilks' λ=0.496; Table 2). Phenolic peak richness did not show a response to experimental treatment (Table 2, Fig. 3). However, the total phenolic concentration was slightly supressed in the herbivory simulating treatment (Table 2, Fig. 3). We identified no treatment x range x latitude interactions (Table 2, Fig. 3), suggesting there is no range difference in inducibility clines. Also, the absence of treatment x latitude interactions (Table 2, Fig. 3), suggests an overall lack of latitudinal clines in inducibility. Moreover, no treatment x range interactions (Table 2, Fig. 3) suggests the inducible response did not differ between ranges. We did not find range differences in the variation of inducible phenolic peak richness (c_v=1.401, p=0.496) or concentration (c_v=2.297, p=0.317).

Table 2. *Ambrosia artemisiifolia* defence-related trait responses (population means) to range, latitude, treatment, their interactions and latitude^2 in the inducible experiment in multivariate (individual phenolic compounds) and univariate analyses. Range, latitude, their interaction or latitude^2 were included as covariates and significant results were not explored further. Reported Wald type III F-test values, Kenward-Roger degrees of freedom (subscript), significance (symbols) and Wilk's λ (multivariate analysis only). Models were step-wise reduced starting with the highest order non-significant interaction and univariate analyses included neutral population genetic structure as a random effect.

	Range	Latitude	Latitude^2	Range:Latitude	Treatment	Treatment:Range	Treatment:Latitude	Treatment:Range: Latitude
Individual phenolic compounds concentration	$7.591_{10,118}$***, $\lambda=0.370$	$10.637_{5,59}$***, $\lambda=0.526$	$4.818_{5,59}$**, $\lambda=0.710$	$2.311_{10,118}$*, $\lambda=0.699$	$12.014_{5,59}$***, $\lambda=0.496$	$0.326_{10,112}$(ns), $\lambda=0.944$	$0.977_{5,58}$(ns), $\lambda=0.922$	$1.357_{10,108}$(ns), $\lambda=0.789$
Phenolic richness	$3.764_{2,29.93}$*	$4.700_{1,31.08}$*	$1.340_{2,28.24}$(ns)	$6.030_{1,30.95}$*	$0.850_{1,35.33}$(ns)	$0.091_{2,32.1}$(ns)	$0.825_{1,34.89}$(ns)	$1.923_{2,30.78}$ (ns)
Phenolic concentration	$4.970_{2,30.905}$*	$5.932_{1,31.505}$*	$1.577_{2,28.556}$(ns)	$1.745_{1,31.505}$(ns)	$4.241_{1,35.628}$*	$0.005_{2,32.285}$(ns)	$1.077_{1,35.428}$(ns)	$1.417_{2,31.192}$(ns)

*ns: p>0.1; #: p<0.1, *: p<0.05; **: p<0.01; ***: p< 0.001*

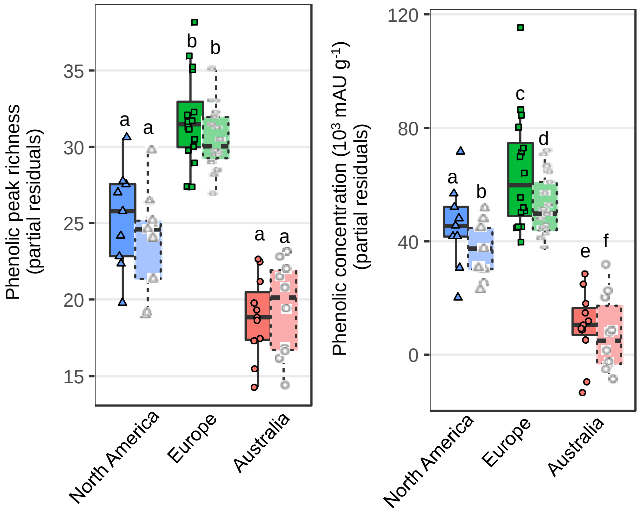


Fig. 3. Partial residual defence trait responses (phenolic peak richness and concentration) of *A. artemisiifolia* populations to control (solid symbols) and herbivore simulating treatment (wounding + MeJA, dashed transparent symbols), with covariates of range, accounting for latitudinal clines and neutral population structure. Letters indicate significance of effect (Table 2).

Associations between defence, biomass and specific leaf area (SLA)

Within each range, phenolic richness and total concentration was positively correlated with shoot biomass (Table 3, Fig. 4a-b), whereas we found a negative association between trichome density and shoot biomass (Table 3, Fig. 4c). We found high-SLA leaves had lower phenolic peak richness (Fig. 4d) and concentration (Fig. 4e), yet higher trichome density (Table 3, Fig. 4f). No interactions were significant between range and predictor variables (shoot biomass or SLA), suggesting these associations among traits were consistent between ranges (Table 3, Fig. 4). These results emphasize the close relationship between plant growth, morphology and defence.

Table 3. Constitutive defence trait response of *Ambrosia artemisiifolia* individuals to shoot biomass, specific leaf area and their interaction with range (a), with dissection of significant range effects ($p<0.05$) in post-hoc tests (b). We reported Wald type III F (a) or χ^2 test values (b), Kenward-Roger degrees of freedom (subscript) and significance (symbols). Models were step-wise reduced starting with the highest order non-significant interaction and included population origin and neutral population genetic structure as random effects.

a.

Predictor	Response	Range	Predictor	Range:Predictor
Shoot biomass	Phenolic richness	$18.96_{2,79.29}$***	$53.389_{1,180.51}$***	$1.565_{2,187.82}$(ns)
	Phenolic concentration	$19.321_{2,79.435}$***	$31.098_{1,181.299}$***	$1.441_{2,184.26}$(ns)
	Trichome density	$10.242_{2,79.4}$***	$18.49_{1,174.06}$***	$0.525_{2,180.84}$(ns)
Specific leaf area	Phenolic richness	$5.349_{2,69.16}$**	$42.692_{1,217.97}$***	$0.32_{2,202.6}$(ns)
	Phenolic concentration	$6.162_{2,71.167}$**	$38.464_{1,208.912}$***	$0.98_{2,202.53}$(ns)
	Trichome density	$1.828_{2,71.7}$(ns)	$10.994_{1,204.66}$**	$1.196_{2,206.12}$(ns)

b.

Predictor	Response	**North America - Europe**	**North America - Australia**	**Europe - Australia**
Shoot biomass	Phenolic richness	2.546_1(ns)	26.667_1***	37.964_1***
	Phenolic concentration	5.846_1*	22.155_1***	40.087_1***
	Trichome density	2.255_1(ns)	13.049_1***	21.047_1***
Specific leaf area	Phenolic richness	0.459_1(ns)	6.655_1*	10.643_1**
	Phenolic concentration	2.152_1(ns)	5.032_1*	12.485_1**
	Trichome density	-	-	-

*ns: $p>0.1$; #: $p<0.1$, *: $p<0.05$; **: $p<0.01$; ***: $p< 0.001$*

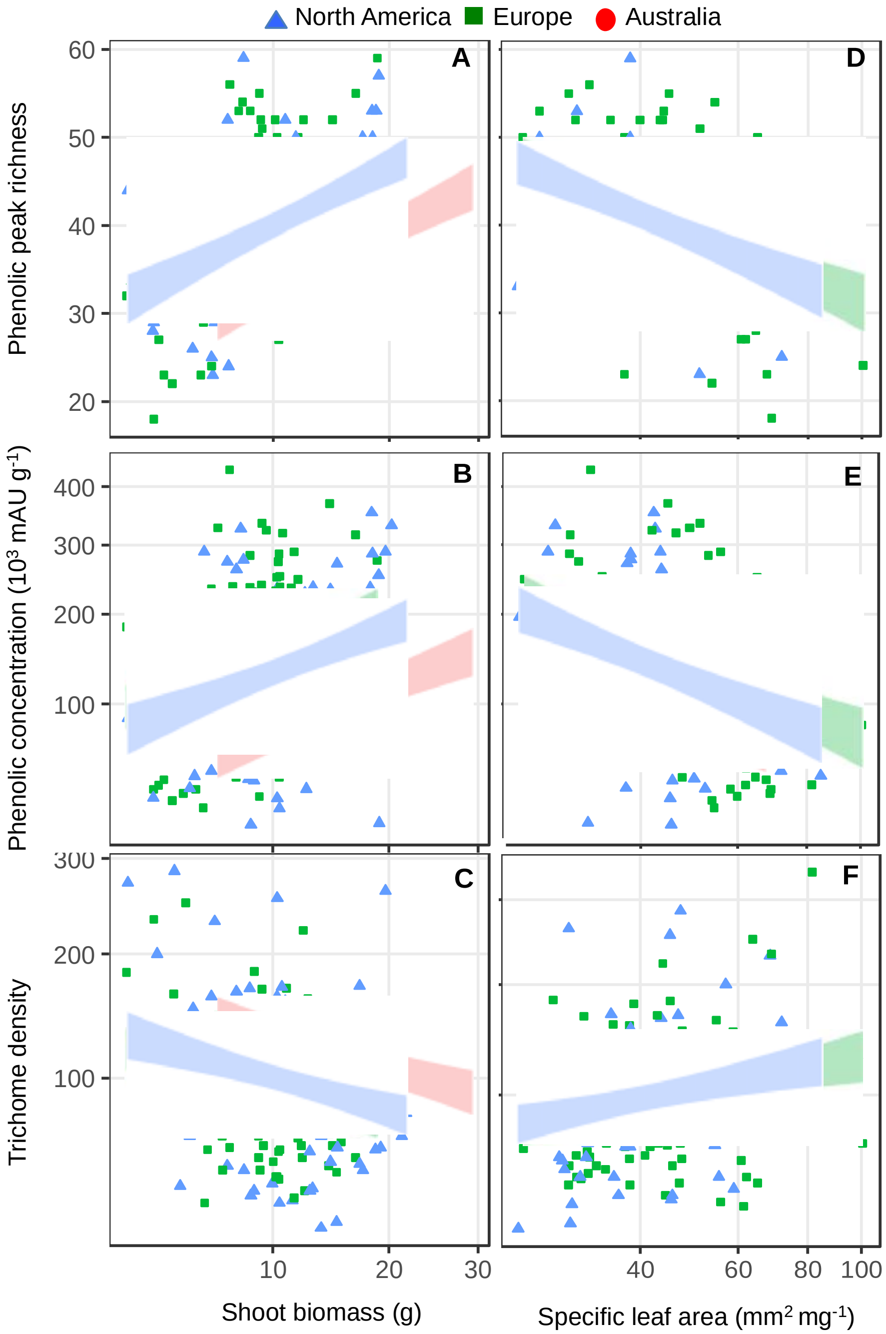


Fig 4. Defence trait responses (phenolic peak richness, concentration and trichome density) of *A. artemisiifolia* individuals to range (native North America (blue triangles); Europe (green squares); Australia (red circles)), shoot biomass (a-c) or specific leaf area (d-f) with model predictions (+/- 95% confidence interval, Table 4).

At comparable shoot biomass or SLA, phenolic peak richness was no longer significantly different between North America and Europe (Table 3, Fig. 4a & d) compared to range comparisons accounting for latitude (Table 1, Fig. 1). Conversely, total phenolic concentration in European plants was higher compared to North American individuals of comparable weight (Table 3, Fig. 4b), whereas no difference existed in latitude models (Table 1, Fig. 1). Australian plants exhibited lower phenolic peak richness and concentration compared to native or European plants of comparable weight or SLA. Yet, at the same plant weight, Australian plants had higher trichome densities than in the other ranges (Table 3, Fig. 4c). These patterns match previous analyses including latitude (Table 1, Fig. 1).

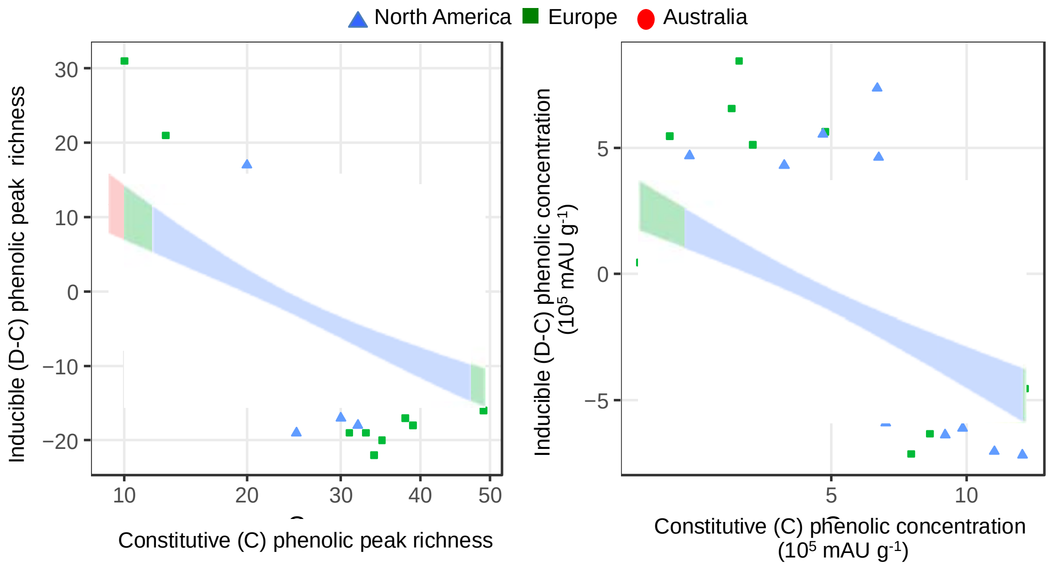


Fig. 5. Inducible (D: wounding + MeJA; C: control) versus constitutive (control) defence trait responses (phenolic peak richness and concentration) of *A. artemisiifolia* populations among ranges (native North America: blue triangles; Europe: green squares; Australia: red circles) with model predictions (+/- 95% confidence interval).

Constitutive-inducible trade-offs

Induced levels of phenolic richness and total concentration were negatively associated with constitutive levels (richness: $F_{1,123.81}=78.126$, $p<0.001$; concentration: $F_{1,141.88}=76.286$, $p<0.001$; Fig. 5). We found no range differences in either trait (richness: $F_{2,31.518}=1.719$, $p=0.196$; concentration: $F_{2,31.07}=0.265$, $p=0.769$; Fig. 5), nor did we identify interactions between range and the phenolic concentration

($F_{2,141.11}$=0.866, p=0.423). Range x phenolic peak richness ($F_{2,129.82}$=3.045, p=0.051) was marginally significant. These results suggest that constitutive and inducible defence trade off, although there is no difference between ranges.

DISCUSSION

In this study, we show the Evolution of Increased Competitive Ability (EICA) hypothesis fails as a general explanation of repeated intraspecific defence-related trait divergence across multiple introduced ranges. Though we observed reduced phenolic richness and concentration in introduced Australia compared to the native plants while controlling for genetic structure, levels were similar or slightly higher in the introduced Europe compared to native populations at comparable latitudes and energy budgets. In addition, trichome density did not differ among ranges. In line with predictions, a trade-off between the constitutive and inducible phenolics was observed together with similar phenolic inducibility among ranges. Inconclusive support for EICA has been shown in inter- (Felker□Quinn et al., 2013) and intraspecific comparisons (Colomer□Ventura et al., 2015). To our knowledge however, this is the first study testing EICA across multiple introductions while exploring the predicted confounding of latitudinal clines, population substructure or genotypic differences in individual energy budgets. Therefore, the apparent absence of the predicted repeated selection against high defence investment following introduction is unlikely to be entirely masked by these factors. We examine these processes in detail and suggest alternative mechanisms driving defence-trait divergence within and among native and introduced ranges.

Divergence in constitutive defence-related traits

We found conflicting patterns of defence-related trait divergence between the native and two introduced ranges. Biotic and abiotic latitudinal clines in focal traits (Colautti et al., 2009), genotypic differences in resource acquisition (Van Noordwijk & de Jong, 1986; Agrawal, 2011; Züst & Agrawal, 2017) and historical contingency (Lee, 2002; Facon et al., 2006; Prentis et al., 2008; Rius & Darling, 2014; Estoup et al., 2016) can obscure trade-offs predicted under EICA. Accordingly, in addition to latitudinal clines in phenolic compound composition and peak richness (Fig. 2), we show trichome density, phenolic peak richness and concentration were strongly associated with plant biomass and specific leaf area (SLA)(Fig. 4). Contrary to EICA predictions, phenolic richness was significantly higher in Europe than North America at equivalent latitudes, but this likely reflects the larger size and lower SLA of European plants at similar latitudes (van Boheemen et al., 2018). Similarly, phenolic peak concentration was significantly higher in Europe compared to native North America at comparable shoot biomass, although this difference disappeared when controlling for latitude or SLA. However, lower phenolic peak richness and concentration in Australia was still present at similar latitude, biomass or SLA compared to North America. Invasion history is unlikely a major factor in this observed defence-related trait divergence as we accounted for population genetic structure in our analysis.

An adaptive reduction of constitutive defence traits following introduction to Europe and Australia was predicted due to a general release from natural enemies. However, levels of chemical defence-related traits (phenolic richness and concentration) were not consistently lower in introduced ranges compared to native populations. Such unexpected findings could have resulted from variation in contemporary herbivory among introduced ranges. Of particular relevance to the

EICA hypothesis are specialist herbivores, as herbivory by specialists, but not necessarily generalists, is hypothesized to consistently decline during invasion (Muller-Scharer et al., 2004; Joshi & Vrieling, 2005; Felker□Quinn et al., 2013). Indeed, introduced Japanese *A. artemisiifolia* populations re-exposed to specialist leaf beetle *Ophraella communa* for >10 years were more resistant than herbivore-free populations (Fukano & Yahara, 2012). However, rapid adaptation to *O. communa* is unlikely to have led to the observed elevated European phenolic concentration and richness, as the seeds used in our experiment were collected in 2014 and this beetle is constrained to southern Europe since introduction in 2013 (Sun et al., 2017).

Alternatively, differences in generalist load between introduced ranges could have resulted in variation in quantitative digestibility-reducing chemicals (e.g. phenolics), which defend against both generalist and specialists (Muller-Scharer et al., 2004). Surveys describe a high diversity of generalist species in Europe (Gerber et al., 2011; Essl et al., 2015) not identified in Australia (Palmer & McFadyen, 2012) suggesting herbivory in this species is higher in Europe than Australia. However, Genton et al. (2005) previously found that compared to native Ontario, the most common forms of damage (chewing and perforation) together with the generalist herbivore load was reduced in introduced France populations consistent with enemy escape in Europe compared to native North America. Contradicting EICA expectations, but consistent with our findings for Europe, the French plants showed no evolutionary loss of defence (Genton et al., 2005). Therefore, although reductions in both specialists and generalist herbivores have been documented in both introduced ranges, we did not find parallel changes in defence-related traits as predicted by EICA, suggesting such predictions are perhaps too simplistic. Nevertheless, a more detailed survey of herbivory, resistance and the mechanisms of resistance across all three ranges is warranted, particularly given the contrasting patterns of divergence in phenolics identified among the two introduced ranges.

We found no support for the EICA-predicted resource allocation trade-off between defence and growth in Europe. However, even when these traits have evolved in the EICA predicted direction, negative genetic correlations have yet to be detected (Franks et al., 2008; Schrieber et al., 2017; Hodgins et al., 2018). Furthermore, a direct trade-off might not be evident as resource reallocation from other traits, drawing from the same resource pool, could allow for the elevated investment in defence related traits and growth simultaneously (Züst & Agrawal, 2017; Hodgins et al., 2018). For instance, an analysis of climate niche shifts in *A. artemisiifolia* has revealed that Eurasian and Australasian ranges on average experience warmer, wetter climates compared to the North American range (van Boheemen et al., 2018). Therefore, reduced investment in abiotic stress tolerance could have allowed for resource reallocation to defence and growth simultaneously. These recently acknowledged complex dynamics underlying competitive ability call for more integrative tests of invasive spread.

The rapid and repeated latitudinal divergence in phenolic compound composition and richness populations suggests direct or indirect selection of latitude-associated factors. Corresponding to our findings, typical reported patterns include high growth and low defence at more productive high-resource (Coley et al., 1985; Zandt, 2007; Endara & Coley, 2011), low-latitude (Woods et al., 2012; Moreira et al., 2014; Hahn & Maron, 2016) environments (Blumenthal, 2006). Native clines in herbivore load could result in such observations, though the predicted herbivore absence following introduction should lead to non-parallel defence clines among native and introduced ranges (Cronin et al., 2015; Allen et al., 2017). However, in our data, latitudinal clines in defence-related traits (phenolic compound composition and peak richness) were parallel, suggesting consistent patterns of selection with latitude in all three ranges. The absence of the predicted patterns could result from parallel clines in herbivore loads in each range or the presence of alternative evolutionary forces driving latitudinal trait divergence in the multiple ranges. Clinal variation in herbivory is not as common as previously thought (Moles et al., 2011a) and geographic information on *A. artemisiifolia* herbivore pressure is needed.

Alternatively, latitudinal clines could arise through direct selection on the alternative functions of phenolic compounds (e.g. antioxidant activity, Sakihama et al., 2002) or indirect selection through genetic covariance with traits under climate mediated selection. Climate was previously shown to be a more important driver of trait divergence compared to enemy release (Colautti et al., 2009; Colautti & Barrett, 2013; Colomer□Ventura et al., 2015). Accordingly, climatic differences between the ranges not captured by latitude could contribute to patterns of divergence in Australian defence-related traits. For instance, trichomes protect plants from UV (Bassman, 2004; Hauser, 2014) and selection for this alternate function in high-UV Australia (WHO, 1998) could potentially explain the higher density of trichomes in this range when controlling for plant size. Herbivore exclusion experiments at various latitudes and environments would be required to disentangle how resource availability, herbivory and other climatic factors might interact during invasion and impact the evolution of growth and defence traits.

Constitutive versus induced range divergence

We observed a negative association between constitutive and inducible defence-related traits suggesting a trade-off (Koricheva et al., 2004; Agrawal et al., 2010). A decrease in the level and predictability of attack in the introduced range is expected to cause a reduction in constitutive defence and the maintenance or increase in inducible defence (Cipollini et al., 2005; Orians & Ward, 2010; Lande, 2015). In agreement with this prediction constitutive phenolic levels were reduced in Australia, while inducible response did not differ among ranges. Such maintenance of mean inducibility could result from insufficient herbivore pressure, where a selection-drift imbalance could increase inducible variability (Eigenbrode et al., 2008). Although analysis of neutral markers suggests genetic drift has been particularly strong in Australia (van Boheemen et al., 2017), we did not reveal any increase in inducible variation. The growing body of literature testing constitutive versus inducible defence in native and introduced ranges frequently report inconsistent results varying from reductions, to maintenance, to increases in either defence (Cipollini et al., 2005; Eigenbrode et al., 2008; Beaton et al., 2011; Carrillo et al., 2012; Cipollini & Lieurance, 2012; Wang et al., 2012; Wang et al., 2013; Fortuna et al., 2014; Gu et al., 2014; Agrawal et al., 2015; Macel et al., 2017) and calls for more detailed research on the costs-benefit trade-offs of the various responses.

Remarkably, we found evidence of a suppression of phenolics in response to herbivore simulation for some populations, especially those with high constitutive levels, in contrast to some previous studies (Lee et al., 1997; Constabel & Ryan, 1998; Keinänen et al., 2001; Heredia & Cisneros-Zevallos, 2009). Conversely, cardenolide suppression was found in various *Asclepias* species at high constitutive levels (Rasmann et al., 2009), though the mechanistic cause was not discussed (Agrawal et al., 2010). We propose that the retraction of phenolics from damaged leaves could indicate a cost-reducing response when the inducible phenolic compounds have alternative functions (e.g. allelopathic interactions and plant structure; Bhattacharya et al., 2010; Li et al., 2010), or function only in particular aspects of defence response, not induced by the treatment. Nevertheless, gaining insight into such cost-benefit associations might prove difficult due to, for instance, issues identifying and addressing all factors influencing the investment of defence-related traits (Neilson et al., 2013).

Conclusion

We show that escape from natural enemies is not likely the single force driving the evolution of increased competitive ability in this invasive, as enhanced growth in European populations was not in lieu of defence-related trait reduction. Trait evolution in Australian populations, derived from European founders, occurred rapidly (~80 generations), seemingly unconstrained by strong genetic bottleneck identified in this range (van Boheemen et al., 2017), as measured traits in these two invaded ranges are primarily on opposing ends of the phenotypic spectrum of values. Evidence is growing that adaptation to climate might explain the alarming spread and success of non-natives to a greater extent than release from natural enemies (Colautti et al., 2009; Colautti & Barrett, 2013; Colomer-Ventura et al., 2015). This study emphasizes that intraspecific multi-introduction tests of adaptive trait divergence of invasive species are helpful for understand contemporary evolutionary process during range expansion.

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AUTHOR CONTRIBUTIONS

All authors developed the project, with data collection and analyses carried out by LAB and SB, refined by AU and KH. All authors discussed the results, contributed to the MS writing and gave final approval for publication.

DATA ACCESSBILITY

Sequence data are available at the National Center for Biotechnology Information **(**NCBI) Sequence Read Archive under Bioproject PRJNA449949.

REFERENCES

Agrawal, A. A. (2011) Current trends in the evolutionary ecology of plant defence. Functional Ecology, 25(2), 420-432.

Agrawal, A. A., Conner, J. K. & Rasmann, S. (2010) Tradeoffs and negative correlations in evolutionary ecology. *Evolution since Darwin: the first 150 years* (eds M. A. Bell, D. J. Futuyma, W. F. Eanes & J. S. Levinton), pp. 243-268. Sinauer Associates, Sunderland, Massachusetts, USA.

Agrawal, A. A., Hastings, A. P., Bradburd, G. S., Woods, E. C., Züst, T., Harvey, J. A. & Bukovinszky, T. (2015) Evolution of plant growth and defense in a continental introduction. The American Naturalist, 186(1), E1-E15.

Agrawal, A. A. & Karban, R. (1999) Why induced defenses may be favored over constitutive strategies in plants. *The ecology and evolution of inducible defenses* (eds R. Tollrian & C. D. Harvell), pp. 45-61. Princeton University Press, Princeton, New Jersey.

Agrawal, A. A. & Kotanen, P. M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. Ecology Letters, 6(8), 712-715.

Allen, W. J., Meyerson, L. A., Cummings, D., Anderson, J., Bhattarai, G. P. & Cronin, J. T. (2017) Biogeography of a plant invasion: drivers of latitudinal variation in enemy release. Global Ecology and Biogeography, 26(4), 435-446.

Allendorf, F. W. & Lundquist, L. L. (2003) Introduction: population biology, evolution, and control of invasive species. Conservation Biology, 17(1), 24-30.

Bassett, I. J. & Crompton, C. W. (1975) The biology of Canadian weeds. Canadian Journal of Plant Science, 55, 463-476.

Bassman, J. H. (2004) Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. Photochemistry and Photobiology, 79(5), 382-398.

Beaton, L. L., Van Zandt, P. A., Esselman, E. J. & Knight, T. M. (2011) Comparison of the herbivore defense and competitive ability of ancestral and modern genotypes of an invasive plant, *Lespedeza cuneata*. Oikos, 120(9), 1413-1419.

Bhattacharya, A., Sood, P. & Citovsky, V. (2010) The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. Molecular Plant Pathology, 11(5), 705-719.

Bixenmann, R. J., Coley, P. D., Weinhold, A. & Kursar, T. A. (2016) High herbivore pressure favors constitutive over induced defense. Ecology and Evolution, 6(17), 6037-6049.

Blossey, B. & Notzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. Journal of Ecology, 83(5), 887-889.

Blumenthal, D. M. (2006) Interactions between resource availability and enemy release in plant invasion. Ecology Letters, 9(7), 887-895.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E. & Prati, D. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia, 144(1), 1-11.

Brandes, D. & Nitzsche, J. (2006) Biology, introduction, dispersal and distribution of common ragweed (*Ambrosia artemisiifolia* L.) with special regard to Germany. Nachrichtenblatt des Deutschen Planzenschutzdienstes, 58, 286-291.

Campos□Vargas, R. & Saltveit, M. E. (2002) Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. Physiologia Plantarum, 114(1), 73-84.

Cardarelli, E., Musacchio, A., Montagnani, C., Bogliani, G., Citterio, S. & Gentili, R. (2018) *Ambrosia artemisiifolia* control in agricultural areas: effect of grassland seeding and herbivory by the exotic leaf beetle *Ophraella communa*. NeoBiota, 38, 1.

Carrillo, J., Wang, Y., Ding, J., Klootwyk, K. & Siemann, E. (2012) Decreased indirect defense in the invasive tree, *Triadica sebifera*. Plant Ecology, 213(6), 945-954.

Chapman, D. S., Haynes, T., Beal, S., Essl, F. & Bullock, J. M. (2014) Phenology predicts the native and invasive range limits of common ragweed. Global Change Biology, 20(1), 192-202.

Chauvel, B., Dessaint, F., Cardinal-Legrand, C. & Bretagnolle, F. (2006) The historical spread of *Ambrosia artemisiifolia* L. in France from herbarium records. Journal of Biogeography, 33(4), 665-673.

Chown, S. L., Hodgins, K. A., Griffin, P. C., Oakeshott, J. G., Byrne, M. & Hoffmann, A. A. (2014) Biological invasions, climate change and genomics. Evolutionary Applications, 8(1), 23-46.

Chun, Y. J., Fumanal, B., Laitung, B. & Bretagnolle, F. (2010) Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. New Phytologist, 185(4), 1100-1107.

Cipollini, D. & Lieurance, D. M. (2012) Expression and costs of induced defense traits in *Alliaria petiolata*, a widespread invasive plant. Basic and Applied Ecology, 13(5), 432-440.

Cipollini, D., Mbagwu, J., Barto, K., Hillstrom, C. & Enright, S. (2005) Expression of constitutive and inducible chemical defenses in native and invasive populations of *Alliaria petiolata*. Journal of Chemical Ecology, 31(6), 1255-1267.

Colautti, R. I. & Barrett, S. C. (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science, 342(6156), 364-366.

Colautti, R. I. & Lau, J. A. (2015) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Molecular Ecology, 24(9), 1999-2017.

Colautti, R. I., Maron, J. L. & Barrett, S. C. H. (2009) Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. Evolutionary Applications, 2(2), 187-199.

Coley, P. D., Bryant, J. P. & Chapin, F. S. (1985) Resource availability and plant antiherbivore defense. Science, 230(4728), 895-899.

Colomer□Ventura, F., Martínez□Vilalta, J., Zuccarini, P., Escolà, A., Armengot, L. & Castells, E. (2015) Contemporary evolution of an invasive plant is associated with climate but not with herbivory. Functional Ecology, 29(11), 1475-1485.

Constabel, C. P. & Ryan, C. A. (1998) A survey of wound-and methyl jasmonate-induced leaf polyphenol oxidase in crop plants. Phytochemistry, 47(4), 507-511.

Cronin, J. T., Bhattarai, G. P., Allen, W. J. & Meyerson, L. A. (2015) Biogeography of a plant invasion: plant–herbivore interactions. Ecology, 96(4), 1115-1127.

Dalin, P., Ågren, J., Björkman, C., Huttunen, P. & Kärkkäinen, K. (2008) Leaf trichome formation and plant resistance to herbivory. *Induced plant resistance to herbivory* (ed A. Schaller), pp. 89-105. Springer.

De Rosario-Martinez, H. (2013) Phia: post-hoc interaction analysis. R package (version 0.1-3)

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A. & Gillette, H. D. (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Molecular Ecology, 24(9), 2095-111.

Eigenbrode, S. D., Andreas, J. E., Cripps, M. G., Ding, H., Biggam, R. C. & Schwarzländer, M. (2008) Induced chemical defenses in invasive plants: a case study with *Cynoglossum officinale* L. Biological Invasions, 10(8), 1373-1379.

Endara, M. J. & Coley, P. D. (2011) The resource availability hypothesis revisited: A meta□analysis. Functional Ecology, 25(2), 389-398.

Essl, F., Biró, K., Brandes, D., Broennimann, O., Bullock, J. M., Chapman, D. S., . . . Guisan, A. (2015) Biological flora of the British Isles: *Ambrosia artemisiifolia*. Journal of Ecology, 103(4), 1069-1098.

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M. & Facon, B. (2016) Is There a Genetic Paradox of Biological Invasion? Annual Review of Ecology, Evolution, and Systematics, 47(1), 51-72.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A. & David, P. (2006) A general eco-evolutionary framework for understanding bioinvasions. Trends in Ecology & Evolution, 21(3), 130-135.

Felker□Quinn, E., Schweitzer, J. A. & Bailey, J. K. (2013) Meta□analysis reveals evolution in invasive plant species but little support for Evolution of Increased Competitive Ability (EICA). Ecology and Evolution, 3(3), 739-751.

Fortuna, T. M., Eckert, S., Harvey, J. A., Vet, L. E., Müller, C. & Gols, R. (2014) Variation in plant defences among populations of a range□expanding plant: consequences for trophic interactions. New Phytologist, 204(4), 989-999.

Franks, S. J., Pratt, P. D., Dray, F. A. & Simms, E. L. (2008) Selection on herbivory resistance and growth rate in an invasive plant. The American Naturalist, 171(5), 678-691.

Fukano, Y. & Yahara, T. (2012) Changes in defense of an alien plant *Ambrosia artemisiifolia* before and after the invasion of a native specialist enemy *Ophraella communa*. PloS One, 7(11), e49114.

Gaudeul, M., Giraud, T., Kiss, L. & Shykoff, J. A. (2011) Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, *Ambrosia artemisiifolia*. PLoS One, 6(3), e17658.

Genton, B. J., Kotanen, P. M., Cheptou, P. O., Adolphe, C. & Shykoff, J. A. (2005) Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment. Oecologia, 146(3), 404-414.

Gerber, E., Schaffner, U., Gassmann, A., Hinz, H., Seier, M. & MÜLLER□SCHÄRER, H. (2011) Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. Weed Research, 51(6), 559-573.

Gladieux, P., Giraud, T., Kiss, L., Genton, B. J., Jonot, O. & Shykoff, J. A. (2010) Distinct invasion sources of common ragweed (*Ambrosia artemisiifolia*) in Eastern and Western Europe. Biological Invasions, 13(4), 933-944.

Gu, X., Siemann, E., Zhu, L., Gao, S., Wang, Y. & Ding, J. (2014) Invasive plant population and herbivore identity affect latex induction. Ecological Entomology, 39(1), 1-9.

Hahn, P. G. & Maron, J. L. (2016) A framework for predicting intraspecific variation in plant defense. Trends in Ecology & Evolution, 31(8), 646-656.

Hauser, M.-T. (2014) Molecular basis of natural variation and environmental control of trichome patterning. Frontiers in Plant Science, 5, 320.

Heredia, J. B. & Cisneros-Zevallos, L. (2009) The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (*Daucus carota*) under different wounding intensities. Postharvest Biology and Technology, 51(2), 242-249.

Hodgins, K. A., Bock, D. G. & Rieseberg, L. (2018) Trait Evolution in Invasive Species. Annual Plant Reviews online, 1, 1-37.

Hodgins, K. A. & Rieseberg, L. (2011) Genetic differentiation in life-history traits of introduced and native common ragweed (*Ambrosia artemisiifolia*) populations. Journal of Evolutionary Biology, 24(12), 2731-2749.

Ito, K. & Sakai, S. (2009) Optimal defense strategy against herbivory in plants: Conditions selecting for induced defense, constitutive defense, and no-defense. Journal of Theoretical Biology, 260(3), 453-459.

Joshi, J. & Vrieling, K. (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. Ecology Letters, 8(7), 704-714.

Katabuchi, M. (2015) LeafArea: an R package for rapid digital image analysis of leaf area. Ecological Research, 30(6), 1073-1077.

Kazinczi, G., Béres, I., Novák, R., Bíró, K. & Pathy, Z. (2008) Common ragweed (*Ambrosia artemisiifolia*): a review with special regards to the results in Hungary. I. Taxonomy, origin and distribution, morphology, life cycle and reproduction strategy. Herbologia, 9(1), 55-91.

Keinänen, M., Oldham, N. J. & Baldwin, I. T. (2001) Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. Journal of agricultural and food chemistry, 49(8), 3553-3558.

Kessler, A. & Baldwin, I. T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology, 53(1), 299-328.

Koricheva, J., Nykänen, H. & Gianoli, E. (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? The American Naturalist, 163(4), E64-E75.

Krishnamoorthy, K. & Lee, M. (2014) Improved tests for the equality of normal coefficients of variation. Computational Statistics, 29(1-2), 215-232.

Lande, R. (2015) Evolution of phenotypic plasticity in colonizing species. Molecular Ecology, 24(9), 2038-2045.

Lee, C. E. (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution, 17(8), 386-391.

Lee, J., Vogt, T., Schmidt, J., Parthier, B. & Löbler, M. (1997) Methyljasmonate-induced accumulation of coumaroyl conjugates in barley leaf segments. Phytochemistry, 44(4), 589-592.

Li, Z.-H., Wang, Q., Ruan, X., Pan, C.-D. & Jiang, D.-A. (2010) Phenolics and plant allelopathy. Molecules, 15(12), 8933-8952.

Lommen, S. T. E., Hallmann, C. A., Jongejans, E., Chauvel, B., Leitsch-Vitalos, M., Aleksanyan, A., . . . Müller-Schärer, H. (2017) Explaining variability in the production of seed and allergenic pollen by invasive *Ambrosia artemisiifolia* across Europe. Biological Invasions, 20(6), 1475-1491.

Macel, M., Dostálek, T., Esch, S., Bucharová, A., van Dam, N. M., Tielbörger, K., . . . Münzbergová, Z. (2017) Evolutionary responses to climate change in a range expanding plant. Oecologia, 184(2), 543-554.

Maron, J. L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos, 95(3), 361-373.

Marwick, B. & Krishnamoorth, K. (2018) cvequality: Tests for the Equality of Coefficients of Variation from Multiple Groups. R package (version 0.1.3)

Moles, A. T., Bonser, S. P., Poore, A. G., Wallis, I. R. & Foley, W. J. (2011a) Assessing the evidence for latitudinal gradients in plant defence and herbivory. Functional Ecology, 25(2), 380-388.

Moles, A. T., Wallis, I. R., Foley, W. J., Warton, D. I., Stegen, J. C., Bisigato, A. J., . . . Cornwell, W. K. (2011b) Putting plant resistance traits on the map: a test of the idea that plants are better defended at lower latitudes. New Phytologist, 191(3), 777-788.

Moreira, X., Mooney, K. A., Rasmann, S., Petry, W. K., Carrillo‐Gavilán, A., Zas, R. & Sampedro, L. (2014) Trade‐offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters, 17(5), 537-546.

Morris, W. F., Traw, M. B. & Bergelson, J. (2006) On testing for a tradeoff between constitutive and induced resistance. Oikos, 112(1), 102-110.

Muller-Scharer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. Trends in Ecology & Evolution, 19(8), 417-422.

Müller‐Schärer, H., Lommen, S. T., Rossinelli, M., Bonini, M., Boriani, M., Bosio, G. & Schaffner, U. (2014) *Ophraella communa*, the ragweed leaf beetle, has successfully landed in Europe: fortunate coincidence or threat? Weed research, 54(2), 109-119.

Neilson, E. H., Goodger, J. Q., Woodrow, I. E. & Møller, B. L. (2013) Plant chemical defense: at what cost? Trends in plant science, 18(5), 250-258.

Orians, C. M. & Ward, D. (2010) Evolution of plant defenses in nonindigenous environments. Annual Review of Entomology, 55, 439-459.

Orrock, J. L., Sih, A., Ferrari, M. C., Karban, R., Preisser, E. L., Sheriff, M. J. & Thaler, J. S. (2015) Error management in plant allocation to herbivore defense. Trends in Ecology & Evolution, 30(8), 441-445.

Oswalt, M. L. & Marshall, G. D. (2008) Ragweed as an example of worldwide allergen expansion. Allergy, Asthma and Clinical Immunology, 4(3), 130-135.

Palmer, B. & McFadyen, R. C. (2012) *Ambrosia artemisiifolia* L. - annual ragweed. *Biological control of weeds in Australia*pp. 52-59. CSIRO, Collingwood.

Parker, J. D., Torchin, M. E., Hufbauer, R. A., Lemoine, N. P., Alba, C., Blumenthal, D. M., . . . Heckman, R. W. (2013) Do invasive species perform better in their new ranges? Ecology, 94(5), 985-994.

Poorter, H. & Remkes, C. (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia, 83(4), 553-559.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M. & Lowe, A. J. (2008) Adaptive evolution in invasive species. Trends in Plant Sciences, 13(6), 288-294.

R Core Team (2017) R: a language and environment for statistical computing. (version 3.4.3 "Kite-Eating Tree")

Rasmann, S. & Agrawal, A. A. (2011) Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. Ecology Letters, 14(5), 476-483.

Rasmann, S., Agrawal, A. A., Cook, S. C. & Erwin, A. C. (2009) Cardenolides, induced responses, and interactions between above□and belowground herbivores of milkweed (*Asclepias spp.*). Ecology, 90(9), 2393-2404.

Ricciardi, A. (2007) Are modern biological invasions an unprecedented form of global change? Conservation Biology, 21(2), 329-336.

Rius, M. & Darling, J. A. (2014) How important is intraspecific genetic admixture to the success of colonising populations? Trends in Ecology & Evolution, 29(4), 233-242.

Sakihama, Y., Cohen, M. F., Grace, S. C. & Yamasaki, H. (2002) Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. Toxicology, 177(1), 67-80.

Sax, D. F. & Brown, J. H. (2000) The paradox of invasion. Global Ecology and Biogeography, 9(5), 363-371.

Scheiner, S. M. (2001) Multiple response variables and multi-species interactions. *Design and analysis of ecological experiments*pp. 99-133. Chapman & Hall, New York.

Schrieber, K., Wolf, S., Wypior, C., Höhlig, D., Hensen, I. & Lachmuth, S. (2017) Adaptive and non□adaptive evolution of trait means and genetic trait correlations for herbivory resistance and performance in an invasive plant. Oikos, 126(4), 572-582.

Sun, Y., Brönnimann, O., Roderick, G. K., Poltavsky, A., Lommen, S. T. & Müller□Schärer, H. (2017) Climatic suitability ranking of biological control candidates: a biogeographic approach for ragweed management in Europe. Ecosphere, 8(4), e01731.

Taramarcaz, P., Lambelet, C., Clot, B., Keimer, C. & Hauser, C. (2005) Ragweed (*Ambrosia*) progression and its health risks: will Switzerland resist this invasion? Swiss Medical Weekly, 135(37-38), 538-548.

Thébaud & Simberloff (2001) Are plants really larger in their introduced ranges? The American Naturalist, 157(2), 231-236.

Throop, H. L. (2005) Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. Oikos, 111(1), 91-100.

Tian, D., Tooker, J., Peiffer, M., Chung, S. H. & Felton, G. W. (2012) Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). Planta, 236(4), 1053-1066.

Uesugi, A., Connallon, T., Kessler, A. & Monro, K. (2017) Relaxation of herbivore‐mediated selection drives the evolution of genetic covariances between plant competitive and defense traits. Evolution, 71(6), 1700-1709.

Uesugi, A. & Kessler, A. (2016) Herbivore release drives parallel patterns of evolutionary divergence in invasive plant phenotypes. Journal of Ecology, 104(3), 876-886.

van Boheemen, L. A., Atwater, D. Z. & Hodgins, K. A. (2018) Rapid and repeated local adaptation to climate in an invasive plant.

van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H. & Hodgins, K. A. (2017) Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia. Molecular Ecology, 26(20), 5421-5434.

Van Noordwijk, A. J. & de Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. The American Naturalist, 128(1), 137-142.

Wang, Y., Carrillo, J., Siemann, E., Wheeler, G. S., Zhu, L., Gu, X. & Ding, J. (2013) Specificity of extrafloral nectar induction by herbivores differs among native and invasive populations of tallow tree. Annals of Botany, 112(4), 751-756.

Wang, Y., Siemann, E., Wheeler, G. S., Zhu, L., Gu, X. & Ding, J. (2012) Genetic variation in anti‐herbivore chemical defences in an invasive plant. Journal of Ecology, 100(4), 894-904.

War, A. R., Kumar Taggar, G., Hussain, B., Sachdeva Taggar, M., Nair, R. M. & Sharma, H. C. (2018) Plant Defense Against Herbivory and Insect Adaptations. AoB Plants.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S. & Sharma, H. C. (2012) Mechanisms of plant defense against insect herbivores. Plant signaling & behavior, 7(10), 1306-1320.

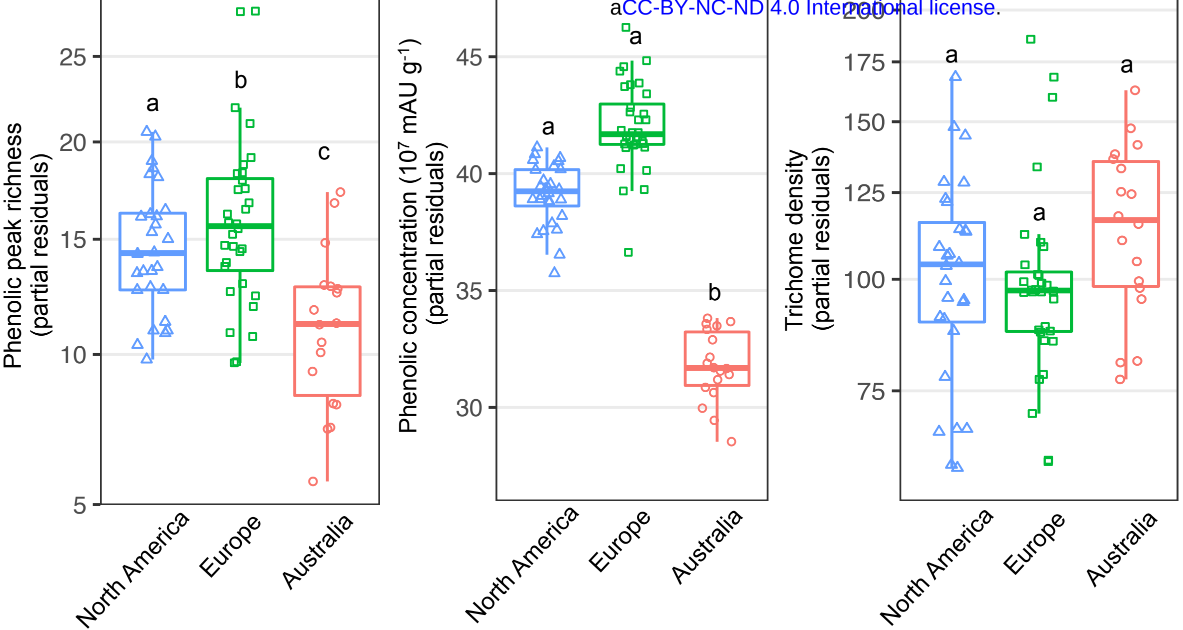
WHO, W. H. O. (1998) Global solar UV index. *Global Solar UV Index*.

Willemsen, R. W. (1975) Dormancy and germination of common ragweed seeds in the field. American Journal of Botany, 62(6), 639-643.

Woods, E. C., Hastings, A. P., Turley, N. E., Heard, S. B. & Agrawal, A. A. (2012) Adaptive geographical clines in the growth and defense of a native plant. Ecological Monographs, 82(2), 149-168.

Zandt, P. A. V. (2007) Plant defense, growth, and habitat: a comparative assessment of constitutive and induced resistance. Ecology, 88(8), 1984-1993.

Züst, T. & Agrawal, A. A. (2017) Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. Annual Review of Plant Biology, 68, 513-534.


Phenolic peak richness
(partial residuals)
25
20
15
10
5
a
b
c
North America
Europe
Australia
Phenolic concentration (10^7 mAU g^{-1})
(partial residuals)
45
40
35
30
a
a
b
North America
Europe
Australia
Trichome density
(partial residuals)
200
175
150
125
100
75
a
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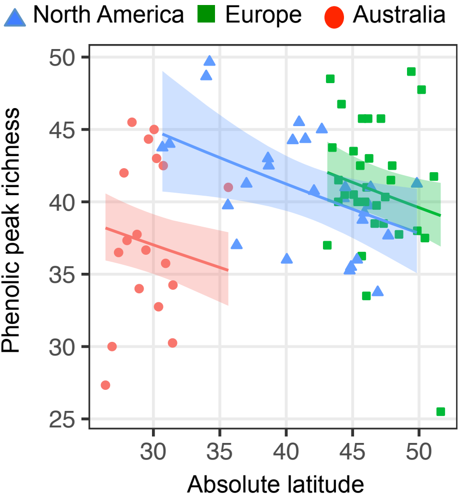
a
North America
Europe
Australia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Phenolic peak richness
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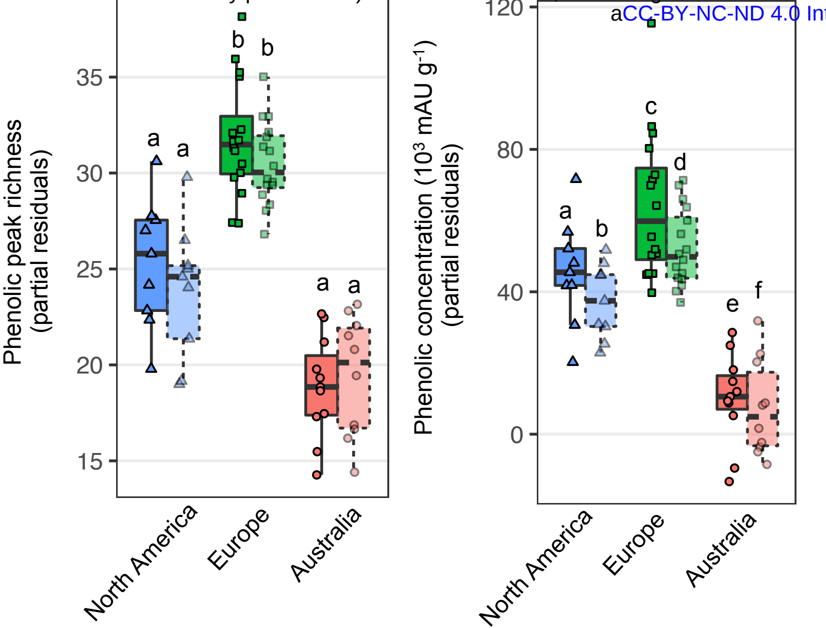
Phenolic peak richness
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35
30
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a
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North America
Europe
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Phenolic concentration (10^3 mAU g^{-1})
(partial residuals)
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80
40
0
a
b
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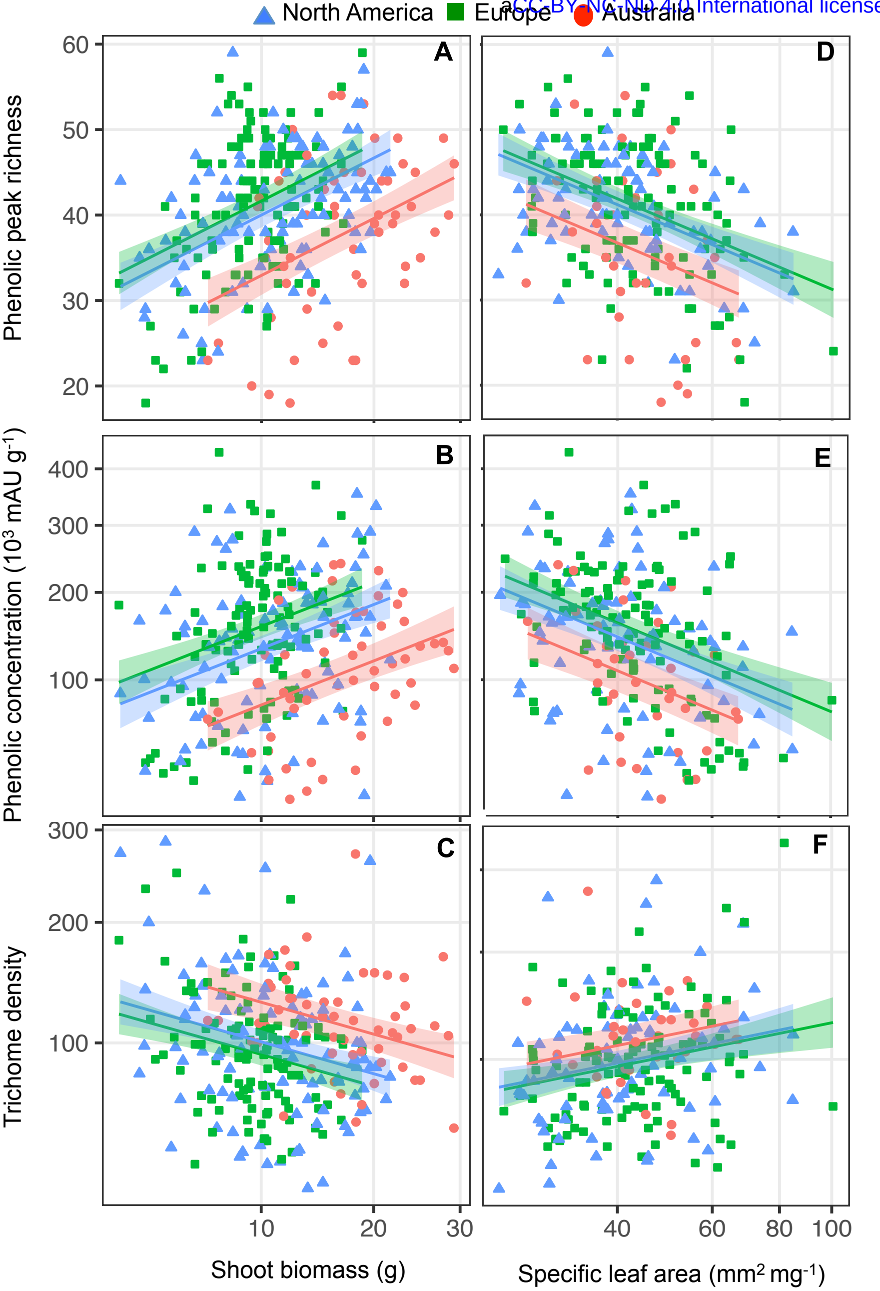
f
North America
Europe
Australia


▲ North America
■ Europe
● Australia
A
B
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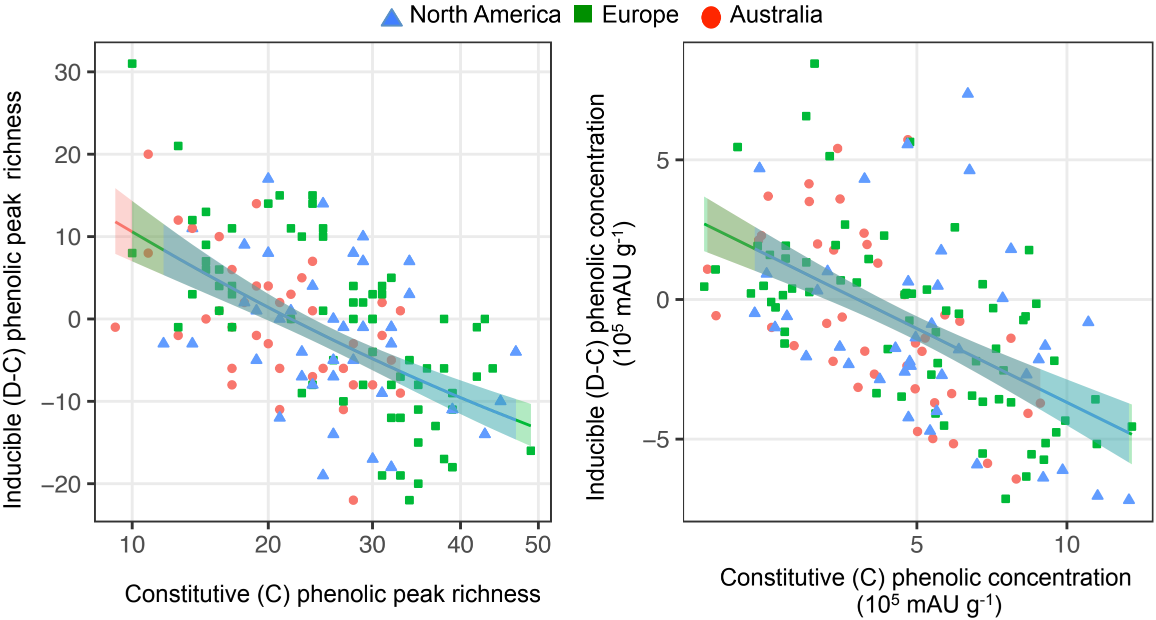
D
E
F
Phenolic peak richness
Phenolic concentration (10^3 mAU g^{-1})
Trichome density
60
50
40
30
20
400
300
200
100
300
200
100
10
20
30
40
60
80
100
Shoot biomass (g)
Specific leaf area (mm^2 mg^{-1})


North America
Europe
Australia
Inducible (D-C) phenolic peak richness
30
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0
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Constitutive (C) phenolic peak richness
Inducible (D-C) phenolic concentration (10^5 mAU g^{-1})
5
0
−5
5
10
Constitutive (C) phenolic concentration (10^5 mAU g^{-1})