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Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment

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Abstract When invading new regions exotic species may escape from some of their natural enemies. Reduced top-down control (“enemy release”) following this escape is often invoked to explain demographic expansion of invasive species and also may alter the selective regime for invasive species: reduced damage can allow resources previously allocated to defence to be reallocated to other functions like growth and reproduction. This reallocation may provide invaders with an “evolution of increased competitive ability” over natives that defend themselves against specialist enemies. We tested for enemy release and the evolution of increased competitive ability in the North American native ragweed (*Ambrosia artemisiifolia*: Asteraceae), which currently is invading France. We found evidence of enemy release in natural field populations from the invaded and native ranges. Further we carried out a reciprocal transplant experiment, comparing several life history traits of plants from two North American (Ontario and South Carolina) and one French population in four common gardens on both continents. French and Canadian plants had similar flowering phenologies, flowering earlier than plants from further south in the native range. This may suggest that invasive French plants

originated from similar latitudes to the Canadian population sampled. As with natural populations, experimental plants suffered far less herbivore damage in France than in Ontario. This difference in herbivory translated into increased growth but not into increased size or vigour. Moreover, we found that native genotypes were as damaged as invading ones in all experimental sites, suggesting no evolutionary loss of defence against herbivores.

Keywords *Ambrosia artemisiifolia* · Bioinvasion · Herbivory · Plant defence · Weed

Introduction

Worldwide trade, agriculture and many other human activities promote displacements of species beyond their native ranges. Although most of these introduced species probably fail to establish, some become invasive (Williamson 1996). They can then threaten local biodiversity, disrupt important ecosystem functions and have major economic consequences (Vitousek et al. 1997). Understanding biological invasions, with an ultimate goal of predicting and preventing them, is therefore of great importance. One hypothesis, which has gained popularity is the “enemy release” (ER) hypothesis: species escape many of their natural enemies in moving to new areas, giving them an advantage over natives, and leading to their spread (Elton 1958; Crawley 1987; Maron and Vilà 2001; Keane and Crawley 2002). Escape from control by enemies may also explain the increased vigour and size of some invasive plants (Crawley 1987 but see Thébaud and Simberloff 2001).

Invasive species may adapt to their new environments, as suggested by genetic differences between native and introduced populations (e.g. Maron et al. 2004). Indeed, many of the best examples of rapid evolution involve invasive species (Reznick and Ghalambor 2001). Not only are the migrants introduced into a new environment generally few in number, resulting in founder

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effects and increased genetic drift (e.g. Eckert et al. 1996; Amsellem et al. 2000), but they also face changes in the selective regime (e.g. Weber and Schmid 1998; Maron et al. 2004). One example of a selective change may be the relaxation in enemy pressure predicted by the ER hypothesis. It has been suggested that natural selection could favour individuals that reallocate resources from defence to other functions, resulting in better growth and competitive ability of invaders in their new ranges (the evolution of increased competitive ability, or EICA hypothesis: Blossey and Nötzold 1995). To date, good evidence exists for escape of some invaders from natural enemies (Memmott et al. 2000; Wolfe 2002; Torchin and Mitchell 2004 but see Agrawal and Kotanen 2003). Evidence for the EICA hypothesis is more equivocal. There is an evidence for reduced allocation to defence by *Spartina alterniflora* (Daehler and Strong 1997) and by *Lythrum salicaria*, though in the latter case no increase in susceptibility to herbivores results (Willis et al. 1999). In *Sapium sebiferum*, an Asian native tree invasive in the US, invasive populations produce less herbivore defence, are larger and flower more than those from the native range (Siemann and Rogers 2001) and are preferentially consumed by generalist herbivores (Siemann and Rogers 2003). Similarly, North American introduced *Silene latifolia* populations were more susceptible to fungal attack, fruit predation and aphid infestation and had a higher reproductive potential than native European ones (Wolfe et al. 2004). However, other studies have provided evidence suggesting that exotic populations do not contain larger or more competitive individuals than the native ones (Willis et al. 2000; Thébaud and Simberloff 2001; Maron et al. 2004). For example, invasive populations of *Alliaria petiolata* are more damaged than native ones by a specialist herbivore when tested in the native range (Bossdorf et al. 2004b), but are less competitive in the absence of herbivores (Bossdorf et al. 2004a). Thus although the ER and the EICA hypotheses are logical and appealing, no clear picture yet exists regarding their contribution to plant invasions.

To investigate the role of ER and EICA in the invasion of common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae) in France, we combine field surveys in 29 natural populations and transplant experiments in four common gardens in the native and in the introduced range. *A. artemisiifolia* is a North American native weed currently invading the valleys of the Rhône and Saône rivers. This species presents an interesting subject for testing these hypotheses. Indeed, it possesses elaborate systems of chemical (e.g. sesquiterpene lactones: David et al. 1999) and structural defence, which could be modified during the invasion. In addition, no other species of *Ambrosia* is native in the invaded area, making it unlikely that specialist herbivores or pathogens could shift from close relatives to this species. *A. maritima*, the only congener native to Europe, grows in the Mediterranean region.

We addressed the following questions to investigate the role of natural enemies in the invasion of this plant: (1) Are ragweed plants less damaged by herbivores and

parasites in the introduced area as the ER hypothesis predicts? (2) Is there evidence for loss of genetically determined defences against natural enemies in this plant, a basic assumption of the EICA hypothesis? Because many characters are likely to evolve over the course of an invasion, we also compared other life history traits, asking: (3) Are there genetic differences in plant life history characteristics, such as growth, final biomass and age at flowering, between the native and introduced ranges? Our experimental design allowed us to test for genetic differences among populations of origin, ecological differences among sites of the different experimental gardens and non-parallel reaction norms for plastic responses by comparing plants from the same populations of origin across the different experimental common gardens (origin \times continent of experimental site interaction effect).

Material and methods

Study species

A. artemisiifolia (Asteraceae) is a common annual native to North America. This anemophilous species is protandrous and monoecious (Bassett and Crompton 1975). It flowers in summer and starts producing mature seeds in September, which germinate from April to July (Bazzaz 1968). During the eighteenth century, the plant was cultivated in numerous botanical gardens in France (Paris, Lyon and Montpellier). The first French records of this plant in natural habitats include a species list of the Beaujolais region near Lyon from 1863 and a written record of its presence in a *Trifolium pratense* field about 150 km to the northwest of Lyon from 1865 (Anonymous 1876). Subsequently, the plant was sporadically recorded until the Second World War after which it became far more common on species lists and in herbarium collections (Bonnot 1967). More recently *A. artemisiifolia* has spread to the north into Burgundy (Carvin et al. 2003) and south into Provence (BJG, personal observation, Fig. 1). In parallel, and probably independently, this plant also invaded other parts of Europe, from Northern Italy to Russia, and parts of Asia and Australia (Igrc et al. 1995).

Ragweed is an aggressive weed causing yield losses to many crops (Clewley et al. 2001), and is particularly common and abundant in disturbed sites such as abandoned or fallow fields (Bazzaz 1968), road verges (Bassett and Crompton 1975) and construction areas. Besides its economic importance as a weed, this plant represents an important public health concern, for its pollen is highly allergenic (Harf et al. 1992).

Field survey

We compared enemy damage in introduced and native populations of *A. artemisiifolia* in the summer of 2003. We sampled 14 populations in France and 15

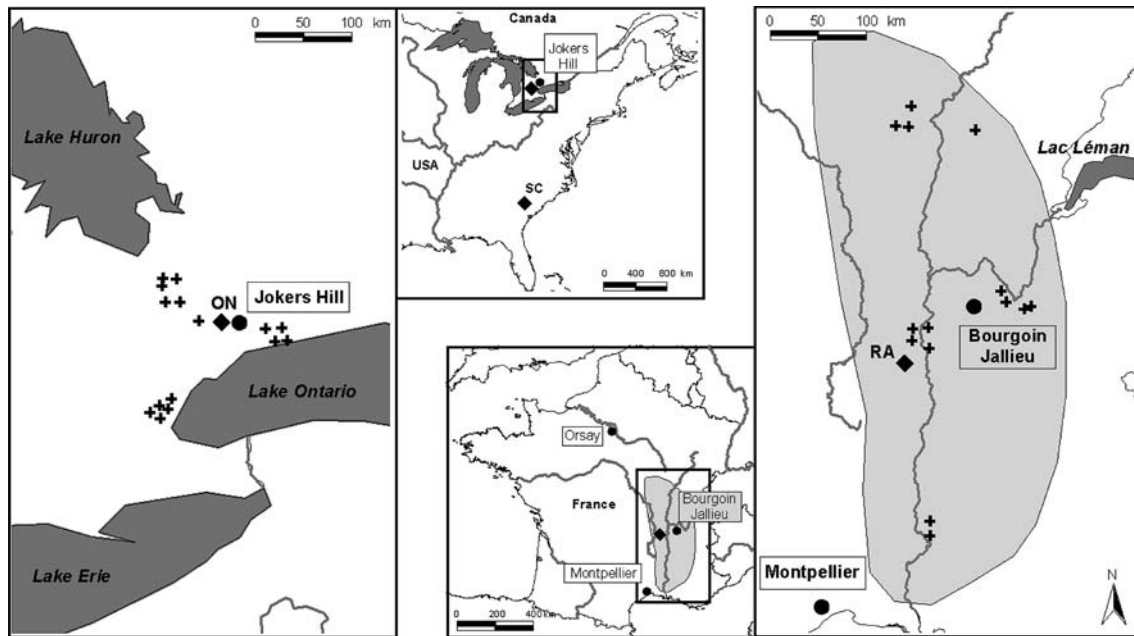


Fig. 1 Locations of natural populations surveyed (+), seed source populations in France (RA, filled diamond) and North America (SC, filled diamond and ON, filled diamond), and experimental sites

for the reciprocal transplant experiment (filled circle). Common ragweed distribution area in France is indicated in grey

populations in Ontario (Fig. 1), along rural roads and river banks. In each population 15 individuals were randomly chosen and assessed for damage. Because plants commonly reached over a metre in height with several hundreds of leaves by mid August, estimating the proportion of damaged leaves was unfeasible for such a large number of populations. Therefore we inspected each plant, selected the five most damaged leaves and noted their damage type: herbivore effects including chewing, perforation, scraping and leaf mines, and pathogen effects including a “white rust” fungus (probably *Albugo tragopogonis* [Pers.] S.F. Gray) and discolouration, which could be due to pathogen infection or perhaps abiotic stresses. From preliminary measures we knew that there was a little damage in French populations, so sampling the most damaged leaves was a conservative method for detecting a difference among the continents.

Reciprocal transplant experiment

In 2003, we conducted an intercontinental reciprocal transplant experiment with four common gardens to investigate possible evolutionary change between native and invasive populations in genetically determined defence, vigour and life history. Since French population sources are unknown and seem to be multiple (Genton et al. 2005) we used plants collected from two North American sites from the north (Ontario) and the centre (South Carolina) of its North American distribution, and plants from one site within the Rhône-Alpes region in France (Fig. 1). At each of these three sites we

collected seeds from at least ten plants. We soaked seeds from each of these mother plants in a solution of 1 mg GA3 gibberelic acid per l for 24 h and sowed them in pots filled with potting compost. Pots were placed in greenhouses at the Université Paris-Sud at Orsay and the Centre d’Ecologie Fonctionnelle et Evolutive (CEFE) at Montpellier in France, and the University of Toronto at Mississauga, Ontario, Canada. At the two to six-leaf stage, two or three seedlings per mother plant, depending on germination success, were transplanted directly to a randomly assigned point on a grid, with 1 m space between each point, at each of the four following experimental fields (Fig. 1): (1) the Koffler Scientific Reserve at Jokers Hill, near Newmarket, Ontario, where in total we planted 63 plants between 2 June and 9 July, (2) Bourgoin-Jallieu, France, with 73 plants planted between 30 May and 25 June, (3) CEFE, Montpellier, France, 61 plants between 11 June and 24 June and (4) Université Paris-Sud, Orsay, France, 57 plants between 13 June and 7 July.

The plants were watered once a week for 4 weeks. Each week, size, flowering status and herbivore damage were recorded for each plant. Because chewing and perforation damage were the most frequent types of damage recorded in natural populations on both continents (see *Results* that follow), we concentrated only on these in the reciprocal transplant experiment. On each plant we selected the five most chewed and perforated leaves and scored their level of chewing or perforation on a scale ranging from 0 (no damage) to 3 (more than half the leaf surface affected). These weekly leaf scores were summed within plants to generate weekly chewing and perforation scores. All invertebrate herbivores

present on the plants were collected and identified. The sites at Orsay and Bourgoin-Jallieu were managed by the same person but different observers collected data at each of the other sites. The use of transplant experiments in the study of invasions, though necessary for understanding their mechanisms, is rife with ethical problems. Translocations may reinforce or alter the genetic composition of introduced as well as native populations through genetic pollution. Here we reduced these risks by destructively sampling this annual plant before it completed its reproductive cycle. Although this approach somewhat restricts the scope of our experiments, we believe it was required for ethical and environmental reasons. Plants were thus harvested just before flowering in Bourgoin-Jallieu where naturalised populations exist around our field site. In Orsay and Montpellier, where no wild populations are known within the immediate vicinity, the plants were allowed to flower, but were harvested before the seed setting to prevent seed contamination. Plants were harvested on 1 October 2003 in Montpellier and on 3 September 2003 in Orsay. In Ontario plants were harvested on 6 September 2003, also before seed set. Because these are annuals that store no resources for survival or subsequent growth, only above-ground biomass were assessed, drying and weighing aerial plant parts after harvest.

Analyses

To compare the intensity of damage between natural populations on the two continents generalised linear mixed models were used as implemented by the GLIMMIX macro (Wolfinger 1998) in SAS version 8 (SAS Institute 2000). A binomial distribution and a logit link function were used since data represent number of damaged leaves out of five. Of the explanatory variables, continent was considered as a fixed effect and population (nested within continent) as a random effect. To analyse data from the transplant experiment, mixed models were also employed, with origin of the seeds and continent of the experimental field as fixed effects, and mother plants (nested within origins) and experimental field sites (nested within continents) as random effects. This last effect was only relevant for the French sites. Because weekly measures (damage scores and plant height) are not independent, the plant was treated as a repeated measurement factor and the age of the plant (in days since germination for the analysis of height and in days since transplantation for the analysis of damage scores) as a covariate. Growth data were only considered until flowering. To investigate whether damage influenced plant growth we also included a total damage score (sum of chewing and perforation scores) as a covariate in the analysis of plant height. To compare final biomass, age of the plant at harvest (in days since germination) was also considered as a covariate. We tested for all possible interactions between fixed effects with stepwise elimination of non-significant interactions.

Chewing damage was analysed with a Poisson distribution and a log link function, whereas for perforation damage a binomial distribution with a logit link function fit the data better (GLIMMIX macro). Since height and biomass data were continuous we assume a normal error distribution (general linear mixed models, PROC MIXED, SAS Institute 2000). To stabilise the variance of the residuals, heights and biomasses were square-root transformed. For age at male flowering (in days since germination), we compared origins, continents of the experimental field, their interaction and sites (nested within continent—only relevant for the French sites). Since plants originating from South Carolina did not flower at two of the sites they were not included in this analysis. A few plants from other origins had not flowered by harvest date, so we used survival analyses that are designed for time—truncated data by fitting models with the PROC LIFEREG in SAS version 8 (SAS Institute 2000) considering a Weibull distribution. However, it is not possible with such models to stipulate that an effect is random. Age at female flowering was only available for the plants growing at Jokers Hill and Montpellier because these completed flowering before harvest. This was analysed as earlier but without the continent term.

Results

Herbivore and pathogen damage

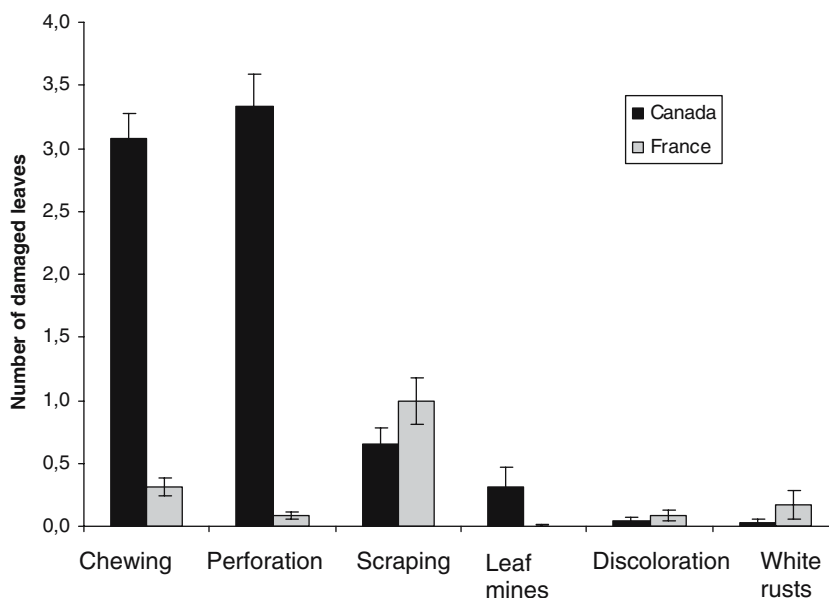
Our surveys of natural enemy damage revealed that natural populations in Canada suffered more damage than those in France. Canadian populations were far more attacked by herbivores than were French populations, whereas no differences were found for pathogen damage. Chewing and perforation were by far the most common forms of damage observed in Canada, greatly exceeding any other type of damage at any location. These types of damage as well as leaf mines were much more common in Canada than in France, while frequencies of other types of damage (scraping, discolouration and white rust) were not significantly different between the two continents. Populations varied for all measures of damage (Table 1, Fig. 2).

Damage levels for chewing and perforation in the transplant experiment were also far greater at the Canadian site than at the French ones. Chewing damage was more frequent in Canada than in France but did not significantly differ among seed origins, sites or mother plants. No interaction between origin and continent was found (Table 2, Fig. 3). Data clearly showed more perforation damage in Canada than France, though this effect was marginally non-significant (Table 2), likely because of a significant interaction between continent and origin. Comparisons of least square mean estimates (lsmeans option in SAS) revealed that, no difference was significant in France whereas, there was a difference among origins in

Table 1 Differences in damage caused by herbivores and pathogens in natural populations of France and Canada

Response variables	<i>df</i>	<i>F</i> value	<i>P</i> value	Direction of difference
Chewing damage	1,27	116.04	<.0001	C > F
Perforation	1,27	114.37	<.0001	C > F
Scraping damage	1,27	2.26	0.14	
Leaf mines	1,27	8.28	0.0077	C > F
Discoloration	1,27	1.51	0.2	
White rust	1,27	0.27	0.6	

Presence of herbivore damage on five leaves was analysed using generalised linear mixed models with logit link function and binomial distribution (GLIMMIX macro in SAS). Populations, defined as a random effect in the models, were heterogeneous for all damage types (all *P* values < 0.01). *C* Canada, *F* France

Fig. 2 Mean (\pm SE) number of damaged leaves, separated by type of damage, recorded on the five most damaged leaves in natural populations in France and Canada**Table 2** Differences in chewing and perforation damage caused by herbivores in the reciprocal transplant experiment

Variables and fixed effects	<i>df</i>	<i>F</i>	<i>P</i>	Direction of difference (least square means)
Chewing score				
Origin	2,26	0.46	0.6	
Continent	1,2	44.89	0.022	C > F
Origin \times Continent	2,224	0.08	0.9	
Age (in days since transplantation)	1,600	34.92	<.0001	+
Perforation score				
Origin	2,26	1.64	0.2	
Continent	1,2	13.40	0.067	C > F
Origin \times Continent	2,224	7.20	0.0009	F: No diff; C: SC > (ON, RA)
Age (in days since transplantation)	1,600	234.8	<.0001	+

Data were analysed using generalised linear mixed models (GLIMMIX macro in SAS) with log link function and Poisson distribution for chewing damage and with logit link function and binomial distribution for perforations; plant was defined as a repeated factor. The random site effect was not significant (*P* values > 0.2) while mother plant as a random effect was significant for perforation (*P* = 0.015), but not for chewing (*P* = 0.28).

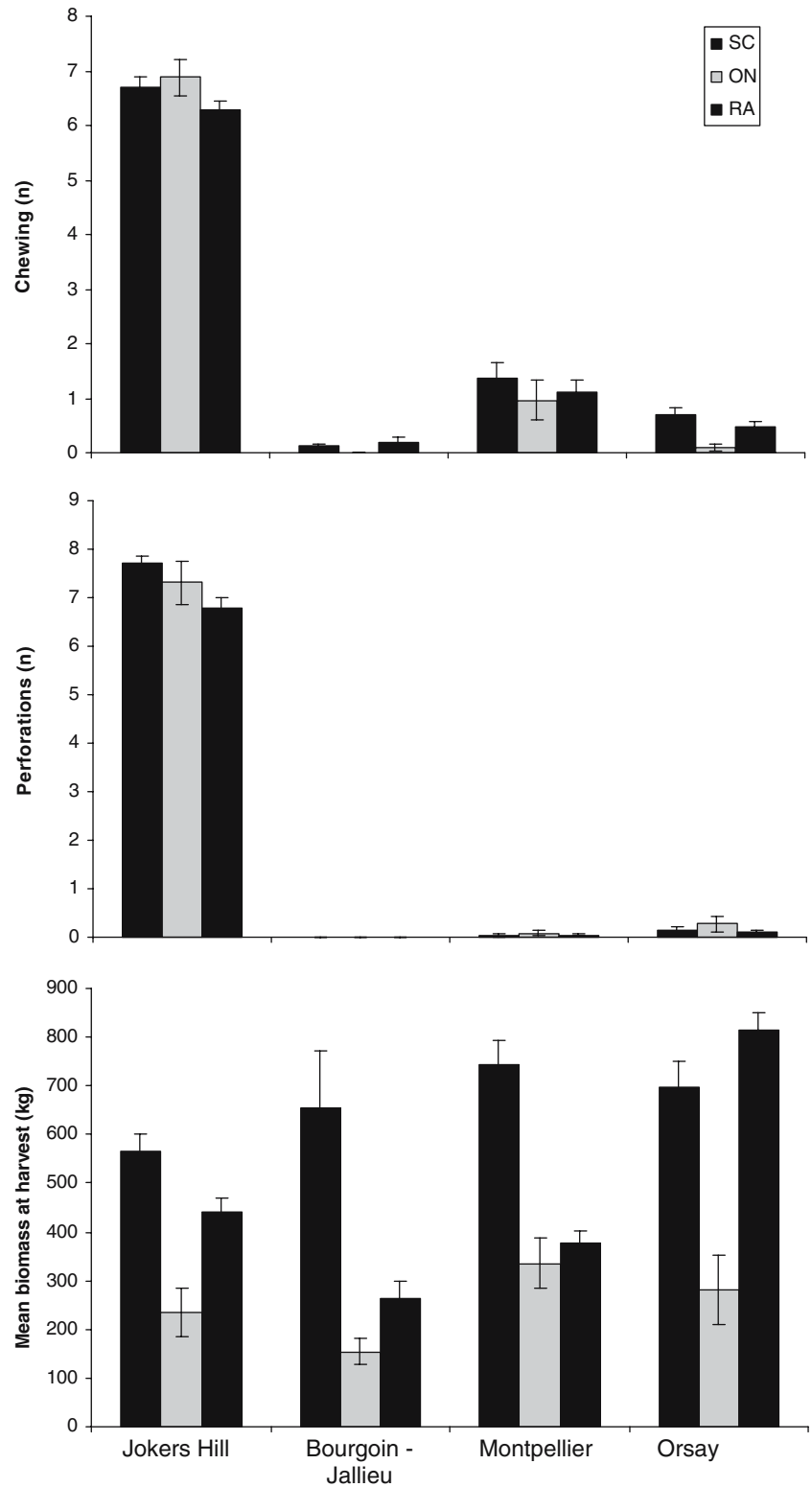
C Canada, *F* France, *SC* South Carolina, *ON* Ontario, *RA* Rhône-Alpes, *No diff* No difference

Canada: plants from South Carolina were more perforated than plants from Ontario or Rhône-Alpes. Perforation level differed significantly among mother plants, but not among sites. Finally, chewing and

perforation damage increased with time from transplantation (Table 2).

In the transplant experiment, a limited variety of insects, mostly aphids and grasshoppers, were found on

Fig. 3 Mean (\pm SE) over all weeks of (a) chewing and (b) perforation scores (potentially ranging from 0 to 15). (c) Mean (\pm SE) of biomass of plants originated from South Carolina (SC), Ontario (ON) and Rhône-Alpes (RA) in the four sites of the reciprocal transplant experiment: Jokers Hill in Canada, Bourgoin-Jallieu, Montpellier and Orsay in three different regions of France



the plants at the French sites, whereas, at the Canadian site, we recorded the presence of many herbivores known to attack ragweed including *Systema blanda* (Coleoptera: Chrysomelidae), *Corythucha* (Heteroptera: Tingidae) and *Tarachidia* (Lepidoptera: Noctuidae).

Life history characters

Table 3 and Fig. 3 summarise results on plant size. Briefly, plants grown in France did not differ in size from plants grown in Ontario but plant size varied with

Table 3 Differences in height/growth and final biomass in the reciprocal transplant experiment

Variables and fixed effects	<i>df</i>	<i>F</i>	<i>P</i>	Direction of difference (least square means)
Square root (Height)				
Origin	2,27	4.87	0.016	SC > RA > ON
Continent	1,2	8.01	0.11	
Origin × Continent	2,253	3.37	0.036	F: No diff; C: SC > (RA, ON)
Total damage score	1,1323	6.24	0.013	–
Age (in days since germination)	1,1323	4076	< .0001	+
Age × Origin	2,1323	15.87	< .0001	SC > RA > ON
Age × Continent	1,1323	134	< .0001	F > C
Square root (Biomass)				
Origin	2,27	14.92	< .0001	SC > RA > ON
Continent	1,2	0.03	0.9	
Origin × Continent	2,201	4.94	0.008	F: RA > ON; C: SC > RA > ON
Age (in days since germination)	1,201	125	< .0001	+
Age × Origin	2,201	15.71	< .0001	SC > ON > RA

Data were analysed using general linear mixed models (proc MIXED in SAS), with site and mother as random effects, and plant as a repeated factor. Site was not significant (P values > 0.16), while mother was significant for height ($P = 0.002$), but not estimable for biomass.

C Canada, F France, SC South-Carolina, ON Ontario, RA Rhône-Alpes

site of origin, with plants from South Carolina being tallest and with greatest biomass at harvest and those from Ontario smallest. We found a significant interaction between continent and origin, with plants from Rhône-Alpes being smallest when grown in Ontario but largest when grown in France. Overall there was a negative relationship between height and intensity of damage. The rate of increasing height and biomass with plant age differed among the populations of origin, with plants from South Carolina showing most rapid growth rate for both characters. Rate of increase in plant height also differed between continents, being more rapid in France than Canada (Table 3, Fig. 3).

Plants from South Carolina flowered much later than those of French and Canadian origin. Indeed they failed to flower at the Jokers Hill and Orsay sites before these were harvested (Fig. 4). Survival analyses on plants of French and Canadian origin revealed no difference between continents of experimental fields or seed origins in age at first flowering (first male inflorescences). Plants flowered at about the same age at the Jokers Hill and the Montpellier sites, whereas plants at Bourgoin-Jallieu and Orsay flowered somewhat later (significant site effect; Table 4, Fig. 4). Further, female flowering occurred at a more advanced age for plants of French origin than those of Canadian origin, and for plants in Montpellier than in Canada (Table 4, Fig. 4).

Discussion

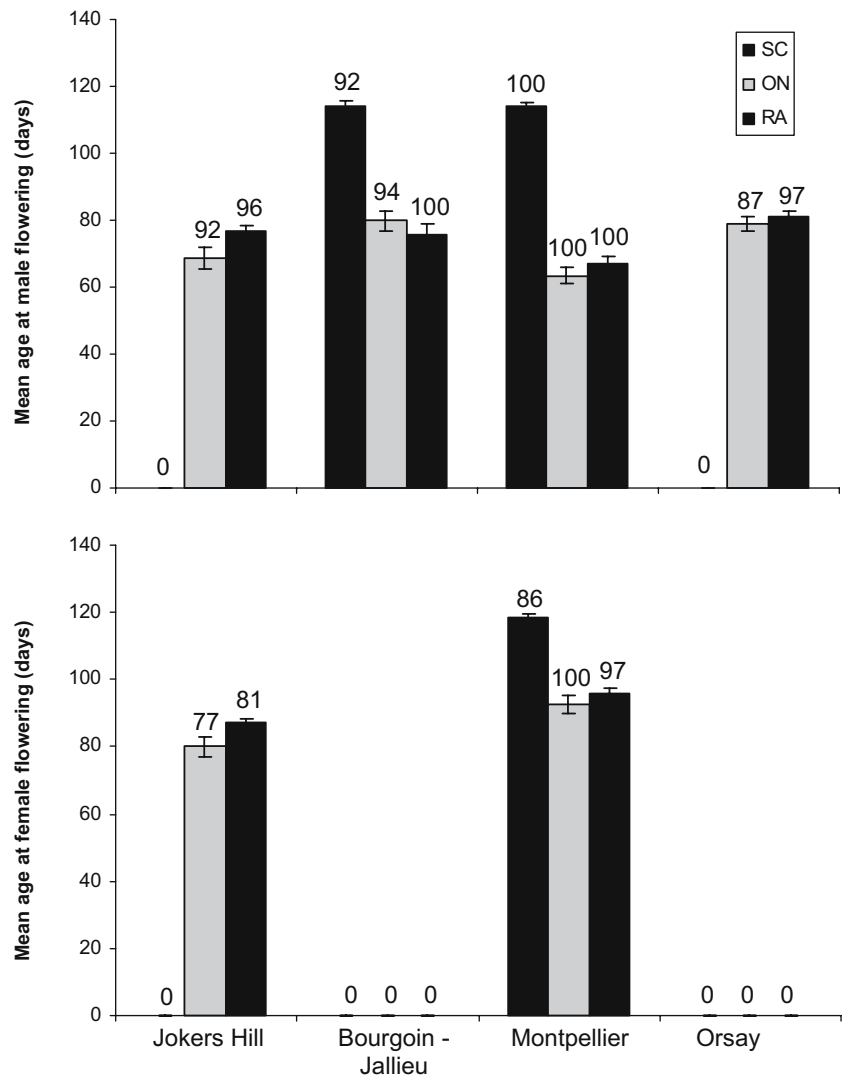
Evidence for enemy release

Damage caused by leaf herbivores in both natural and experimental populations was far greater in Canada, the native range, than in France, an introduced area for this plant (Figs. 2, 3). We recorded numerous individuals of

several North American herbivore species that are known to prefer ragweed (primarily *Systema blanda* and *Tarachidia* sp.) at the Canadian experimental site, whereas fewer species of herbivores were observed at the French sites. Thus it appears that the introduced populations of ragweed in France have indeed escaped from oligophagous herbivores, as has been noted for other plant invasions (Memmott et al. 2000; Wolfe 2002; DeWalt et al. 2004). However, in contrast to general patterns observed for both plants and animals (Torchin and Mitchell 2004), pathogen damage was overall very low and did not differ between France and Canada (Fig. 2). This could be explained by (1) presence of generalist pathogens able to infect *A. artemisiifolia* in France, (2) transport of systemic pathogens in ragweed seeds, or (3) a switch of specialist pathogens from European relatives such as *Artemisia* or other Asteraceae (e.g. *Albugo tragopogonis* attacks numerous other Asteraceae).

The greatly reduced rates of herbivore attack suggest that ER could play a role in invasion of France. Although reductions in damage do not necessarily translate directly into individual, population or community-level consequences (Keane and Crawley 2002), it was indeed found that invading individuals gained an advantage by escaping herbivores. Indeed, severely damaged plants were shorter overall, and herbivore escape may explain why plants gained height faster at experimental sites in France than in Canada. However, this benefit in growth rate in France did not translate into an increase in biomass, which we expect to be a good predictor of seed production in this annual plant. Moreover, populations must be “top-down”-regulated for ER to result in population increase (Maron and Vilà 2001). Although classical biological control does not provide a rigorous test of the ER hypothesis (Keane and Crawley 2002; Colautti et al. 2004), it is still suggestive

Fig. 4 Mean (\pm SE) (a) male and (b) female flowering age (time from germination to flowering) of plants from South Carolina (SC), Ontario (ON) and Rhône-Alpes region (RA) in the four experimental sites: Jokers Hill in Canada, Bourgoin-Jallieu, Montpellier and Orsay in France. Numbers represent the percentage of plants that flowered before harvest



that control attempts of *A. artemisiifolia* by the beetle *Zygogramma suturalis* F. in Russia, Croatia and China failed (Reznik 2000), despite optimistic beginnings (Igrc et al. 1995).

Differences in vigour

Two findings on plant growth and vigour are noteworthy. First, South Carolina plants grew faster and

Table 4 Age at male and female flowering in the reciprocal transplant experiment

Variables and sources of variation	df	χ^2	P	Direction of difference
Age at male flowering				
Origin	1	1.44	0.23	
Continent	1	0.078	0.8	
Origin \times Continent	1	0.072	0.8	
Site (Continent).	2	39.72	< .0001	
Age at female flowering				
Origin	1	10.54	0.0012	RA > ON
Site	1	10.22	0.0014	Montp > JH
Origin \times Site	1	1.36	0.2	

Data were analysed using PROC LIFEREG with a Weibull distribution for the error term (survival analyses). Data for plants of SC origin were excluded because they failed to flower at two sites. Age at female flowering was compared only between Montpellier and Jokers Hill because plants were harvested before female flowering at the two other sites. C Canada, F France, SC South Carolina, ON Ontario, RA Rhône Alpes, Montp Montpellier, JH Jokers Hill (Ontario)

attained greater height and biomass than either the French or Canadian plants, especially when grown in Canada. These differences are probably due to life history differences, with South Carolina plants inducing flowering significantly later and thereby growing for an additional month before diverting meristems to flowering. Second, considering French and Canadian plants that flowered at approximately the same age, French plants grew faster and achieved significantly greater biomass than the Canadian plants, consistent with the common finding that invasive plants are larger than plants from their native area (Siemann and Rogers 2001; Wolfe et al. 2004; but see Willis et al. 2000).

No evidence for evolution in defence against leaf herbivores

Invasive species can lose defences as predicted by the EICA hypothesis (Siemann and Rogers 2001; Wolfe et al. 2004). This is consistent with optimal defence theory, which predicts that exotic plants should diminish their allocation to costly resistance (Strauss et al. 2002). Since few ragweed herbivores were found in France we expected French populations to have lost resistance, which would have shown up as a significant origin by continent of experimental site interaction in the reciprocal transplant experiment. No such interaction was detected, however. French plants did not suffer more damage than North American plants in the Canadian experimental site (Fig. 2), indicating no decrease in defence against herbivores (Willis et al. 1999; Maron et al. 2004). The EICA mechanism, with resource reallocation from defence to growth or reproduction, is thus unlikely to have contributed greatly to the successful invasion of France. Loss of defence may not be selected if (1) defences are not costly, or (2) other selective pressures maintain these mechanisms in the invaded environment. Indeed, *A. artemisiifolia* produces toxic secondary plant metabolites (sesquiterpene lactones: David et al. 1999) whose direct associated costs have only been shown in few studies (see qualitative defence in Müller-Schärer et al. 2004). If these toxins deter generalists, they might be maintained or even increase (if they also act as attractants for specialists) in new areas where specialists are lacking but generalists are present (Müller-Schärer et al. 2004). No differences in herbivore damage between plants from different origins were detected in the French sites of the reciprocal transplant experiment, suggesting that invasive French plants maintained defence against generalists (but also that they were not better defended). Finally, sesquiterpene lactones that have phytotoxic properties (Kelsey and Locken 1987) are also involved in ragweed allelopathy (Béres et al. 1998). For all of these reasons reallocation of resources from at least this class of defences to growth and reproduction may not have been selected in *A. artemisiifolia*. Furthermore, even though *A. artemisiifolia* was sporadically recorded from France for over 100 years most populations may derive

from recent introductions and thus may not have had sufficient time to lose defence (Keane and Crawley 2002).

Tolerance is also an important response to attack by herbivores (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999). In the reciprocal transplant experiment, we detected no significant interaction between damage and origin in explaining variation in plant height, suggesting that tolerance has not decreased during the invasion process. Abiotic stress and disturbance factors can lead to the maintenance of tolerance (Rosenthal and Kotanen 1994; Müller-Schärer et al. 2004) and tolerance traits may also directly increase competitive ability of plants even in the absence of herbivory (Strauss and Agrawal 1999). Moreover, costs associated with tolerance are difficult to estimate; to date there is no clear evidence that tolerance is actually a costly strategy (Strauss and Agrawal 1999).

Phenological adaptation to local conditions

A. artemisiifolia is a short-day species (Deen et al. 1998), initiating flowering with decreasing day length in July in the northern hemisphere. Site effects are consistent with the idea that flowering induction is associated with a threshold day length, with flowering occurring earlier at the two southernmost sites (Jokers Hill and Montpellier) and significantly later at the more northern sites (Bourgoin-Jallieu and Orsay). Furthermore, plants differed in behaviour across site of origin, with plants from South Carolina producing male and female flowers when older than plants from Canada or France. Adaptation to local conditions could explain the timing of flowering of South Carolina plants. Day length in summer is much shorter in South Carolina than at our experimental sites in Canada and France. South Carolina plants should thus be adapted to induce flowering at a shorter day length, and therefore wait until this short day threshold has been reached when they are grown under northern conditions.

Similar flowering behaviour of plants from French and Canadian origins could be due to adaptation to similar latitudinal and hence photoperiodic conditions as has been observed in other invasive species (Maron et al. 2004; Weber and Schmid 1998). Alternatively, the current French populations may have originated from populations at similar latitudes in southern Canada or the northern US, and appropriate flowering behaviour may have been a prerequisite for successful invasion.

Conclusion

In summary, common ragweed was far less attacked by herbivores in France than in Canada. Lower rates of attack partly explain the higher growth rate of plants in France, though they may not translate into an advantage at the population level. Despite reduced enemy

pressure in France no evidence for loss of defence was found in introduced French *A. artemisiifolia* populations. Differences in height or biomass detected in common gardens between plants of different origins were mainly due to phenological patterns. However, French plants produced higher biomass than the Canadian plants did even though they were phenologically similar. Similar phenologies may suggest that the source of the French invasion was from the same geographic region that supplied the Canadian seeds (Genton et al. 2005). In this case, the Canadian population that provided seeds for the common garden experiment would represent the appropriate comparison for post invasion populations, which then appear to have increased in vigour without accompanying loss of defence. Alternatively, French populations of *A. artemisiifolia* may have multiple origins, as suggested by the pattern of genetic variation of *A. artemisiifolia* in France (Genton et al. 2005). In this case, phenological similarity between French and Canadian populations may suggest post-invasion evolution in France.

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