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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 29 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 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 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 exotic populations do not contain larger or more competitive individuals than 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 Enemy Release 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?

MATERIAL AND METHODS

Study species

Ambrosia 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 June (Bazzaz 1968).

During the 18th 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 150km to the north west 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, pers. 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 such as sunflower, 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 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 meter in height with several hundreds of leaves by mid August, estimating the proportion of damaged leaves as was done in 2002 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 “tar spot” fungus, a “white rust” fungus (probably *Albugo tragopogonis* (Pers.) S.F. Gray), and discoloration, which could be due to pathogen infection and perhaps abiotic stresses. From preliminary measures in 2002 we knew that there was little damage in French populations so sampling the most damaged leaf was a conservative method for detecting a difference between the continents, while still allowing us to compare differences among genotypes.

Reciprocal transplant experiment

In 2003, we conducted an inter-continental 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 (BJ Genton and T Giraud, unpublished data) 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 10 plants. We soaked seeds from each of these mother plants in a solution of 1mg.L^{-1} GA3 gibberelic acid for 24h 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 2- to 6-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 1m 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 four 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 below) we concentrated only on these in the reciprocal transplant

experiment. On each plant we selected the 5 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, we let the plants flower, but harvested them before seed set 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, we assessed only above-ground biomass, drying and weighing aerial plant parts after harvest.

Analyses

To compare the intensity of damage between natural populations on the two continents we used generalised linear mixed models as implemented by the GLIMMIX macro (Wolfinger 1998) in SAS version 8 (SAS Institute 2000). We used a binomial distribution and a logit link function since data represent number of damaged leaves out of 5. 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, we also employed mixed models, 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, we treated plant 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 above 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, tar spots, discoloration 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. 3a). 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, whereas no difference was significant in France, there was a difference among origins in 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 the plants at the French sites, whereas, at the Canadian site, we recorded the presence of many herbivores known to attack ragweed including *Systema* (Coleoptera: Chrysomelidae), *Corythucha* (Heteroptera: Tingidae), *Tarachidia* (Lepidoptera: Noctuidae), and some Curculionidae (Coleoptera).

Life history characters

Plants grown in France did not differ in height from plants grown in Ontario. Even if origin seemed to be a significant effect, pair-wise differences of least square means revealed no significant difference between plants from South Carolina, the tallest plants, plants from Rhône-Alpes or plants from Ontario, the smallest ones. There was a significant interaction between continent and origin, with plants from Rhône-Alpes being the smallest plants when grown in Ontario and the tallest when grown in France, as well as a strong negative relationship between height and intensity of damage. Though we found no interaction between damage and continent or seed origin, we did find significant origin by age and continent by age interactions, indicating that the increase of height with increasing age (i.e. the growth rate) differed significantly among origins and between continents. Plants from South Carolina grew more rapidly than French plants, that in turn grew more rapidly than plants from Ontario. Finally, plants grew more quickly in France than in Canada (Table 3).

South Carolina plants had greater biomass at harvest than French plants that were more vigorous than Ontario plants (Table 3, Fig. 3c). A significant interaction between continent of experimental field and seed origin revealed that French plants were the most vigorous in France followed by South Carolina plants, while South Carolina plants were the most vigorous in the Canadian experimental field. Age at harvest influenced biomass, with older plants weighting more. We found a significant interaction between age and seed origin, with South Carolina plants increasing in biomass more rapidly than did French plants, which increased in biomass more rapidly than Ontario plants.

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 (Fig. 2 and 3a-b). We recorded numerous individuals of several North American herbivore species that are known to prefer ragweed (primarily *Systema* cf. *elongata* 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), we detected no differences in pathogen-induced damage 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 that we documented suggest enemy release 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), we did find that invading individuals gained an advantage by escaping herbivores. Indeed, height was reduced in more severely damaged plants; this may explain why plants in France grew faster than plants 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 E. R. 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 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

Vigour differed among populations of origin, with South Carolina plants growing faster and attaining greater heights and biomasses than both French and 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. However, French plants grew faster and achieved significantly greater biomass than did Canadian plants although they flowered at the same age. This increased vigour of invasive genotypes is consistent with the common finding that invasive plants are larger than plants from their native area (Crawley 1987 but see Thébaud and Simberloff 2001). Still, the difference in biomass between French and Canadian plants again could be partly due to the fact that not all Canadian plants flowered before harvest and were thus still growing: this difference was the highest in the site of Orsay where the percentage of Canadian plants that flowered is the lowest whereas this difference was not significant in Montpellier where all plants flowered (Fig. 2c). Consequently, these results do not convincingly demonstrate a growth advantage in introduced populations.

No evidence for evolution in defence against leaf herbivores

Some studies have indicated that invasive species can lose defences as predicted by the EICA hypothesis. Examples include reduced tannin production in the invasive species *Sapium sebiferum* in North America (Siemann and Rogers 2001), reduced herbivore resistance in introduced populations of *Spartina alterniflora* (Daehler and Strong 1997), and higher susceptibility to fungal infection, fruit predation and aphid infestation in *Silene latifolia* (Wolfe et al. 2004). These results are consistent with optimal defence theory which

predicts that exotic plants should diminish their allocation to defence because herbivore resistance is costly (Strauss et al. 2002).

Since few ragweed herbivores were found in France, we expected a decrease in resistance in French populations as predicted by the EICA hypothesis. However, French plants suffered no 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) if 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 in fact be expected to 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 defences 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 at a more advanced age than plants from Canada or France, starting flowering when the latter two were already finishing. Adaptation to local conditions could explain the timing of flowering of South Carolina plants. Because of latitudinal differences (around 34.0° in Columbia, South Carolina; 43.6° in Montpellier, France; 44.0° at Newmarket, Ontario; 45.6° in Bourgoin-Jallieu, France and 48.7° in Orsay, France) day length in summer is 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; Weher 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 we found no evidence for loss of defence 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 did Canadian plants 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 our Canadian seeds. In this case the Canadian population that provided seeds for our 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 (B Genton and T Giraud, unpublished data). In this case phenological similarity between French and Canadian populations may suggest post-invasion evolution in France.

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Table 1. Differences in damage caused by herbivores and pathogens in natural populations of France and Canada. Data were 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 and F: France.

Response variables	df	<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
Tar spots	1, 27	0.11	0.7	
Discoloration	1, 27	1.51	0.2	
White rust	1, 27	0.27	0.6	

Table 2. Differences in chewing and perforation damage caused by herbivores in the reciprocal transplant experiment. 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 and, No diff: No difference.

Variables and fixed effects	df	<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 x 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 x Continent	2, 224	7.20	0.0009	F: No diff; C: SC>(ON,RA)
Age (in days since transplantation)	1, 600	234.8	<.0001	+

Table 3. Differences in height/growth and final biomass in the reciprocal transplant experiment. 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 and RA: Rhône-Alpes.

Variables and fixed effects	df	F	P	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 x 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 x Origin	2, 1323	15.87	<.0001	SC > RA > ON
Age x 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 x Continent	2, 201	4.94	0.008	F: RA > ON; C: SC > RA > ON
Age (in days since germination)	1, 201	125	<.0001	+
Age x Origin	2, 201	15.71	<.0001	SC > ON > RA

Table 4. Age at male and female flowering in the reciprocal transplant experiment. 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 and JH: Jokers Hill (Ontario).

Variables and sources of variation	df	ChiSq	<i>P</i>	Direction of difference
Age at male flowering				
Origin	1	1.44	0.23	
Continent	1	0.078	0.8	
Origin x 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 x Site	1	1.36	0.2	

Figure 1. Locations of natural populations surveyed, experimental fields for the reciprocal transplant experiment, and seed source populations in France and North America.

Figure 2. Number of damaged leaves, separated by type of damage, recorded on the five most damaged leaves in natural populations in France and Canada. 10, 25, 50, 75, 90th percentiles are represented

Figure 3. Mean ($\pm$ s.e.) over all weeks of (a) chewing and (b) perforation scores (potentially ranging from 0 to 15). (c) Mean ($\pm$ s.e.) 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.

Figure 4. Mean ($\pm$ s.e.) (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.