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2 **The effects of disturbance and enemy exclusion on performance of**
3 **an invasive species, common ragweed, in its native range**

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Abstract

Common ragweed (*Ambrosia artemisiifolia*) is an abundant weed in its native North America, despite supporting a wide range of natural enemies. Here, we tested whether these enemies have significant impacts on the performance of this plant in its native range. We excluded enemies from the three principal life-history stages (seed, seedling and adult) of this annual in a series of field experiments; at the adult stage, we also manipulated soil disturbance and conspecific density. We then measured the consequences of these treatments for growth, survival, and reproduction.

Excluding fungi and vertebrate granivores from seeds on the soil surface did not increase germination relative to control plots. Seedling survivorship was only slightly increased by the exclusion of molluscs and other herbivores. Insecticide reduced damage to leaves of adult plants, but did not improve growth or reproduction. Growth and survivorship of adults were strongly increased by disturbance, while higher conspecific density reduced performance in disturbed plots.

These results indicate ragweed is insensitive to attack by many of its natural enemies, helping to explain its native-range success. As well, they suggest that even though ragweed lost most of its insect folivores while invading Europe, escape from these enemies is unlikely to have provided a significant demographic advantage; instead, disturbance is likely to have been a much more important factor in its invasion. Escape from enemies should not be assumed to explain the success of exotic species unless improved performance also can be demonstrated; native-range studies can help achieve this goal.

Key words: *Ambrosia artemisiifolia*, biological invasions, disturbance, Enemy Release Hypothesis, natural enemies

Introduction

Natural enemies have the potential to control populations of plants (Maron and Crone 2006); nonetheless, attack by enemies often fails to prevent weedy species from reaching problem levels. One such example is provided by common ragweed (*Ambrosia artemisiifolia*, hereafter "ragweed" or "*Ambrosia*"). This North American annual is highly abundant in its native range, and is considered a severe weed of agricultural and urban areas as well as a principal source of pollen allergies (Bassett and Teresmae 1962; Bassett and Crompton 1975; Teshler et al. 2002; Ziska et al. 2007), despite supporting a rich herbivore community including both generalists and specialists (Futuyma and McCafferty 1990; Funk *et al.* 1995; Teshler *et al.* 2002; MacKay and Kotanen 2008). These observations suggest that ragweed may respond weakly to natural levels of damage by its enemies; existing studies provide indirect evidence both supporting (Genton *et al.* 2005a; MacKay and Kotanen 2008) and apparently contradicting (Throop 2005) this suggestion. However, there still has been no direct, manipulative test of the effects of natural levels of herbivore damage to ragweed.

Information about ragweed's response to natural enemies might help to explain its invasiveness, or to rule out potential explanations. This plant has been widely introduced throughout Eurasia, and now is abundant in parts of both eastern and western Europe, where it is considered a severe problem (Kiss 2007). *Ambrosia* is much less attacked by enemies in its invaded European range than in North America (Genton *et al.* 2005a). This would seem to suggest invasive populations have benefitted as predicted by the Enemy Release Hypothesis (Elton 1958; Keane and Crawley 2002; Torchin and Mitchell 2004; Liu and Stiling 2006), which suggests that introduced plants are able to become invasive because their usual herbivores and pathogens are absent in their new range. However, this explanation assumes that invaders previously were limited by enemies within their home range, so that escaping these enemies

during invasion provides a significant demographic benefit (Maron and Vilà 2001; Colautti *et al.* 2004; Hierro *et al.* 2005). The limiting effects of natural enemies on invaders therefore need to be studied in their native range (Guo 2006), as well as in invaded areas (Hierro *et al.* 2005). If a plant such as ragweed is not susceptible to control by enemies even in its native range, then its success in invaded regions must be explained by other factors (Maron and Vilà 2001).

Observations of enemy effects at all life stages are necessary to understand the long-term consequences for a population (Halpern and Underwood 2006; Mitchell *et al.* 2006). Therefore, to understand the importance of biotic interactions to *Ambrosia*, we excluded enemies in a series of field experiments from all three major stages of this annual's life cycle: seeds (technically achenes), seedlings and adult plants. We also manipulated disturbance and intraspecific density to investigate whether these interacted with herbivory to affect reproduction, growth, and survival at the adult stage. Disturbance and population density might be important both because herbivores could respond in a density-dependent fashion, and because herbivory could make plants less able to compete with either conspecifics or surrounding vegetation (Halpern and Underwood 2006). Together, our experiments represent the most complete study to date of ragweed's responses to its natural enemies.

Materials and Methods

Site and study species

These experiments were conducted at the Koffler Scientific Reserve (KSR) at Joker's Hill, a 350 ha field station owned by the University of Toronto and situated 50km north of Toronto, Ontario (44°03'N, 79°29'W: <http://www.ksr.utoronto.ca>). Plots were established in old fields dominated by perennial vegetation, notably *Bromus inermis* Leyss. and *Solidago* cf. *canadensis*. This site has previously hosted other ragweed research, including MacKay and Kotanen (2008) and components of Genton *et al.* (1995).

Ragweed is common at the site in general and around our experimental plots in particular. Plants are scarce in well-vegetated old fields, but reach high densities in recently-disturbed sites such as plowed fields and roadsides. This species is highly disturbance-dependent (Allard 1943; Bazzaz 1968; Bazzaz and Mezga 1973; Bazzaz 1974; Kosola and Gross 1999; Fumanal *et al.* 2008): its growth is strongly suppressed by shade (Raynal and Bazzaz 1975; Paquin and Aarssen 2004) and by root competition (Kosola and Gross 1999). The competitive effect on ragweed is so pronounced that it has been suggested that competitors be included in biological control efforts (Teshler *et al.* 2002).

Ragweed is frequently attacked by numerous species at our study site, including leaf-chewing insects (Noctuidae: *Tarachidia candefacta* Hbn., *Tarachidia erastrionides* Hbn.; Chrysomelidae: *Zygogramma suturalis* F., *Systema blanda* Mels.), stem-galling moths (Tortricidae: *Epiblema* sp.), invertebrate and vertebrate seed and seedling predators (Carabidae: *Harpalis* sp., rodents: *Microtus pennsylvanicus* (Ord), *Peromyscus leucopus* (Raf.)), and snails (MacKay and Kotanen 2008); *Ophraella* spp. are uncommon at our site. Data from France indicate that this plant has escaped most of these enemies during its invasion (Genton *et al.* 2005a); however, it is not clear that this has resulted in substantially improved performance or higher population densities as might be expected if this escape led to a marked demographic benefit. Although it is likely that populations in other regions of North America are attacked by different suites of enemies, French populations seem to have originated from multiple sources, potentially including Ontario populations (Genton *et al.* 1995a,b); thus, there is no reason to believe our study site is in any way unrepresentative.

Experimental design

Seed survival

The effects of vertebrate (bird and mammal) granivory and fungal pathogens on seed

mortality were assessed in a seed survival experiment. We established this experiment in early November 2007, just after natural ragweed plants had dispersed seeds, using a mixture of seeds collected in 2005 from four mother plants from each of 10 populations in Southern Ontario. We ploughed six blocks (*ca.* 4m x 20m), and within each we marked nine small plots (approx. 10cm x 10cm, in a 3x3 grid) separated from one another and the block's edge by a 1m buffer. Within each plot we scattered 10 seeds on the ground surface. Adding these seeds in November ensured that they would have been naturally cold-stratified before germination began next spring. Plots were then randomly assigned one of three nested treatments: control (no treatment), cage protection, and cage protection plus fungicide addition. The cages had a mesh size of approx. 1cm² and were meant to exclude large (vertebrate) granivores, but not granivorous insects, and only minimally reduced light intensity. Fungal seed pathogens were controlled by soaking seeds and surrounding soil with a 10g/L solution of Captan (Zeneca Agro, Stoney Creek, ON, Canada, a heterocyclic phthalimide fungicide which is very effective against Oomycetes, Ascomycetes, and Basidiomycetes (Sharvelle 1961; Torgeson 1969) and has been successfully used at this site to exclude fungal pathogens from seeds (*e.g.*, Blaney and Kotanen 2001; Schafer and Kotanen 2003). Low densities of seeds do occur in the soil in previously undisturbed sites; however, since we treated soil (by fungicide addition or caging) rather than only treating our added seeds, any pre-existing seeds in the seed bank should have received the same protection as our added seeds. MacKay and Kotanen (2008) also used similar methods to examine the effects of fungi on buried seeds; however, our experiment represents a significant departure both in that it examines other possible sources of mortality, and in that it simulates impacts on newly-dispersed seeds, rather than seeds stored below-ground in the seed bank.

In May and June 2008, emerging seedlings were marked and censused weekly; we counted seedlings until the end of June, when new seedlings had stopped appearing. Our response

variable is the number of germinated seeds; although such seedlings clearly survived the seed stage, ungerminated seeds could have been moved by earthworms, washed away, or have entered secondary dormancy (Baskin and Baskin 1980), and therefore cannot be presumed to have died.

Seedling survival

The effects of attack by slugs, snails, and other herbivores on seedling survival were tested in a seedling survival experiment in spring 2008. On June 7, we transplanted wild ragweed seedlings (approx 2-4cm high, with at least 2 pairs of adult leaves) from a population at KSR to five of the blocks also used for the seed survival experiment. In each block we planted 10 rows of 3 plots (approx. 10cm diameter) with ten ragweed seedlings per plot (300 plants/block). Three nested treatments were then randomly applied within rows: control (no enemy exclusion), aluminum barrier plus slug pellets (mollusc exclusion), and aluminum barrier, slug pellets, plus a covering of 1mm² nylon netting (complete herbivore exclusion). We used about 60ml of slug pellets per plot (Spectrum Brands IP inc., Brantford, Ontario); these contained 2.75% metaldehyde, which kills slugs and snails when ingested. Aluminum barriers were made of a sheet of aluminum flashing open at the bottom and protruding a few cm from the soil; such barriers are effective against molluscs with added protection from slug pellets (Schüder *et al.* 2003). Netting excluded all but the smallest herbivores, including most insects, but minimally reduced light intensity. We counted the seedlings throughout the seedling stage (until June 25) to determine the rate of seedling mortality from these enemies.

Adult survival and reproduction

This experiment was conducted twice at KSR, once in 2007 and again in 2008. In both years we used seeds from the same populations as the seed survival experiment. The seeds were stratified in moist soil at approx. 5°C for one week, before being transferred to cell packs filled

with standard potting soil and germinated in the greenhouse on the campus of the University of Toronto at Mississauga. When experimental plants had developed two pairs of adult leaves and were 6-10 cm high (approximately matching the size of naturally-occurring plants in the field) they were brought to the field site and allowed to acclimatize in a sheltered location for at least 3 nights.

In both years, 5 experimental blocks were marked within old field vegetation, each containing twelve 1m² plots separated by about 2m. Blocks were at least 30m apart. Each plot within a block was randomly subjected to one of 8 factorial combinations of three treatments: a conspecific density treatment (9 vs. 25 plants/m²), an insect exclusion treatment (insecticide vs. water spray), and a disturbance treatment (plots cleared of existing vegetation vs. not cleared). Plots were disturbed by completely hand-removing existing sod and stirring the underlying soil; this likely represents a less severe disturbance than agricultural ploughing, though it may more completely remove the seed bank. In the conspecific density treatments, plants were laid out in a grid with approximately equal distances between each other and from the edge of the plot. Finally, in 2007, insects were excluded by spraying the upper and lower surfaces of all leaves with a weekly spray of the insecticidal plant extract Rotenone (5% Rotenone, 30ml in 3L water, Wilson Laboratories, Dundas, ON). In 2008 we followed the same procedure, but used the organophosphate pesticide Malathion (50% Malathion, 25ml in 3L water, Wilson Laboratories, Ontario). We changed the pesticide treatment because Rotenone proved less effective (see Results). In both years control plots were sprayed with an equivalent amount of water.

In both years, the proportion of leaves with visible herbivore damage (tissue removal) and two measures of plant size (plant height and leaf number) were measured in August, close to the end of the growing season. We estimated the proportion of leaves damaged rather than leaf area removed because the complex, dissected morphology of ragweed leaves makes it impossible to

estimate the area of missing leaf segments. In 2007, damage and plant size were measured for all plants; in 2008, they were measured for six randomly chosen focal individuals per plot, and the proportion of plants attacked by stem borers was determined as well. As well, at the end of the summer (September), when flowering was complete, stem biomass and fecundity were measured. In 2007, fecundity was determined by counting all seeds on three randomly-chosen plants per plot. In 2008, we estimated fecundity by weighing all seeds produced by each individual: a regression of 38 plants subsampled from all treatments of one of our experimental blocks indicated that weight is an excellent predictor of seed number (linear regression: $F_{1,36} = 947.94$, $P < 0.001$, $r^2 = 0.96$, both variables log-transformed).

Statistical analyses

We tested for the effects of enemy exclusion on the number of germinated seeds and surviving seedlings with randomized-block ANOVAs (Kirk 1995). To simplify our design, avoid pseudoreplication, and improve estimates of damage, we first calculated the means of all within-block replicates (3 for the seed experiment, 10 for the seedling experiment) and performed our analysis on these; patterns of significance were essentially unchanged if individual plants were instead used as datapoints. Block means were log-transformed to normalize residual variance, and block was treated as a random effect in our analyses. In each experiment, we tested the *a priori* hypothesis that a more complete exclusion of enemies would increase survival. Specifically, we hypothesized the following ranks of magnitude in our response variables: control < cage < cage & fungicide (seeds germinating), control < barrier < barrier & net (seedlings surviving). The analysis of these ordered factors was conducted with orthogonal polynomial contrasts (Sokal and Rohlf 1994; Quinn and Keough 2002).

For analyses of adult plants, we tested for treatment differences in plant size, fecundity, and damage with randomized block factorial ANOVAs (Kirk 1995). All statistical models were

mixed, including the three treatments (disturbance, density and pesticide spray) as fixed effects and block as random. Again, we used plot means in our analyses; again, this did not qualitatively alter our results. In contrast, survivorship and stem gall incidence were measured as proportions of plants within each plot. We analyzed these data with Generalized Linear Mixed Models (GLMM: Crawley 2007; Bolker 2008), using the same structure of fixed and random effects as in the ANOVAs. To accommodate proportional data, the GLMM uses a logit link and assumes binomial errors. The significance of terms was determined by simplifying the model and checking for a significant ($p < 0.05$) loss of explanatory power with a likelihood-ratio test. Two-way interactions were not deleted sequentially but were replaced after testing, so that each interaction was tested against a model containing all other 2-way interactions (Crawley 2007). Analyses were performed in R 2.8.1 (R Development Core Team 2008).

Results

Seeds and seedlings

About 50% of seeds germinated *in situ* (out of 10 seeds, 4.78 ± 0.5 , mean $\pm$ SEM). Protection from both vertebrate herbivores and fungi had no effect: by the end of June there were no significant differences among treatments in the number of germinated seeds (Table 1).

Seedling survivorship was very high; between early and late June an average of 7.8 out of 10 seedlings survived in each plot. Treatments differed significantly (at $P = 0.10$), and our *a priori* hypothesis of increasing survivorship with increasing protection was supported (linear contrast $P = 0.04$) (Table 1). Increasing levels of enemy exclusion (control, molluscs, all herbivores) resulted in increasingly greater seedling survival: 7.14 ± 0.95 , 7.76 ± 0.56 and 8.6 ± 0.44 of 10 seedlings, respectively. Excluding molluscs therefore created an 8.7% relative increase in survivorship; excluding other herbivores added a further 10.8%.

Adults

In 2007, the proportion of leaves damaged was high: on average 81.9% of leaves in the control treatment were damaged. This figure reflects the net herbivore damage from all sources; the principal herbivores responsible are believed to be those listed in our Methods section. This level of damage was greater than that reported by MacKay and Kotanen (2008), who found 40% to 58% of leaves were damaged, depending on the degree of isolation from conspecifics. Our pesticide treatment was weakly effective, reducing relative damage by 16.7% (Table 2, Fig. 1). There was no significant pattern of increasing damage with density or disturbance, and no significant interactions (Table 2, Fig. 1).

Survivorship over the summer was strongly enhanced by disturbance, with 93.5% of plants surviving in disturbed sites and 42.6% in undisturbed sites (Fig. 2); GLMM model simplification confirmed that survivorship increased in disturbed plots ($|z| = 6.70$, $p < 0.001$), though neither density nor pesticide had a significant effect ($p > 0.05$) (Fig. 2). Plant growth (number of leaves and stem biomass) and fecundity were not affected by insecticide but significantly increased in response to disturbance, and also showed a strong interaction with density (Table 2, Fig. 2). Greater conspecific density was important only in the disturbed treatment, where it relatively reduced average leaf number by 38.9%, stem biomass by 67%, and seed production by 74%.

The following summer damage rates were much lower: on average, 33.4% of leaves per plant were damaged in control plots in 2008 (Fig. 1). Again, this figure represents net damage, rather than damage by a specific herbivore. This level of damage was less than that reported by MacKay and Kotanen (2008), but differences between treatments were much more extreme: Malathion was very effective, reducing the relative damage level by 72% (Table 2). However, in contrast to 2007, we found significant interactions of insecticide application with both density and disturbance: denser plots experienced relatively 31% less leaf damage per plant in control

plots only, while disturbance lowered leaf damage in both pesticide and control plots but had a proportionally greater effect in pesticide-sprayed plots where overall damage rates were lower. While stem galling (likely by *Epiblema strenuata* Walk.) also was effectively controlled by Malathion (GLMM $|z| = 3.80$, $p < 0.001$), average rates of attack across both herbivory treatments instead were higher in disturbed plots ($|z| = 4.06$, $P < 0.001$), though this result depended on the water controls (Fig. 3). Conspecific density did not have a significant effect on stem galling ($P > 0.05$).

Survivorship over the summer of 2008 was very high: only 10 focal plants died in the entire year (4.2% mortality), making it impossible to detect treatment effects on survivorship (Fig. 4). However, plant growth and fecundity followed a pattern similar to the 2007 results (Table 2, Fig. 4). Disturbance strongly increased the number of leaves, stem biomass, and seed production. Density had a clear effect only in disturbed plots, where it reduced average leaf number by 41.4%, stem biomass by 63.8%, and seed production by 65.6%. Finally, insect exclusion, while effective, did not affect either growth or fecundity.

Discussion

The major findings of our study are that enemies have i) no effect on seed germination, ii) only a slight effect on seedling survivorship and iii) no effect on adult survivorship, growth or fecundity. These results suggest that natural enemies are likely to be ineffective in controlling this species even in its native range. As a result, enemy release is not likely to have encouraged its invasiveness in Europe. Comparatively, disturbance is likely to be much more important than herbivory for populations in both native and non-native regions.

Seed germination

Ragweed seeds have high survivorship between growing seasons (Baskin and Baskin 1980), and our results suggest that protection did not cause an increase in this already high rate.

In fact, we probably underestimated survival, since at least some of the seeds that failed to germinate likely remained dormant, or were moved out of the plot. We were unable to detect any losses to vertebrate granivores, though these are known to be important for related species (Harrison and Regnier 2003). As well, we failed to find evidence of fungal pathogens; MacKay and Kotanen (2008) also failed to detect seed pathogens for ragweed, though they did provide evidence that feedback by soil pathogens may reduce plant growth over time. We did not attempt to manipulate seed losses to insects, but post-dispersal insect predation may be low because of ragweed's hard pericarp: once mature, this is very difficult to crack and resists breaking by even strong-jawed organisms such as crickets and ground beetles (pers. obs.). In contrast, pre-dispersal seeds at this site are vulnerable to granivores such as *Harpalus* spp. (Coleoptera: Carabidae) (MacKay and Kotanen 2008).

Our study contrasts with work in a related species, *Ambrosia trifida* (Harrison and Regnier 2003): in this species, rodent and invertebrate seed predation was as high as 88%. We may have underestimated rodent predation since seed mortality is likely to be low in disturbed areas where small mammals themselves risk predation (e.g., Manson and Stiles 1998). Nonetheless, Harrison and Regnier (2003) point out that even with a high rate of seed predation, population limitation of *A. trifida* by this means alone is unlikely; our results suggest that this is even more true for *A. artemisiifolia*.

Seedling survival

Although excluding herbivores increased survival at the seedling stage, this effect was small, and the survivorship of unprotected seedlings remained high. This is despite the local abundance of snails (MacKay and Kotanen 2003), which can have a very large effect on seedling survival (e.g., Hill and Silvertown 1997; Ehrlén 2003); as well, many leaf-eating insects are present in spring, including overwintered adults and newly-laid eggs of the specialist

Zygogramma suturalis F. Since ragweed plants, once grown, are highly tolerant to leaf damage (see below), high seedling survivorship will likely result in dense adult populations and a correspondingly high level of seed production. Our results from this experiment are consistent with our observations of germinated seeds in the seed protection experiment: of 257 germinated seeds, only 8 resulting seedlings died before the end of June.

Adult survival and reproduction

Two years of data cannot capture the full range of variability in herbivore damage; nonetheless, the contrast between 2007 and 2008 gives some indication of the magnitude of variability that may be expected. In both years, ragweed plants were insensitive to reductions in natural levels of herbivory: neither when unprotected plants were heavily damaged in 2007, nor when we were able to better control damage levels in 2008, did we find treatment effects on growth or reproduction. The absence of strong enemy effects at the adult stage was robust to manipulations of density and the presence of competitors.

These results are consistent with most previous evidence, which has not consistently suggested a strong negative effect of herbivores. For instance, in ragweed's native region, MacKay and Kotanen (2008) but did not find evidence of improved performance by less-damaged plants; however herbivores were not experimentally removed from plants; thus, variation in damage was relatively small, and likely was confounded with other factors such as habitat and host-choice. In fact, some measures of damage were positively correlated with performance, likely because larger plants were more attractive to herbivores. In both native and invaded regions, Genton *et al.* (2005a) found foliar damage was negatively correlated with plant height, but this did not result in greater biomass in non-native areas despite greatly reduced herbivore pressure; again, herbivore pressure was not directly manipulated. Irwin and Aarssen (1996) also reported that ragweed was very tolerant of artificial apical meristem removal, at least

in open sites. On the other hand, Throop (2005) reported that damage by the leaf beetle *Ophraella communa* LeSage significantly reduced seed production (but not vegetative growth); a specialist aphid (*Uroleucon tuataiae* Olive) caused much milder damage. It is possible that *O. communa* is a more damaging herbivore than those observed in our study; however, a possible bias may result from the methods used by Throop (2005): multiple beetles were added to caged experimental plants, potentially resulting in unusually elevated levels of damage. No information was provided regarding natural beetle densities or levels of herbivory, though the author noted that *O. communa* can cause high levels of damage. Even so, a field study of spatial patterns of abundance of both ragweed and *O. communa* in Japan suggested that even large numbers of beetles were ineffective at reducing population density (Yamanaka *et al.* 2007). Ours remains the only study to experimentally document the effects of natural levels of herbivore damage to ragweed.

Disturbance effects

Ragweed is a disturbance-dependent ruderal (Bazzaz 1968; Bazzaz and Mezga 1973; Kosola and Gross 1999; Ziska *et al.* 2007; Fumanal *et al.* 2008); every measurement of performance was higher when our plots were cleared of sod. Interestingly, clearing vegetation also reduced the amount of leaf damage plants incurred, at least in 2008; this could have contributed to the improved performance of plants in these plots. However, any such effect would be confounded with the direct effects of reduced competition, again indicating a need to directly assess the effects of herbivores by experimental removal; as indicated above, such tests failed to find a direct benefit of herbivore exclusion. Historically, this strong response to disturbance is believed to have resulted in rapid growth of ragweed populations over much of eastern North America following deforestation and agriculture by European settlers, as indicated by pollen records (Bassett and Terasmae 1962; McAndrews 1988). Likewise, invasion of disturbed sites is

believed to be largely responsible for ragweed's successful invasion of Europe (Chauvel *et al.* 2006; Kiss 2007; Fumanal *et al.* 2008).

Density-dependence

Ragweed can grow at very high densities. The highest density we used (25 plants/m²) is representative of natural many populations (*e.g.*, Foster *et al.* 1980); in comparison, we can consider 9 plants/m² treatment to represent reduced intraspecific competition, as might be found in newly-founded populations. Higher conspecific densities decreased most measures of plant performance, including reproduction, but only when plots were disturbed. In such disturbed locations, individuals were able to grow to sufficiently large sizes to directly interact with one another, whereas interspecific competition kept individual plants too small to interact in undisturbed sites, and likely masked any intraspecific effects. Nonetheless, while density-dependence in disturbed sites makes population regulation possible in principle (Halpern and Underwood 2006), the very strong direct response of ragweed to disturbance is likely to greatly reduce this potential control.

Implications

Our results indicate that natural enemies have weak effects on ragweed even in its native range. Unless damage in our area is atypically low, this also suggests that escape from its natural enemies may have played only a minor role in ragweed's invasion of Europe. Instead, even though European populations experience much lower levels of herbivore damage than Ontario populations (Genton *et al.* 2005a), the benefits of this escape may be minor. This interpretation agrees with earlier results correlating damage with performance (Genton *et al.* 2005a; MacKay and Kotanen 2008). As well, this may suggest why European ragweed apparently has not lost anti-herbivore defences (Genton *et al.* 2005a), contrary to the Evolution of Increased Competitive Ability Hypothesis (Blossey and Nötzold 1995): if herbivore pressures were small even in native

377 areas, one would expect little evolutionary response when these pressures were removed.

378 Our results also have implications for biological control. In both its native (Teshler *et al.*
379 2002) and invaded range (Igrc 1995; Reznik *et al.* 1994; Kiss 2007), there have been numerous
380 attempts to control *Ambrosia artemisiifolia* by introducing ragweed-feeding invertebrates, but
381 few successes. There likely are a number of reasons for this result, including failure of biocontrol
382 organisms to establish viable populations; however, our experiments suggest that one factor
383 simply may be a weak effect of most natural enemies of plant performance. Even in its native
384 range, natural consumer damage did not significantly reduce *Ambrosia* survival or seed
385 production; consequently, biological control programs seem unlikely to provide an effective
386 means of control unless they can produce damage well in excess of the natural levels we have
387 documented.

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Table 1. Effects of protection on seed germination and seedling survival. For seed germination, ordered treatments levels are: no protection (control), cage (vertebrate exclusion), and cage + fungicide (vertebrate & fungal exclusion); for seedlings, they are: no protection (control), barrier + slug pellets (mollusc exclusion), and barrier + slug pellets + netting (complete herbivore exclusion). ANOVAs have been used to partition variation into orthogonal polynomial contrasts to test for an increase in survivorship with more complete enemy exclusion. Data were log-transformed to normalize variance

Source	Seed germination				Seedling survival			
	df	MS	F	p	df	MS	F	p
Treatment	2	0.025	0.354	0.71	2	0.06	3.13	0.10
Linear	1	0.031	0.442	0.52	1	0.12	6.25	0.04
Quadratic	1	0.019	0.265	0.62	1	<0.01	<0.01	0.97
Residual	10	0.071			8	0.16		

Table 2. Effects of experimental treatments on adult plants. Factorial ANOVAs have been used to examine the effects of conspecific density, disturbance, and insecticide application. Leaf number and seed number were log-transformed before analysis to normalize residuals, as was 2008 stem biomass. F-statistics and p-values are shown in bold typeface when significant

Year	Source	Damage		Leaf Number		Stem Biomass		Fecundity ¹	
		F _{1,28}	p	F _{1,28}	p	F _{1,28}	p	F _{1,28}	p
2007	Density	1.25	0.28	77.19	<0.01	62.51	<0.01	2.73	0.11
	Disturbance	<0.01	0.97	1109.8	<0.01	62.18	<0.01	64.87	<0.01
	Insecticide	6.35	0.02	2.95	0.10	<0.01	0.99	0.72	0.40
	Density x Disturbance	1.26	0.27	5.72	0.03	16.61	<0.01	5.23	0.03
	Density x Insecticide	0.20	0.66	1.09	0.31	0.03	0.86	0.40	0.53
	Disturbance x Insecticide	0.16	0.69	0.21	0.65	0.18	0.67	0.09	0.76
	Density x Disturbance x Insecticide	0.07	0.79	2.31	0.14	0.11	0.74	1.11	0.30
2008	Density	5.57	0.03	2.12	0.16	12.97	<0.01	2.21	0.15
	Disturbance	45.12	<0.01	152.05	<0.01	235.96	<0.01	56.34	<0.01
	Insecticide	84.62	<0.01	1.98	0.17	0.12	0.73	2.16	0.15
	Density x Disturbance	1.05	0.31	6.95	0.01	19.77	<0.01	10.39	<0.01
	Density x Insecticide	5.83	0.02	0.04	0.83	0.37	0.55	0.29	0.59
	Disturbance x Insecticide	5.53	0.03	4.29	0.05	0.00	0.96	0.54	0.47
	Density x Disturbance x Insecticide	1.36	0.25	0.84	0.37	1.03	0.32	0.53	0.47

¹ 2007: seed number; 2008: total seed mass

Figure legends

Fig. 1 Leaf damage to adult plants during the summer of (A) 2007 and (B) 2008.

Treatments are disturbed *vs.* undisturbed plots, high *vs.* low conspecific density, and insecticide application (2007: Rotenone; 2008: Malathion) *vs.* controls (water). Points are means $\pm$ SE ($n = 5$); see Table 2 for ANOVA results

Fig. 2 Performance of adult plants during the summer of 2007. Treatments are disturbed *vs.* undisturbed plots, high *vs.* low conspecific density, and insecticide application (Rotenone) *vs.* controls (water). Disturbed plots are indicated by filled circles; undisturbed controls by open circles. Points are means $\pm$ SE ($n = 5$). See Table 2 for ANOVA results (growth, reproduction); GLMM analysis indicated survival was increased by disturbance ($P < 0.001$)

Fig. 3 The incidence of stem-galling insects on adult plants during the summer of 2008. Treatments are disturbed *vs.* undisturbed plots, high *vs.* low conspecific density, and insecticide application (Malathion) *vs.* controls (water). Points are means $\pm$ SE ($n = 5$). GLMM analysis indicated galling was increased by disturbance, but decreased by insecticide application ($P < 0.001$)

Fig. 4 Performance of adult plants during the summer of 2008. Treatments are disturbed *vs.* undisturbed plots, high *vs.* low conspecific density, and insecticide application (Malathion) *vs.* controls (water). Disturbed plots are indicated by filled circles; undisturbed controls by open circles. Points are means $\pm$ SE ($n = 5$); see Table 2 for ANOVA results (growth, reproduction); survival was not analyzed because almost all plants survived

Figure 1.

A

B

563 **Figure 2.**

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566 **Figure 3.**

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569 **Figure 4.**

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