

Local escape of an invasive plant, common ragweed (*Ambrosia artemisiifolia* L.), from above-ground and below-ground enemies in its native area

James MacKay and Peter M. Kotanen*

Department of Ecology and Evolutionary Biology, University of Toronto at Mississauga, 3359 Mississauga Road North, Mississauga, Ontario, L5L 1C6 Canada

Summary

1. The Enemy Release Hypothesis proposes that exotic species gain an advantage in new regions because their natural enemies are lost during invasion; however, enemy release could also occur as a result of much smaller scale movements within an invasive plant's native range, i.e. if it escapes enemies while colonizing new sites. Few studies have considered this possibility, and none has compared escape from multiple guilds of enemies.

2. We studied an invasive North American weed, *Ambrosia artemisiifolia* (common ragweed), to determine whether it escapes above-ground or below-ground enemies at a local scale in its native range. Exotic populations of this plant are known to have escaped above-ground enemies in Europe.

3. Experimental populations of ragweed isolated from existing populations by just 100 m experienced reduced levels of damage by invertebrate folivores and seed predators; however, there was no clear evidence that this escape led to improved performance.

4. Plants inoculated with unsterilized soil grew better than plants in sterile soil, likely reflecting a need for mycorrhizal colonization. Inoculum from ragweed populations was as beneficial as inoculum from nearby ragweed-free sites, suggesting only a small initial role for site-specific pathogens.

5. Performance declined over time when plants were serially inoculated with non-sterile soil, indicating negative feedback with the soil biota. This feedback was stronger for inocula derived from ragweed populations vs. those from nearby ragweed-free sites.

6. Seeds buried in ragweed populations were less likely to germinate subsequently than seeds buried in nearby ragweed-free sites. This difference was not reduced by treatment with fungicide; consequently, the mechanism is unclear.

7. *Synthesis.* These results indicate that ragweed can escape both above-ground and below-ground enemies by dispersing to new sites, resulting in reduced levels of folivory, seed predation, soil feedback and perhaps losses to seed pathogens. Although consequences often may be small, this demonstrates that enemy release can occur for native as well as exotic populations of this species, linking possible mechanisms of local spread with long-distance invasion.

Key-words: *Ambrosia artemisiifolia*, annual ragweed, biological invasions, common ragweed, Enemy Release Hypothesis, fungal pathogens, insect herbivores, Janzen-Connell Hypothesis, natural enemies

Introduction

Most plant communities now contain non-indigenous species (di Castri 1989; Vitousek *et al.* 1996; Mack *et al.* 2000), yet our ability to predict invasions by such species remains limited (Mack *et al.* 2000; Hierro *et al.* 2005). One reason may be that most studies of exotic species take place solely within

their introduced range, without knowledge of the basic ecology of the study species within its native range (Hierro *et al.* 2005). Such knowledge can help indicate both abiotic limiting factors and the potential of competitors and natural enemies to hold invaders in check (Guo 2006). For instance, one of the most popular and widely cited theories in invasion biology is the Enemy Release Hypothesis, which postulates that invasiveness can be attributed to a loss of natural enemies during the invasion process (Maron & Vilà 2001; Keane &

*Correspondence author. E-mail: peter.kotanen@utoronto.ca

Crawley 2002; Torchin & Mitchell 2004; Mitchell *et al.* 2006). Recent reviews (Colautii *et al.* 2004; Bossdorf *et al.* 2005; Liu & Stiling 2006) provide mixed support for this hypothesis, providing positive evidence but also indicating many exceptions and limitations. Studies within the native range can help to clarify this uncertainty, for instance by indicating whether enemy pressure is a significant factor in the native-region ecology of a potential invader, and thus whether the potential for enemy release exists (e.g. Maron & Vilà 2001; Hierro *et al.* 2005).

Enemy release need not be restricted to long-distance dispersal, but can in fact occur for isolated plants within their native range. For example, experimental populations of *Vicia sepium* L. experience less insect damage to their seed pods when isolated from natural populations by just 100–500 m (Kruess & Tschardt 2000). On a geographic scale, isolated natural populations of *Carex blanda* Dewey are less likely than more central populations to be attacked by fungal pathogens and pre-dispersal seed predators (Alexander *et al.* 2007). Perhaps the best-known examples are provided by tests of the Janzen-Connell hypothesis (Janzen 1970; Connell 1971), which posits that seeds and seedlings of tropical trees can escape enemies by dispersing away from their parents; studies providing evidence of density- or distance-dependent recruitment suggest that this model may be widely applicable (e.g. Condit *et al.* 1992; Wills *et al.* 1997; Harms *et al.* 2000; Wright 2002; but see Hyatt *et al.* 2003). The same species that escape enemies at a local or regional scale may escape on a much larger spatial scale as well. For example, Packer & Clay (2000, 2003) found evidence that within its native range the temperate tree *Prunus serotina* Ehrh. escapes soil pathogens by dispersing its seedlings away from the parent; the same species has also escaped soil pathogens on a much larger scale by invading Europe, where it has become a pest species (Reinhart *et al.* 2003). As another example, the tropical shrub *Clidemia hirta* (L.) D. Don has escaped enemies (insect herbivores and fungi) in Hawaii, where it is introduced, but also escapes them in its native range by colonizing open habitats (DeWalt *et al.* 2004). Thus, native-range escape may provide clues to invasiveness in non-native areas.

These studies also emphasize the point that invaders can escape from a range of natural enemies. Most studies of enemy release have focussed on above-ground interactions, particularly with folivores and granivores (Levine *et al.* 2003; Bossdorf *et al.* 2005; Hierro *et al.* 2005; Liu & Stiling 2006), but recent research suggests below-ground interactions may be even more important (Levine *et al.* 2003; Wolfe & Klironomos 2005; Reinhart & Callaway 2006). Soil organisms including root pathogens (Packer & Clay 2000; Reinhart *et al.* 2003; Callaway *et al.* 2004b; Reinhart & Callaway 2004), mycorrhizas (Klironomos 2002; Bray *et al.* 2003) and seed pathogens (Blaney & Kotanen 2001, 2002) have all been shown to affect plant invasions. In particular, feedback between a plant and the soil community can be either positive (as beneficial mycorrhizas and other mutualists accumulate) or negative (as pathogen populations grow) (Bever 1994; Bever *et al.* 1997; Reinhart & Callaway 2006), potentially affecting an invader's

success (Klironomos 2002; Agrawal *et al.* 2005; Van Grunsven *et al.* 2007).

In this study, we investigate enemy release of common ragweed (*Ambrosia artemisiifolia* L.) in its native North American range. This plant has previously been shown to have escaped insect folivores in its invaded European range, as predicted by the Enemy Release Hypothesis (Genton *et al.* 2005). It is highly disturbance dependent, and frequently founds isolated colonizing populations in its native range, suggesting local-scale enemy release frequently might occur here as well. We investigate this possibility for insect herbivores, but also add new dimensions by considering escape from insect granivores, soil pathogens and soil feedback. We combine field and laboratory experiments to address the following specific questions:

1. Do isolated populations of common ragweed experience reduced levels of damage by above-ground herbivores and seed predators?
2. Does common ragweed escape negative effects of soil biota by colonizing new sites?
3. Do common ragweed seeds escape attack by soil-borne fungi in newly colonized sites?

This is one of the few studies to test for enemy release within the native habitat of a species known to escape enemies as a transoceanic invader, and the first to compare release from above-ground and below-ground impacts. It also presents the most detailed picture yet of native-region interactions between this problematic weed and its natural enemies, and provides clues to factors which might contribute to its invasiveness in both its native and introduced range.

Methods

SPECIES AND STUDY SITE

Common ragweed (also known as annual ragweed) is a native North American weed, now common throughout the continent except for northern areas (Bassett & Terasmae 1962; Bassett & Crompton 1975; Teshler *et al.* 2002). It is a spring annual; in Ontario, seedlings are abundantly present in early May. Flowering begins in late summer (early August) and is shortly followed by seed-set, but seeds continue to be dispersed until the plants are killed by frost in October or even later. Ragweed is strongly disturbance-dependant (Bazzaz 1968, 1974; Kosola & Gross 1999), and typically is found in highly perturbed habitats such as agricultural fields, roadsides and waste places (Bassett & Terasmae 1962; Bazzaz 1968; Bassett & Crompton 1975; Maryushkina 1991; Teshler *et al.* 2002). It is a poor competitor, and without repeated disturbance typically is competitively displaced from a newly colonized site after only a few years (Thompson 1943; Bazzaz 1968, 1974; Kosola & Gross 1999).

Ragweed is a very successful long-distance invader: it has occurred in France since at least the 18th century (Chauvel *et al.* 2006) and is common in much of eastern Europe, as well occurring in China, Australia and elsewhere (Kiss 2007). In both its native and introduced range it is a troublesome agricultural weed and a major source of pollen allergies (Bassett & Crompton 1975; Kiss 2007).

The field component of this study was conducted in old fields at the Koffler Scientific Reserve at Jokers Hill, just west of the city of Newmarket, Ontario, Canada (44°03' N, 79°29' W) (<<http://>

www.zoo.utoronto.ca/jokershill/jh.html>). These old fields contain a diverse mixture of native and exotic grasses and dicots, notably *Bromus inermis* Leyss., *Poa pratensis* L. and *Solidago* and *Aster* spp.; a complete checklist is available at the Web site indicated above. Common ragweed currently is common at this site, and likely has been present since significant European settlement began in the early 1800s (McAndrews 1988); dense populations frequently occur on roadsides, recently cultivated plots and other disturbed areas. Greenhouse and growth chamber experiments were conducted at the University of Toronto at Mississauga, Canada.

Seeds for use in this study were collected in the fall of 2005 from 10 natural populations within southern Ontario, between Whitby to Milton (approximately 100 km). Seeds were collected from 15 plants within each population; these seeds were tested for viability (by squeezing) and sources were pooled before use. If seedlings were required, seeds were cold stratified at 4 °C for 5 weeks (Willemsen & Rice 1972; Willemsen 1975) and then were placed for 2 weeks on moistened filter paper in Petri dishes, which were incubated in a growth chamber (SG-30, Econair Ecological Chambers, Winnipeg, MB, Canada) using a 16 h : 8 h, 25 °C : 15 °C light : dark cycle. This cycle mimics late spring conditions and is expected to maximize germination (Willemsen 1975).

EXPERIMENT 1: HERBIVORES AND SEED PREDATORS

Six experimental blocks were established, separated from each other by at least 50 m. Each block consisted of three 1 × 1 m plots from which all above-ground vegetation was removed; root material was also removed and soil was overturned to a depth of 10 cm. Within each block, one plot was drawn from within a pre-existing natural population of common ragweed, one from old-field vegetation adjacent to (10 m away from) this population, and one in old-field vegetation isolated at least 100 m from this or any other ragweed population; the scale of this experiment is similar to many local-scale studies of escape from enemies (e.g. Condit *et al.* 1992; Alexander *et al.* 2007). The small size of these plots ensured that no experimental plant within one of these plots was isolated from surrounding vegetation by more than 50 cm – less than mature plant height, and often less than plant diameter. Although herbivory still might differ between our plots and natural populations, all plots were treated identically, meaning that between-treatment comparisons should be valid; we focus on these comparisons in our analyses.

In preparation for this experiment, laboratory-germinated seedlings were transferred to pots filled with a standard, non-sterile potting soil and maintained in a greenhouse until their size matched that of wild plants in the field. At the end of June of 2006, when both wild and greenhouse-grown plants had 6–12 true leaves, 12 ragweed plants were transplanted into each of the 1-m² experimental plots in a grid format, for a total of 216 plants; plants were spaced roughly 25–40 cm apart, and at least 10 cm from the surrounding vegetation. Plants were watered for 3 weeks to ensure that new root systems effectively established in the surrounding soil, and to prevent transplant shock.

Folivory was assessed between 12–20 August 2006, when plants were mature and damage was conspicuously present in natural populations. Damage was assessed by sampling a subset of stem and branch leaves on each plant, in a stratified procedure designed to produce a consistent, representative index of damage: the top 10 fully formed leaves on each stem were sampled as well as five leaves per branch on five of the 10 top branches. This procedure avoided the oldest leaves, which typically are senescent and difficult to assess. Leaves were visually scored for

1. Scraping, observed to be caused by the native specialist caterpillars *Tarachidia candefacta* Hbn. and *Tarachidia erastrionides* Hbn. (Noctuidae)
2. Chewing, observed to be caused by the native ragweed specialist *Zygogramma suturalis* F. (Chrysomelidae), by larger *Tarachidia* spp., and by generalist snails, primarily the introduced *Trichia striolata* Pfeiffer (Hygromiidae)
3. Perforations, observed to be produced by the native generalist *Systema blanda* Melsheimer (Chrysomelidae)
4. The fraction of leaf area removed on each of 35 leaves sampled per plant
5. The fraction of leaf area removed on the three most damaged leaves per plant.

Other insects likely contribute to the damage observed, but the above species were those most commonly noted in our field surveys.

Granivory by the introduced strawberry seed beetle *Harpalus rufipes* Degeer (Carabidae) also was assessed for each plant in September 2006, when seed set was well advanced but before significant dispersal had occurred. This pre-dispersal seed predator removes the ends and contents from seeds while still attached to the plant, resulting in diagnostic ‘popped tops’; damage by other insect seed predators was extremely scarce. The number of seeds on every other branch for each plant was counted, and the number with popped tops was recorded.

Finally, performance estimates were obtained for each plant in early to mid-September (when annual growth was essentially complete) by measuring height and counting the number of branches, as well as the seed counts described above.

EXPERIMENTS 2 AND 3: SOIL BIOTA

The net effects of soil biota (including both mutualists and pathogens) on plant growth were assessed in two linked experiments.

The first experiment assessed whether ragweed grows better in the presence of soil biota from established ragweed populations vs. biota from undisturbed old fields lacking ragweed. In February 2006, 400 pots were filled with 500 mL of a standard mixture of soil created by collecting, mixing and double autoclaving samples (two consecutive 20-min sterilization treatments) from 10 old fields on the Koffler Reserve. These soils had been collected about 1 month previously, and were stored outdoors until use. Twenty g of inoculum (about 4% of pot volume) also was added to each pot in a balanced factorial design; inoculum addition was kept to a minimum to avoid changing the composition of the soil mix. Half of the pots were inoculated with soil from one of 10 old field sites, and half with soil from one of 10 ragweed populations; in each case, half of these inocula were autoclaved, and half were unsterilized. There were 10 replicates for each site × habitat × sterilization combination. One lab-germinated ragweed seedling was then transplanted into each pot, and pots were transferred to a greenhouse. After 10 weeks, each plant was harvested, and its height, number of true leaves, above-ground dry biomass, and below-ground dry biomass were recorded. The net effect of soil biota was determined by the difference in these growth measurements between sterile vs. non-sterile treatments.

The second experiment was designed to examine feedback between ragweed and its soil biota using a serial inoculation approach. In April 2007, soil samples were collected at the Koffler Reserve from five existing ragweed populations, and five undisturbed old fields at least 50 m from a ragweed population. Twenty g of inoculum drawn from each of these sites was then added to each of ten 500-mL pots containing a double autoclaved mix of field soil, as described above. One lab-germinated seedling was added to each pot, and these plants

were then transferred to an Econair SG-30 growth chamber set to a 16 h : 8 h, 25 °C : 15 °C light : dark cycle. This first 'generation' of plants was grown for a period of 6 weeks, at which time they were harvested and their height, number of true leaves, above-ground dry biomass, and below-ground dry biomass were recorded. Twenty grams of sub-surface soil was then recovered from each individual pot and used to inoculate a single new pot, containing one ragweed seedling, which subsequently was treated and sampled as for the initial 'generation'. This entire procedure was then repeated for a third time; the final result was an experiment with two factorial treatments: 'Generation' (1, 2, or 3) and 'Habitat' (ragweed population or old field). In addition, a set of 40 control pots was established for generation 2 and 3. Each of these pots was inoculated with 20 g of the original inoculum used in generation 1 (stored until use at -2 °C). Comparing these controls with the serial inoculation treatments ensures that changes in plant performance over time are related to serial inoculation, and not to unrelated temporal changes in soil or seed stocks.

EXPERIMENT 4: SEED PATHOGENS

This experiment was designed to assess negative effects of soil fungi on seed germination. Since most common ragweed seeds are dispersed late in the fall and germinate the following spring, this experiment was conducted over the winter to match the timing and duration of exposure to soil fungi that would be experienced by a typical seed. Twelve 1 × 1 m plots were established at the Koffler Reserve, six in pre-existing ragweed populations, and six in old-field vegetation at least 50 m from a common ragweed population. At the end of October 2006, when plants in the field still were dispersing seeds, 20 seed bags were buried 2–4 cm below the surface of each experimental plot in a grid pattern. Each bag was constructed from nylon stocking material and contained 15 ragweed seeds mixed with 25 g of single-autoclaved locally collected glacial sand; half of these bags were dipped in fungicide for 30 s, and half in water, in a factorial design. The fungicide used was a 10 g/L aqueous solution of Maestro 75 DF (Zeneca Agro, Stoney Creek, ON, Canada: active ingredient 75% Captan by weight). Captan is a non-systemic heterocyclic nitrogen fungicide used to protect seeds from a wide range of Oomycetes, Ascomycetes, and Basidiomycetes (Sharville 1961; Torgeson 1969; Neergaard 1977), and has been successfully used in the past in similar experiments at this site (e.g. Blaney & Kotanen 2001; Schafer & Kotanen 2003; O'Hanlon-Manners & Kotanen 2004).

Seed bags were recovered from the field at the beginning of May 2007, when seedlings were observed germinating in natural populations; 221 of the 240 seed bags were successfully recovered, for a total of 3315 seeds. The contents of each bag were emptied into a 500 mL pot filled with standard non-sterile potting soil and placed in a greenhouse; seeds that already had germinated in the seed bags were counted and removed. The number of seeds that subsequently germinated was recorded once per week for 5 weeks, and those seeds removed. Seeds that germinated in either the field or the greenhouse were combined for analysis. This procedure did not determine whether ungerminated seeds were dead or dormant, and therefore measured germinability rather than absolute viability; however, differences between the fungicide treatment and the controls still should provide an estimate of the relative impacts of fungal pathogens.

STATISTICAL ANALYSES

Statistical analyses were conducted using the EMS routine of JMP 5 (SAS Institute, Cary, NC). Analyses of field experiments generally

were done on plot means, rather than on individual plants or seeds; this sacrifices degrees of freedom, but gains precision and avoids problems with non-independent and zero data. Proportional data were arcsin-transformed before analysis. Most analyses were by Analysis of Variance, followed by Tukey HSD tests (Kirk 1995).

For Experiment 1 (*herbivores and seed predators*), a randomized block design was used (with habitat as a fixed factor, blocked by site). The relationship between damage and growth measures also was investigated using simple linear regression. Experiment 2 (*soil source*) also was examined using a randomized block factorial model (with habitat and sterilization treatment as fixed factors, blocked by inoculum source). Experiment 3 (*feedback*) was analyzed using a split plot factorial model (with generation and habitat as fixed factors, and site nested within habitat). Finally, Experiment 4 (*seed pathogens*) also was analyzed with a split-plot factorial approach (with habitat and soil treatment as fixed factors, and site nested within habitat).

Results

EXPERIMENT 1: HERBIVORES AND SEED PREDATORS

The incidence of herbivore damage declined with distance from established ragweed populations (Fig. 1). Plots isolated from ragweed populations experienced significantly less total damage (40% of leaves damaged) relative to within-population plots (58% of leaves damaged). All three types of leaf damage (scraping, chewing and perforation) were reduced in isolated plots (Fig. 1). Plots adjacent to ragweed populations generally were intermediate in damage, and in some cases significantly differed from within-population plots as well. These patterns did not translate to a reduction in leaf area removed in isolated plots, whether measured for all leaves or the three most damaged leaves (Fig. 1). The lack of a measurable effect on area likely is due to the difficulty of estimating area lost from the highly dissected leaves of this species.

Damage by seed predators also varied with distance from established populations ($F_{2,10} = 5.847$, $P = 0.021$). Tukey tests indicated that isolated plots experienced significantly less seed predation than the other two treatments ($P < 0.05$); adjacent plots experienced intermediate levels of seed loss, though they did not significantly differ from within-population plots ($P > 0.05$). In relative terms, this was a large effect, with isolated plots experiencing 1/10 the mortality of within-population plots; however, absolute rates of loss were very low (proportion killed = 0.022 within populations, 0.013 in adjacent plots, 0.002 in isolated plots).

These patterns of damage did not translate to effects on performance. No differences were found between habitats for the total number of seeds produced per plant ($F_{2,10} = 1.342$; $P = 0.305$) or branches/plant ($F_{2,10} = 1.128$; $P = 0.362$). Plant height tended to be lower in isolated plots ($F_{2,10} = 3.373$; $P = 0.076$), even though damage in these sites was reduced. When all plots were pooled, plant height was positively and significantly correlated with overall herbivore damage, chewing damage and perforation damage, and seed production was positively and significantly correlated with perforation damage (Table 1); other correlations were non-significant.

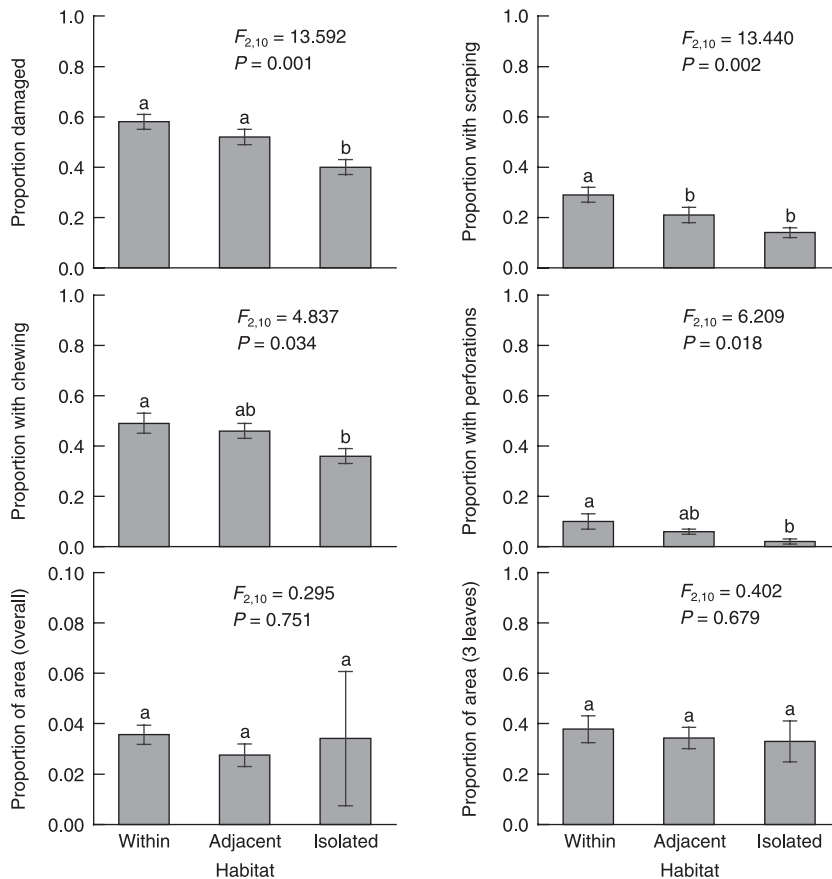


Fig. 1. Mean proportion of leaves damaged per plant, \pm standard error, within, adjacent to, or isolated from existing ragweed populations. The fraction of leaves damaged is subdivided into scraping, chewing, and perforation; also shown is the fraction of leaf area removed per plant, and the fraction removed from the most damaged three leaves. Results of randomized block ANOVAs are shown; bars sharing the same letter are not significantly different ($P > 0.05$; Tukey HSD test). $n = 18$ plots.

Table 1. Effects of damage on plant growth. Sign and strength of correlation (r^2) of plant height, seed production, and number of branches with different measures of herbivore damage ($n = 18$ plots)

Damage type	Plant height		Seed production		Number of branches	
	r^2	P	r^2	P	r^2	P
All	+0.325	0.014	+0.168	0.091	-0.077	0.266
Scraping	+0.066	0.302	+0.167	0.092	+0.004	0.814
Chewing	+0.257	0.032	+0.044	0.403	-0.132	0.139
Perforations	+0.445	0.003	+0.223	0.048	+0.001	0.903
Area (all leaves)	+0.006	0.761	+0.0001	0.966	+0.034	0.462
Area (most damaged)	+0.083	0.247	+0.067	0.301	+0.025	0.535

EXPERIMENT 2: SOIL SOURCE

All growth measures were significantly greater for plants inoculated with unsterilized soil, vs. sterilized controls (Fig. 2; Table 2). This was a large effect: inoculated plants were on average 1.5 times taller, and had 2.1 times more leaves, 2.6 times more above-ground biomass, and 2.6 times more below-ground biomass than sterile plants. In contrast, soil source had only one significant effect: number of leaves was slightly (1.09 times) greater for plants inoculated with soil from ragweed populations vs. soil from old fields (Fig. 2; Table 2). There also was a nearly significant habitat \times sterilization interaction for above-ground biomass: effects of non-sterile inoculum were marginally more positive for old-field soils than ragweed population soils. For both this experiment and

the soil feedback experiment, it is unlikely that our sterilization treatment removed absolutely all pre-existing biota from our experimental soils; however, the existence of statistical differences between sterilized and non-sterilized treatments provides evidence that our procedure was effective in significantly reducing microbial populations.

EXPERIMENT 3: SOIL FEEDBACK

Details varied among the four performance components measured, but the overall response clearly was a decrease in growth over successive serial inoculations (Fig. 3; Table 3: marginally ns for belowground biomass). In contrast, analysis of control plants indicated no significant decline in performance over time (Table 3). The decline was strongest

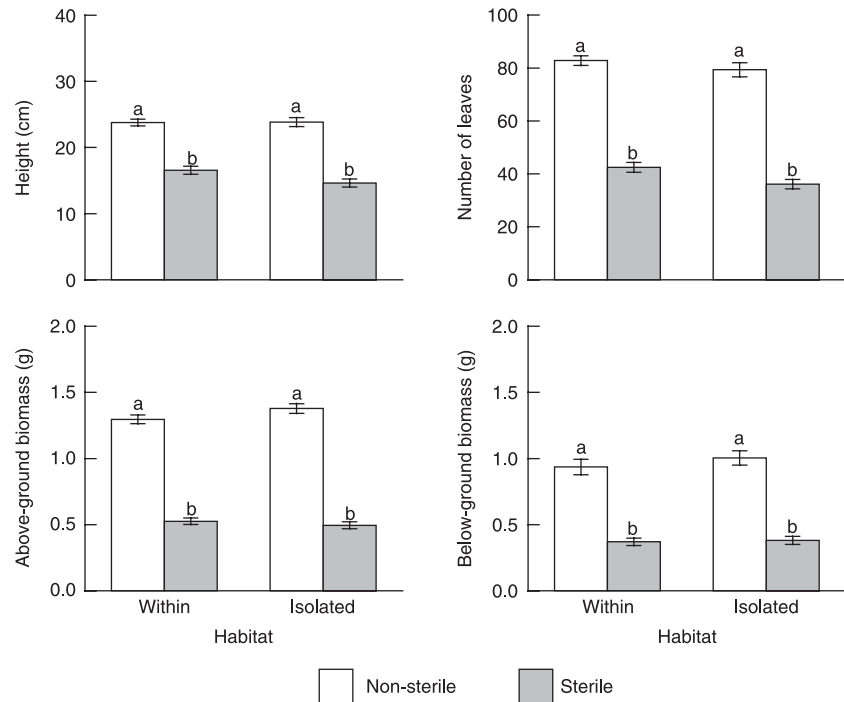


Fig. 2. Effects of soil biota on plant height, number of true leaves, above-ground biomass, and below-ground biomass \pm standard error. Treatments are inoculation with sterilized or non-sterilized soil from within ragweed populations or from old-fields isolated from ragweed. Bars sharing the same letter are not significantly different ($P > 0.05$; Tukey HSD test); see Table 2 for ANOVA results. $n = 400$ plants.

Table 2. Effects of soil biota on ragweed growth. Randomized block factorial ANOVAs for each of the four growth measures. The 'habitat' factor reflects soil drawn either from ragweed populations or from old fields lacking ragweed; the 'sterilization' factor consists of either autoclaved or unsterilized inoculum ($n = 400$ plants)

Factor	d.f.	Plant height		Number of leaves		Above-ground biomass		Below-ground biomass	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Habitat	1,387	2.427	0.120	5.929	0.015	0.723	0.396	0.767	0.382
Sterilization	1,387	189.322	< 0.0001	429.142	< 0.0001	767.047	< 0.0001	178.241	< 0.0001
Sterilization \times habitat	1,387	2.734	0.099	0.513	0.475	3.636	0.057	0.425	0.515

Table 3. Soil feedback results. Split-plot factorial ANOVAs for each of four measures of growth. The 'habitat' factor reflects soil drawn either from ragweed populations or from old fields lacking ragweed. There were three generations; each generation grew for a period of 6 weeks, either as a serial inoculation (experimental) trial or as a control ($n = 100$ plants)

Inoculation type	Factor	d.f.	Plant height		Number of true leaves		Above-ground biomass		Below-ground biomass	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Serial inoculation	Generation	2,16	11.698	0.001	10.337	0.001	6.134	0.011	3.530	0.054
	Habitat	1,8	9.234	0.016	9.287	0.016	4.690	0.062	4.395	0.069
	Generation \times Habitat	2,16	0.005	0.995	2.018	0.165	3.312	0.063	2.335	0.129
Controls	Generation	2,16	0.715	0.504	0.689	0.516	0.356	0.706	1.639	0.225
	Habitat	1,8	0.710	0.424	1.277	0.291	5.273	0.051	2.379	0.162
	Generation \times Habitat	2,16	0.968	0.401	1.074	0.365	1.500	0.253	2.752	0.094

for plants serially inoculated with biota from ragweed populations: a 23% decline for plant height, 31% for number of leaves, 70% for above-ground biomass, and 78% for below-ground biomass by the end of the experiment. Results tended to be weaker for old-field inocula: only plant height significantly

declined (by 26%), though the other variables showed a similar trend (Fig. 3; Table 3). There were no significant generation \times habitat interactions, though results were marginally non-significant for above-ground biomass ($P = 0.063$).

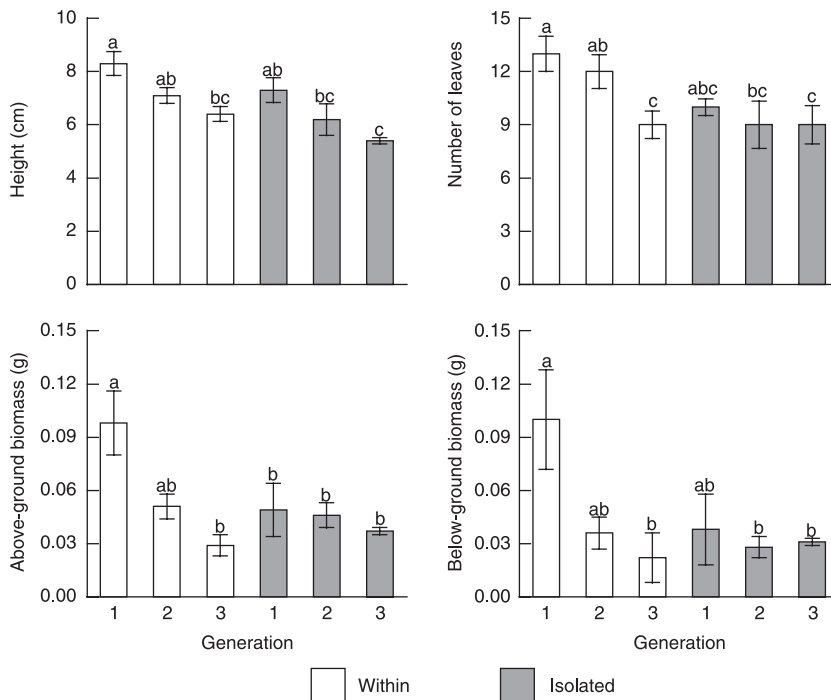


Fig. 3. Effects of soil feedback on plant height, number of true leaves, above-ground biomass, and below-ground biomass, \pm standard error. Shown are results of three 'generations' of serial inoculation with soil from within ragweed populations or from old-fields isolated from ragweed. Bars sharing the same letter are not significantly different ($P > 0.05$: Tukey HSD test); see Table 3 for ANOVA results. $n = 100$ plants.

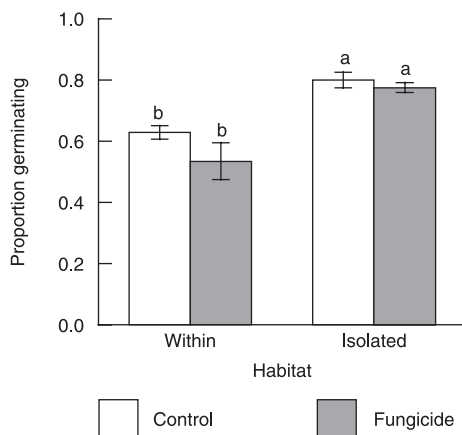


Fig. 4. Effects of soil biota on the mean proportion of seeds germinating at the end of a 5 week period, \pm standard error. Results are shown for seeds in ragweed populations or ragweed-free old fields, treated either with fungicide or water (control). Bars sharing the same letter are not significantly different ($P > 0.05$: Tukey HSD test); see text for ANOVA results. $n = 12$ plots.

EXPERIMENT 4: SEED PATHOGENS

Germination was higher for seeds buried in old fields than for seeds buried in ragweed populations ($F_{1,10} = 23.089$; $P < 0.001$; Fig. 4). Fungicide affected germination, but not in the expected direction: fungicide significantly reduced germination relative to controls ($F_{1,10} = 5.654$; $P = 0.039$; effect not detected by Tukey tests). There was no significant habitat \times fungicide interaction ($F_{1,10} = 1.889$; $P = 0.199$).

Discussion

DO ISOLATED POPULATIONS OF RAGWEED EXPERIENCE REDUCED LEVELS OF DAMAGE BY ABOVE-GROUND HERBIVORES AND SEED PREDATORS?

Leaves and seeds of ragweed were significantly less likely to be damaged by a range of herbivores when plants were isolated from existing populations, providing evidence that ragweed is able to escape enemies by colonizing new sites. The scale of migration required for this escape is small: the 100-m scale of many Janzen-Connell experiments, rather than the thousands of km typically associated with biological invasions. This small-scale escape seems unlikely to be persistent, as enemies ultimately should colonize from nearby ragweed stands, but even a transient effect could be important if it results in improved fitness. Ragweed largely depends on repeated colonization, since plants typically are competitively displaced after only a few years (Thompson 1943; Bazzaz 1968, 1974; Kosola & Gross 1999); a temporary advantage could be important if it occurs during the colonizing phase.

Ragweed also is known to escape enemies at biogeographic scales. Genton *et al.* (2005) quantified damage in natural populations and common gardens in both a non-native region (France) and a native region (Ontario). Their data indicated that French plants experienced a large average reduction (generally $< 90\%$) in both chewing and perforation (the most frequent forms of damage) compared to Ontario plants, though rates of the less-common scraping damage were similar. For chewing damage, the differences they found

between continents were much greater than our among-treatment differences (27% reduction between within vs. isolated treatments); for perforation damage, our estimate was more similar (80% reduction between *within* vs. *isolated* treatments). However, Genton *et al.* (2005) used a sampling procedure that differed significantly from most of our measurements: they sampled the 3–5 most damaged leaves per plant, which likely inflated these treatment effects relative to our approach.

Both our results and those of Genton *et al.* (2005) suggest escape from both specialist and generalist enemies. Although our study was designed to quantify damage to plants, rather than unambiguously identify the source of that damage, observations indicated that the commonest herbivores included both specialists (scraping, chewing: *Tarachidia* spp.; chewing: *Zygogramma suturalis*) and generalists (chewing: snails; perforations: *Systema blanda*; seed predation *Harpalus rufipes*). One might expect that escape from specialists should be more likely than escape from generalists, since specialists are unlikely to occur in areas previously unoccupied by the host (Müller-Schärer *et al.* 2004; Joshi & Vrieling 2005). Contrary to this expectation, all classes of damage declined with distance, even though most were at least partially due to generalists. It may be that the conspicuous generalists observed on ragweed were common on this plant because they preferred it over alternative foods, and thus locally behaved as specialists; for example, *Systema blanda* was rarely observed on other plants at this site, despite a broad host range (Cranshaw 2004).

One criticism of the Enemy Release Hypothesis is that reduced damage may not translate to greater growth and reproduction (Maron & Vilà 2001; Hierro *et al.* 2005). This may be the case for ragweed: despite reduced herbivory, we detected no evidence that isolated populations enjoyed improved performance. In fact, plant height and seed production were positively correlated with some measures of damage, perhaps because larger plants were more attractive to herbivores. We suspect that levels of leaf damage simply were too low to have had strong effects on host performance. Similarly, rates of seed damage likely were insufficient for ecological consequences, although sampling while seeds were still maturing may have underestimated cumulative impacts. In their transatlantic study, Genton *et al.* (2005) found mixed evidence for improved performance in less-damaged plants. In contrast with our results, they found that foliar damage was negatively correlated with plant height, but this did not result in greater size in non-native areas despite the lack of local herbivores; reproduction was not measured in this study. Finally, European efforts to use herbivores for biocontrol (most frequently *Zygogramma suturalis*) have met with very limited success (Kiss 2007). Thus, escape from insect enemies potentially may occur in both native and exotic populations without pronounced population consequences; this may be a common circumstance for biological invaders, and suggests a need for caution in biocontrol efforts.

DOES RAGWEED ESCAPE NEGATIVE EFFECTS OF SOIL BIOTA BY COLONIZING NEW SITES?

Our experimental results indicate a strong positive effect of soil biota on the growth of ragweed, regardless of habitat. Ragweed previously has been reported to be obligately mycorrhizal (Crowell & Boerner 1988; Koide & Li 1991; Fumanal *et al.* 2006), although Fumanal *et al.* (2006) also reported that natural populations of ragweed had surprisingly low mycorrhizal colonization levels: between 1% and 40% of roots infected. However, these studies examined the effects of only a single AM-mycorrhizal fungus (*Glomus etunicatum* Becker & Gerdeman or *Glomus intraradices* Schenck & Smith). In contrast, our study is the first to examine the effects on ragweed of the soil community as a whole, including the potentially conflicting effects of mutualists and pathogens. Such studies can have results quite different from single-species investigations. For example, even though *Centaurea maculosa* L. is AM-mycorrhizal (Callaway *et al.* 2004a), Callaway *et al.* (2004b) found it produced significantly more biomass in sterilized soil vs. non-sterilized soil. This effect was more pronounced in native Eurasian soils than non-native American soils (mean biomass increase of 166% compared to 24%), providing evidence that escape from the native soil community benefited this plant.

Ours also is the only study to examine interactions of ragweed with the soil community of different habitats. Ragweed primarily is found in recently disturbed sites; in principle, this might reflect the availability of more beneficial mycorrhizal associations in disturbed sites. However, other than a very slight increase in leaf number in plants inoculated with soil from ragweed populations, we found no evidence of habitat-related differences in performance. As well, the lack of a habitat × sterilization interaction suggests that even this difference in leaf number may not have resulted from a difference in soil biota between habitats. It may be that suitable mycorrhizal fungi are sufficiently available even in sites lacking ragweed populations, consistent with the generalist nature of many AM associations (Bever *et al.* 1997; Johnson *et al.* 1997; Klironomos 2003).

These results also might seem to suggest that pathogens are no more prevalent in areas dominated by ragweed than in uncolonized sites; however, it is possible that such a difference might develop over time as populations of root pathogens accumulate (Bever 1994; Klironomos 2002). We did find evidence for this sort of negative feedback in our serial inoculation experiment: plant height, number of leaves, and biomass all declined over time. This effect was stronger in plants serially inoculated with soil originating from ragweed populations than in plants treated with old-field soil, and was absent in non-serial controls. These results suggest that soil pathogens present on ragweed-dominated sites may progressively reduce the performance of ragweed populations; on the other hand, colonization of new (old field) sites may provide temporary respite from pathogen attack. Along with competition and herbivory, this negative feedback may contribute to the ephemeral nature of many ragweed

populations (Thompson 1943; Bazzaz 1968, 1974; Kosola & Gross 1999).

DO RAGWEED SEEDS ESCAPE ATTACK BY SOIL-BORNE FUNGI IN NEWLY COLONIZED SITES?

We predicted that seeds buried in ragweed populations would experience higher mortality caused by fungal pathogens than seeds buried in ragweed-free old fields. Although we did find germination was reduced following burial under ragweed, it was not improved by treatment with fungicide. This fails to demonstrate that fungal pathogens were responsible for the reduction in germination, though it still may have been caused by a fungus or other pathogen not controlled by our fungicide treatment.

Fungal pathogens can have important impacts on seed banks (Baskin & Baskin 1998). This is a risk even in winter: recent research has demonstrated considerable microbial activity in snow-covered soils (Schmidt & Lipson 2004), which can result in significant winter mortality of buried seeds (O'Hanlon-Manners & Kotanen 2004, 2006). As an annual, ragweed entirely depends upon over-wintering seeds for survival; as a disturbance-dependent species, its persistent seed bank lets propagules remain dormant until an opportunity for establishment occurs (Willemsen & Rice 1972; Willemsen 1975). For both of these reasons, the ability to avoid attack by seed pathogens may be crucial for this plant. Our results suggest dispersal to new sites may improve seed survival, although it is not clear that escape from pathogens is the explanation.

SYNTHESIS

Overall, our results indicate that *Ambrosia artemisiifolia*, known to escape at least herbivores during long-distance (> 1000 km) invasions, also escapes enemies at the local (100 m) scale. Local-scale escape is a general phenomenon, occurring for both above-ground and below-ground enemies, and results in reduced levels of leaf herbivory, seed predation, soil feedback, and perhaps losses to seed pathogens. These results link native-range ecology with the Enemy Release Hypothesis by demonstrating that escape from enemies need not require a transoceanic invasion; however, they also suggest that for this species, the resulting advantages often may be small. For many species exotic populations may face different selective pressures than native populations (Bossdorf *et al.* 2005), but for colonizing populations of common ragweed, the biotic environment may not be so different at home or abroad.

Acknowledgements

This research was supported by NSERC Research and Equipment Grants (PMK), the Ontario Graduate Scholarship program (JM), and the Koffler Scientific Reserve at Jokers Hill. Thanks to Kateryna Kostyukova for her continuous help, to Steve Hill and Andrew MacDonald for their support, discussions, and field assistance, and to two anonymous reviewers for their suggestions. This is a publication of the Koffler Scientific Reserve.

References

- Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Klironomos, J., Power, A.G. & Godsoe, W. (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. *Ecology*, **86**, 2979–2989.
- Alexander, H.M., Price, S., Houser, R., Finch, D. & Tourtellot, M. (2007) Is there reduction in disease and pre-dispersal seed predation at the border of a host plant's range? Field and herbarium studies of *Carex blanda*. *Journal of Ecology*, **95**, 446–457.
- Baskin, C.C. & Baskin, J.M. (1998) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.
- Bassett, I.J. & Crompton, C.W. (1975) The biology of Canadian weeds. 11. *Ambrosia artemisiifolia* L. and *Ambrosia psilostachya* DC. *Canadian Journal of Plant Science*, **2**, 463–476.
- Bassett, I.J. & Terasmae, J. (1962) Ragweeds, *Ambrosia* species, in Canada and their history in postglacial time. *Canadian Journal of Botany*, **40**, 141–150.
- Bazzaz, F.A. (1968) Succession on abandoned fields in the Shawnee Hills, southern Illinois. *Ecology*, **49**, 924–936.
- Bazzaz, F.A. (1974) Ecophysiology of *Ambrosia artemisiifolia*: a successional dominant. *Ecology*, **55**, 112–119.
- Bever, J.D. (1994) Feedback between plants and their soil communities in an old field community. *Ecology*, **75**, 1965–1977.
- Bever, J.D., Westover, K.M. & Antonovics, J. (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, **85**, 561–573.
- Blaney, C.S. & Kotanen, P.M. (2001) Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology*, **38**, 1104–1113.
- Blaney, C.S. & Kotanen, P.M. (2002) Persistence in the seed bank: an experimental comparison of native and alien plants. *Écoscience*, **9**, 509–517.
- Bosdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1–11.
- Bray, S.R., Kitajima, K. & Sylvia, D.M. (2003) Mycorrhizae differentially alter growth, physiology, and competitive ability of an invasive shrub. *Ecological Applications*, **13**, 565–574.
- Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W. & Gannon, J.E. (2004a) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. *Ecology*, **85**, 1062–1071.
- Callaway, R.M., Thelen, G.C., Rodriguez, A. & Holben, W.E. (2004b) Soil biota and exotic plant invasion. *Nature*, **427**, 731–733.
- Chauvel, B., Dessaint, F., Cardinal-Legrand, C. & Bretagnolle, F. (2006) The historical spread of *Ambrosia artemisiifolia* L. in France from herbarium records. *Journal of Biogeography*, **33**, 665–673.
- Colautii, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, **7**, 721–733.
- Condit, R., Hubbell, S.P. & Foster, R.B. (1992) Recruitment near conspecific adults and the maintenance of tree and shrub diversity in a neotropical forest. *The American Naturalist*, **140**, 261–286.
- Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. *Dynamics of Populations: Proceedings of the Advanced Study Institute on Dynamics of Numbers of Populations* (eds P.J. Den Boer & G. Gradwell), pp. 298–312. Center for Agricultural Publishing and Documentation, Wageningen.
- Cranshaw, W. (2004) *Garden Insects of North America*. Princeton University Press, Princeton.
- Crowell, H.F. & Boerner, R.E. (1988) Influences of mycorrhizal and phosphorus on belowground competition between two old-field species. *Environmental and Experimental Biology*, **28**, 381–392.
- DeWalt, S.J., Denslow, J.S. & Ickes, K. (2004) Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology*, **85**, 471–483.
- di Castri, F. (1989) History of biological invasions with emphasis on the Old World. *Biological Invasions: a Global Perspective* (eds J. Drake, F. di Castri, R. Groves, F. Kruger, H.A. Mooney, M. Rejmánek & M. Williamson), pp. 1–30. Wiley, New York.
- Fumanal, B., Plenchette, C., Chauvel, B. & Bretagnolle, F. (2006) Which role can arbuscular mycorrhizal fungi play in the facilitation of *Ambrosia artemisiifolia* L. invasion in France? *Mycorrhiza*, **17**, 25–35.
- Genton, B.J., Kotanen, P.M., Cheptou, P.-O., Adolphe, C. & Shykoff, J.A. (2005) Enemy release but no evolutionary loss of defence during ragweed invasion of France: an inter-continental reciprocal transplant experiment. *Oecologia*, **146**, 404–414.

- Guo, Q. (2006) Intercontinental biotic invasions: what can we learn from native populations and habitats? *Biological Invasions*, **8**, 1451–1459.
- Harms, K.E., Wright, S.J. & Calderon, O. (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, **404**, 493–495.
- Hierro, L.J., Maron, J.L. & Callaway, R.M. (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, **93**, 5–15.
- Hyatt, L.A., Rosenberg, M.S., Howard, T.G., Bole, G., Fang, W. & Anastasia, J. (2003) The distance dependence prediction of the Janzen–Connell hypothesis: a meta-analysis. *Oikos*, **103**, 590–602.
- Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *The American Naturalist*, **104**, 501–528.
- Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–585.
- Joshi, J. & Vrieling, K. (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters*, **8**, 704–714.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, **17**, 164–170.
- Kirk, R.E. (1995) *Experimental Design: Procedures for the Behavioural Sciences*, 3rd edn. Brooks/Cole, Pacific Grove.
- Kiss, L. (2007) Why is biological control of common ragweed, the most allergenic weed in Eastern Europe, still only a hope? *Biological Control: a Global Perspective* (eds C. Vincent, M.S. Goettel & G. Lazarovits), pp. 80–91. CABI, Wallingford.
- Klironomos, J. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, **84**, 2292–2301.
- Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.
- Koide, R.T. & Li, M. (1991) Mycorrhizal fungi and the nutrient ecology of three old field annual species. *Oecologia*, **85**, 403–412.
- Kosola, K.R. & Gross, K.L. (1999) Resource competition and suppression of plants colonizing early successional old fields. *Oecologia*, **118**, 69–75.
- Kruess, A. & Tschamtkke, T. (2000) Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. *Oecologia*, **122**, 129–137.
- Levine, J.M., Vilà, M., D'Antonio, C.M., Dukes, J.S., Grigulis, K. & Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Biological Sciences*, **270**, 775–781.
- Liu, H. & Stiling, P. (2006) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8**, 1535–1545.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.
- Maron, J.L. & Vilà, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361–373.
- Maryushkina, V.Y. (1991) Peculiarities of common ragweed (*Ambrosia artemisiifolia* L.) strategy. *Agriculture, Ecosystems & Environment*, **36**, 207–216.
- McAndrews, J.H. (1988) Human disturbance of North American forests and grasslands: the fossil pollen record. *Vegetation History* (eds B. Huntley & T. III Webb), pp. 673–697. Kluwer Academic Publishers, Dordrecht.
- Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N. *et al.* (2006) Biotic interactions and plant invasions. *Ecology Letters*, **9**, 726–740.
- Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology and Evolution*, **19**, 417–422.
- Neergaard, P. (1977) *Seed Pathology*, vol. 1. Halsted Press, New York.
- O'Hanlon-Manners, D.L. & Kotanen, P.M. (2004) Logs as refuges from fungal pathogens for seeds of Eastern Hemlock (*Tsuga canadensis*). *Ecology*, **85**, 284–289.
- O'Hanlon-Manners, D.L. & Kotanen, P.M. (2006) Losses of seeds of temperate trees to soil fungi: effects of habitat and host ecology. *Plant Ecology*, **187**, 49–58.
- Packer, A. & Clay, K. (2000) Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature*, **404**, 278–281.
- Packer, A. & Clay, K. (2003) Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees. *Ecology*, **84**, 108–119.
- Reinhart, K.O. & Callaway, R.M. (2004) Soil biota facilitate exotic *Acer* invasions in Europe and North America. *Ecological Applications*, **14**, 1737–1745.
- Reinhart, K.O. & Callaway, R.M. (2006) Soil biota and invasive plants. *New Phytologist*, **170**, 445–457.
- Reinhart, K.O., Packer, A., Van der Putten, W.H. & Clay, K. (2003) Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046–1050.
- Schafer, M. & Kotanen, P.M. (2003) The influence of soil moisture on losses of buried seeds to fungi. *Acta Oecologica*, **24**, 255–263.
- Schmidt, S.K. & Lipson, D.A. (2004) Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant and Soil*, **259**, 1–7.
- Sharville, E.G. (1961) *The Nature and Uses of Modern Fungicides*. Burgess Publishing, Minneapolis.
- Teshler, M.P., DiTommaso, A., Gagnon, J.A. & Watson, A.K. (2002) *Ambrosia artemisiifolia* L. common ragweed (Asteraceae). *Biological Control Programs in Canada 1981–2000* (eds P.G. Mason & J.T. Huber), pp. 290–294. CABI, Wallingford.
- Thompson, J.W. (1943) Plant succession on abandoned fields in the central Wisconsin sand plain area. *Bulletin of the Torrey Botanical Club*, **70**, 34–41.
- Torchin, M.E. & Mitchell, C.E. (2004) Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment*, **2**, 183–190.
- Torgeson, D.C. (1969) *Fungicides: an Advanced Treatise*, vol. 2. Academic Press, New York.
- Van Grunsven, R.H.A., Van der Putten, W.H., Bezemer, T.M., Tamis, W.L.M., Berendse, F. & Veenendaal, E.M. (2007) Reduced plant–soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology*, **95**, 1050–1057.
- Vitousek, P.M., D'Antonio, C.M., Loope, L.L. & Westbrooks, R. (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468–478.
- Willemsen, R.W. (1975) Dormancy and germination of common ragweed seeds in the field. *American Journal of Botany*, **62**, 639–643.
- Willemsen, R.W. & Rice, E.L. (1972) Mechanism of seed dormancy in *Ambrosia artemisiifolia*. *American Journal of Botany*, **59**, 248–257.
- Wills, C., Condit, R., Foster, R.B. & Hubbell, S.P. (1997) Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 1252–1257.
- Wolfe, B.E. & Klironomos, J.N. (2005) Breaking new ground: soil communities and exotic plant invasion. *BioScience*, **55**, 477–487.
- Wright, S.J. (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, **130**, 1–14.

Received 17 March 2008; accepted 26 June 2008

Handling Editor: Rob Brooker