

Testing the directed dispersal hypothesis: are native ant mounds (*Formica* sp.) favorable microhabitats for an invasive plant?

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Abstract Ant-mediated seed dispersal may be a form of directed dispersal if collected seeds are placed in a favorable microhabitat (e.g., in or near an ant nest) that increases plant establishment, growth, and/or reproduction relative to random locations. We investigated whether the native ant community interacts with invasive leafy spurge (*Euphorbia esula*) in a manner consistent with predictions of the directed dispersal hypothesis. Resident ants quickly located and dispersed 60% of experimentally offered *E. esula* seeds. Additionally, 40% of seeds whose final deposition site was observed were either brought inside or placed on top of an ant nest. Seed removal was 100% when seeds were placed experimentally on foraging trails of mound-building *Formica obscuripes*, although the deposition site of these seeds is unknown. Natural density and above-ground biomass of *E. esula* were greater on *Formica* mound edges compared to random locations. However, seedling recruitment and establishment from experimentally planted *E. esula* seeds was not greater on mound edges

than random locations 3 m from the mound. Soil from *Formica* mound edges was greater in available nitrogen and available phosphorus relative to random soil locations 3 m from the mound. These results suggest *Formica* ant mounds are favorable microhabitats for *E. esula* growth following seedling establishment, a likely consequence of nutrient limitation during plant growth. The results also indicate positive species interactions may play an important role in biological invasions.

Keywords Myrmecochory · Seed dispersal · *Euphorbia esula* · Invasive species · Species interactions

Introduction

Ant-mediated seed dispersal, or myrmecochory, is a widespread species interaction that influences plant populations and community structure (Beattie and Culver 1981; Bond and Slingsby 1984; Kalisz et al. 1999; Heinken and Winkler 2009). Occurring in over 11,000 plant species in at least 77 families (Lengyel et al. 2009), myrmecochores typically produce seeds with a lipid-rich structure (elaiosome) that serves as a nutrient-rich food source for ants (Fischer et al. 2008). Ants can serve as secondary seed dispersers (e.g., Beaumont et al. 2009), collecting seeds on the ground and moving them away from the parent plant. Often, collected seeds are brought to the nest where the elaiosome is consumed. Seeds then either remain in the nest or are deposited outside the nest in refuse piles (Beattie 1985; Servigne and Detrain 2008; Renard et al. 2010).

Several mechanisms have been proposed to explain the advantage of myrmecochory from the plant's perspective (Beattie 1985). The dispersal-for-distance hypothesis proposes that ants may decrease parent–offspring conflict or

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sibling competition simply by moving seeds away from the parent's seed shadow (Andersen 1988; Higashi et al. 1989; Kalisz et al. 1999). Other hypotheses consider the advantage of seed burial by ants, including predator-avoidance (Heithaus 1981; Bond and Breytenbach 1985; Boulay et al. 2009), protection from fire (Berg 1975; Bond and Slingsby 1984), and directed dispersal (Howe and Smallwood 1982). The directed dispersal hypothesis proposes that ant nests serve as favorable microhabitats that increase plant growth, survival, and/or fitness relative to random locations (Howe and Smallwood 1982; Wenny 2001).

Support for the directed dispersal hypothesis stems from observations that ant nest soils often have elevated nutrient availability and moisture relative to surrounding areas (Beattie and Culver 1983; Horvitz and Schemske 1986a; Wagner et al. 2004). Subsequently, seeds buried in ant nests have shown greater seedling recruitment and/or establishment (e.g., Culver and Beattie 1980; Hanzawa et al. 1988), as well as greater survival and reproduction of later-staged plants (Hanzawa et al. 1988; Gibson 1993), although support for this outcome is not universal (see Giladi 2006). Support for the directed dispersal hypothesis is also variable across habitats and plant growth forms (Giladi 2006). In addition, studies of the directed dispersal hypothesis, and myrmecochory in particular, often focus on interactions between native species. Many introduced (i.e., non-native) plant species in North America, including several that are considered invasive (i.e., introduced species causing large amounts of economic and/or ecological damage), produce elaiosome-bearing seeds (Pemberton and Irving 1990), and several studies have documented native ants dispersing invasive plant seeds in North America (Pemberton 1988; Bossard 1991; Jensen and Six 2006). However, the role novel interactions with resident ants play in an invasive plant's success at the local scale is relatively unknown.

We tested whether native ants confer benefits consistent with predictions under the directed dispersal hypothesis to an invasive, myrmecochorous plant (*Euphorbia esula* L., leafy spurge, Euphorbiaceae) in Wisconsin, USA. Originally from Europe and Russia (Dunn 1985), *E. esula* was first detected in North America in Massachusetts in 1827 (Dunn 1979), and has since spread throughout much of North America (Dunn 1985). *Euphorbia esula* is a clonal, perennial plant capable of producing over 200 seeds per stem (Selleck et al. 1962). Primary dispersal of seeds occurs by explosive propulsion following fruit dehiscence that can move seeds up to several meters from the parent plant (Selleck et al. 1962). Secondary dispersal occurs via a variety of vectors, including water (Bakke 1936), birds (Noble 1980; Blockstein et al. 1987), mammals (Messer-smith et al. 1985; Lacey et al. 1992; Olson et al. 1997; Wald et al. 2005), and ants (*Formica obscuripes* Forel, Pemberton 1988). The species identity of ant dispersers,

other than *F. obscuripes*, and the fate of collected seeds remain poorly known (Pemberton 1988).

We performed a series of observations and experiments to test how native ants may benefit *E. esula* under predictions of the directed dispersal hypothesis. First, we measured removal of *E. esula* seeds and determined disperser species' identities and deposition sites of dispersed seeds. Second, we investigated whether *Formica* ant mounds are favorable microhabitats for *E. esula* at a variety of development stages. Specifically, we used a series of on- and off-ant mound comparisons to (1) quantify *E. esula* seedling recruitment and establishment for experimentally planted seeds, (2) quantify density and above-ground biomass for naturally occurring *E. esula* plants, and (3) investigate soil characteristics that may influence *E. esula* growth and reproduction. Under predictions of the directed dispersal hypothesis, *F. obscuripes* are expected to deposit *E. esula* seeds within their mounds, and these mounds provide favorable conditions for seedling recruitment, establishment, and/or growth. Focusing on *F. obscuripes* interactions with *E. esula*, this research builds upon previous observations of myrmecochory between an invasive plant and native ant (Pemberton 1988) and tests how this interaction benefits *E. esula*.

Materials and methods

Study site

Research was conducted within the Oak Barrens community natural area (sand oak savanna habitat) at Fort McCoy Military Installation (43°59'N, 90°40–42'W) near Sparta, WI, USA. The site has experienced low levels of disturbance in recent history due to its status as a state natural area. Common plants in the community include: oaks (*Quercus* spp.), *Tephrosia virginiana*, *Tradescantia ohioensis*, and *Euphorbia corollata* and *E. esula* (introduced). The sand soil in this habitat is typically low in nutrient availability, including nitrogen (Grigal et al. 1974). At Fort McCoy, *E. esula* sets seed in mid-July and again in early fall if seasonal conditions are appropriate (M. Berg-Binder, personal observations; personal communications with N. Tucker, 2008).

At least ten ant species are common at Fort McCoy (M. Berg-Binder, personal observations), including the western thatching ant (*F. obscuripes*). *F. obscuripes* is a widespread ant found throughout western North America that creates thatch-covered mounds encircled by a ridge of sand (mound edge) and, often, thick vegetation (typical nest diameter = 60–110 cm; Weber 1935). This thick vegetation ring (Online Resource 1) typically includes *E. esula*, when present, as well as other plants species (e.g., *Ambrosia* sp., *T. virginiana*, and a

variety of graminoids) in varying abundances at Fort McCoy (M. Berg-Binder, personal observations). The pattern of *E. esula* encircling *F. obscuripes* mounds is consistent with observations made by Pemberton (1988) in Montana rangelands where dispersal of *E. esula* by *F. obscuripes* was first observed, and there are no known reports or personal observations of *E. esula* growing in circles when *Formica* mounds are absent (M. Berg-Binder, personal observations). We used 46 *Formica* mounds (45 *F. obscuripes* and 1 *F. exsectoides* as later determined in the laboratory) for this 3-year study (2008–2010). A subset of these 46 mounds was chosen to be included in each of the observations or experiments, based upon selection criteria appropriate for the hypothesis or question being addressed (described below).

Experimental design and analysis

Seed removal by native ants

Fruit/seed collection In both 2009 and 2010, *E. esula* fruits were collected from plants on-site when nearly mature (late June/early July), placed in envelopes, and kept at room temperature to allow for explosive fruit dehiscence. Within several days of fruit dehiscence, seeds were sorted from fruit capsules and refrigerated to maintain elaiosome freshness. Seeds were used for either indirect or direct observations to quantify *E. esula* seed removal by native ants (described below).

Indirect observations (2009) Five seed depots (small wire cage surrounding a covered Petri dish with three entry holes for ant access) and one control depot (same as experimental depots, except the Petri dish was raised on a stake covered with Tree Tanglefoot Insect Barrier) were provided to ants in mid-July (17 July 2009) following fruit maturation and seed set of collected fruits in each of three *Euphorbia esula* patches (total = 15 experimental depots and 3 control depots). The timing of seed provisioning to the ant community approximates when seeds are available from naturally growing *E. esula*. The experimental seed depots were spaced 10–20 m apart in an effort to attract foraging ants from different colonies; ants typically disperse seeds no more than a few meters (Parr et al. 2007). The three *E. esula* patches were over 100 m from each other and considered independent. While *Formica* mounds were present in the vicinity of the depots and variable in density across depot sites, depot placement was random with respect to *Formica* mound location as the objective of these observations was to determine removal rates of *E. esula* seeds by the entire ant community. Each depot started with 20 seeds, a typical number of seeds used in previous studies of myrmecochory (e.g., Pemberton 1988), and the number of seeds removed after 24 and 96 h was recorded.

Direct observations (2010) We complemented the seed depot observations with direct observations of seeds placed near *F. obscuripes* mounds in the summer of 2010. These observations were done to determine (1) the role of *F. obscuripes* in *E. esula* dispersal, and (2) the identity and dispersal behavior of native ant community members dispersing *E. esula* seeds. Mounds with a suitable amount of surrounding *E. esula* plants ripe for seed collection were determined at the field site. A subset of these mounds ($n = 5$ mounds) was randomly selected in July 2010 and seeds were harvested (as described above). Between 13 and 28 July 2010 and again on 3 September 2010, seed removal observations ($n = 9$ observations total) were conducted for 2-h periods (5-min observations with a 1-min rest) during fair weather condition days between 0845 and 1215 hours. Seed set of *E. esula* generally occurs in July and again in early fall (M. Berg-Binder, personal observations; N. Tucker, personal communications). During each observation period, ten seeds were placed on open ground in a randomly selected location 1–2 m from one of five experimental mounds. Caches of ten seeds were used, rather than the 20 seeds provided in the seed depots (above), because a larger seed number would have made conducting observations too difficult. While 3 mounds were used for multiple observation periods to increase the total number of observations, a new location was selected for the placement of ten new seeds. Because the only previously reported observation of *E. esula* seeds being dispersed by ants in North America included seeds placed directly on *F. obscuripes* foraging trails (Pemberton 1988), we conducted additional observations ($n = 3$) of ten seeds each placed directly on high-traffic *Formica* foraging trails at a distance of 3 m from the mounds. The deposition site of removed seeds was recorded as either taken to a nest entrance or abandoned on the ground, and individual workers engaged in myrmecochory (or her nest mates) were collected, stored in 100% ethanol, and identified to species in the laboratory. Ant identification was necessary because a variety of ant species collected the seeds, despite their close location to *Formica* mounds.

Seedling recruitment and establishment of experimentally planted seeds

Seed collection *Euphorbia esula* seeds were collected in fruits at two nearby sites with appropriate *E. esula* and *F. obscuripes* mound abundances for this experiment along sampling transect grids during the summers of 2008 and 2009. Following collection, fruits were pooled within local source populations (small-scale patches of *E. esula* within the study sites) and allowed to mature and dehisce in envelopes to produce seed. Seeds were stored at room temperature for <6 months prior to planting. Care was taken to

plant seeds from the appropriate local source populations so as not to artificially alter genetic variability across *E. esula* patches at Fort McCoy.

Seed planting We used a paired design to compare *E. esula* seedling recruitment and establishment for planted seeds on *Formica* mound edges (hereafter, on-mounds) and randomly chosen locations off-mounds over 2 years ($n = 6$ pairs planted in 2008, $n = 10$ pairs planted in 2009). Planting and monitoring protocols changed slightly across the 2 years and are noted when different. Each seed was planted at a depth of 3 cm (Selleck et al. 1962) at 10-cm intervals on-mound along the edge where *E. esula* can be found growing naturally (Online Resource 1; $n = 17$ –30 seeds, dependent upon mound circumference) and in a ‘circle’ off-mound at a distance of 3 m ($n = 30$ seeds in 2008; $n =$ matched on-mound seed number in 2009). Seeds were planted in October 2008 and July 2009 and approximated when seeds would be available for ants to collect and deposit in nests; fruits typically mature in July and again in early fall at Fort McCoy (M. Berg-Binder, personal observations; N. Tucker, personal communications).

Seedling monitoring The location of each planted seed was marked with a bamboo skewer and monitored the following growing season (mid-April to late July; weekly in 2009, monthly in 2010) for evidence of seedling recruitment (emergence of a seedling with cotyledons) and seedling establishment (survival through a final early September monitoring). Because some markers were inevitably lost during the experiment due to disturbance (likely deer trampling or human foot traffic), only seeds whose markers remained present until September were included in the analysis. In addition, loss of markers past 1 year made monitoring for seedlings following seed dormancy not possible. However, the majority of *E. esula* seedlings emerge during the first year (Selleck et al. 1962), minimizing concerns relating to seed dormancy. The proportion of seeds reaching the seedling recruitment and establishment stages were compared on- and off-mounds using a paired Wilcoxon rank sum test for nonparametric data for both years.

Naturally growing density and above-ground biomass

Euphorbia esula density (stem number in a 1×0.5 m quadrat) for both reproductive (evidence of flowering) and non-reproductive stages were measured on-mound and at distances of 1, 2, and 3 m off-mound in random directions for all mounds ($n = 11$) present in established patches of *E. esula* within one randomly selected site ($=2.30$ ha). Mounds present within the area that had no *E. esula* growing nearby were excluded from the analysis ($n = 5$, total $n = 16$ mounds/2.30 ha). Reproductive and non-reproductive stems were differentiated to assess the possibility of mound effects

on sexual reproductive potential (=reproductive) and clonal spread (=total: reproductive and non-reproductive combined). Similar density protocols were followed in June 2010 (with the exception of off-mound density including additional distances of 5 and 10 m) for all mounds censused in 2009 with *E. esula* present (minus one mound that no longer showed evidence of ant activity in 2010), plus additional mounds from a nearby site of similar size ($n = 24$ mounds/2.39 ha); all mounds located within this nearby site were found within *E. esula* infestations. Therefore, a total of 34 mounds were sampled across the two sites in 2010. Mound thatch diameter, often an indication of colony age (Klimetzek 1981; Tschinkel 1999), was also measured for each mound in 2010 to determine if *E. esula* density increased with mound diameter ($n = 34$ mounds). Above-ground biomass of *E. esula* was determined on- and off-mounds at a distance of 3 m ($n = 8$ mound pairs) by cutting all stems within a 0.5-m^2 quadrat at ground level in mid-July 2010, drying in an oven at 38°C for over 48 h, and weighing on a Mettler PM4800 DeltaRange[®] scale.

We compared density of *E. esula* on- and off-mounds with Friedman’s tests for nonparametric unreplicated blocked data, with mound as the block and distance from each mound as the independent variable. Because the sampling design differed between years, Friedman’s tests were conducted separately for each year and type of density [total plant density, density of reproductive individuals only, and the proportion of reproductive individuals relative to the total (with quadrats of no stems included as proportions equal to zero)], for a total of six tests (2009: reproductive, total, and proportion of reproductive to total; 2010: reproductive, total, and proportion of reproductive to total). Above-ground biomass was compared with a paired Wilcoxon rank sum test for nonparametric data for each mound pair. Mound diameter and both reproductive and total density on-mound were tested for independence using a Spearman’s rank correlation for nonparametric data.

Soil characteristics

We used a paired design to sample soil characteristics on-mounds and 3 m from mounds ($n = 9$ mound pairs) in September 2009. Each soil sample consisted of four subsamples collected at a depth of 0–10 cm around the mound edge (or a ‘circle’ for off-mound collections), sifted in the field through a sieve (2 mm) to remove debris, and combined in a paper soil collection bag prior to storage at room temperature for less than 3 weeks. Samples were shipped to A&L Great Lakes Lab (<http://www.algreatlakes.com/>), Fort Wayne, IN, USA, where they were dried overnight at 40°C , crushed, and sieved (2 mm) prior to analysis. Available phosphorus content (P, ppm) was determined by the Bray-1 method following Mehlich III extraction. Available nitrogen

in the forms of nitrate-nitrogen (NO_3N , ppm) and ammonium-nitrogen (NH_4N , ppm) were found by nitrate reduction and the phenolate method, respectively, following extraction by 1 N KCl extraction. Percent total nitrogen was determined by the Dumas method. Percent organic matter was found by loss on ignition (360°C for 2 h). Available phosphorus, ammonium-nitrogen, nitrate-nitrogen, and percent total nitrogen were compared on- and off-mounds using a paired Wilcoxon rank sum test for nonparametric data, while percent organic matter was compared with a paired t test. We did not adjust p values for multiple comparisons (e.g., Bonferroni adjustments) because variables measured were developed from biologically relevant a priori hypotheses and adjustments would cause an unwarranted reduction in statistical power given the sample sizes (Nakagawa 2004).

All data for this study were analyzed in R 2.1.10.1 (2009-12-14) (<http://www.R-project.org>).

Results

Seed removal by native ants

Native ants quickly detected and removed *E. esula* seeds. In 2009, the majority of seeds offered in depots were removed within 24 h (Fig. 1). Fifty-four of 90 seeds placed off foraging trails near *F. obscuripes* mounds in 2010 were removed by at least four species [*F. obscuripes*, *Aphaenogaster* sp. (*fulva* complex), and two *Myrmica* sp.] within 2 h. The deposition site for 16 of these seeds was observed, with 9 abandoned and 7 taken to an ant nest. We did not determine whether the abandoned seeds were eventually re-collected by ants, although this remains a possibility. All 30 seeds placed directly on *F. obscuripes* foraging trails in 2010 were removed within 2 h. The seed deposition sites were difficult to determine due to the high amount of ant traffic on the trail; however, 3 seeds were observed as abandoned away from the *F. obscuripes* mound and 1 seed was taken to the mound.

Seedling recruitment and establishment

Euphorbia esula seedling recruitment did not differ on-mounds compared to 3 m off-mounds in either year (2009: $V = 7$, $P = 0.56$; 2010: $V = 30$, $P = 0.85$) (Fig. 2) and seedling establishment was no different on- and off-mounds in 2009 ($V = 8$, $P = 0.69$) (Fig. 2a). However, seedling establishment was slightly greater off- than on-mounds in 2010 ($V = 3.5$, $P = 0.05$ with continuity correction) (Fig. 2b).

Density and above-ground biomass

Density of sexually reproductive *E. esula* stems was greater on-mounds in both years (2009: $\chi^2 = 8.08$, $df = 3$,

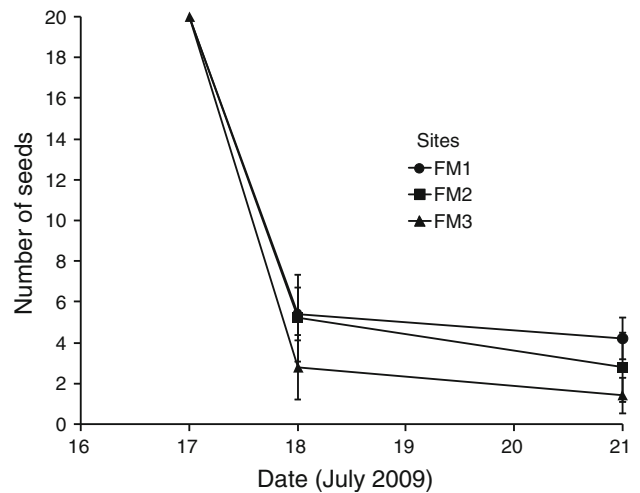


Fig. 1 Ant removal of *E. esula* seeds from depots in July 2009. Values are the mean number of seeds remaining (\pm SE) from 5 sets of 20 seeds placed in three sites (FM1, FM2, FM3)

$P = 0.045$; 2010: $\chi^2 = 51.37$, $df = 5$, $P < 0.0001$) (Fig. 3). However, total density was also greater on-mounds only

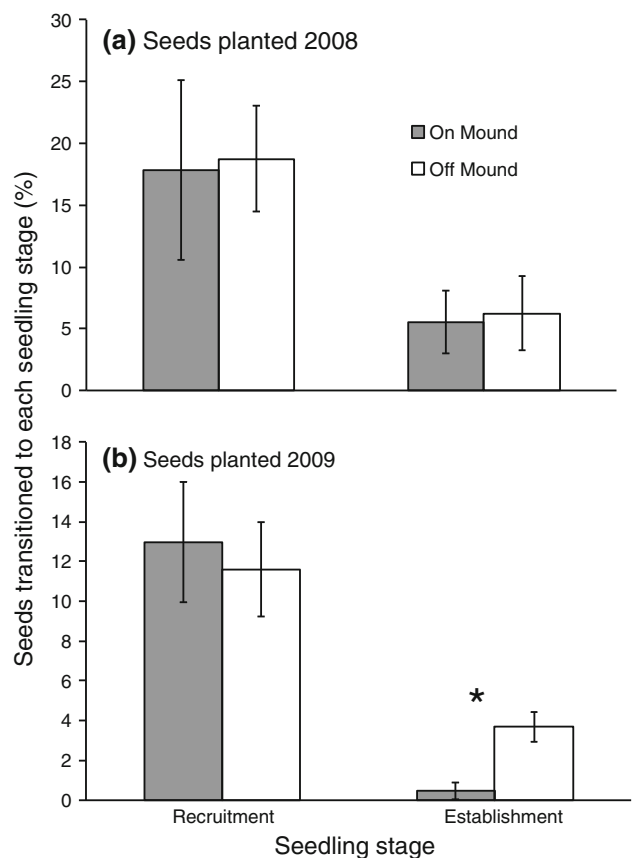


Fig. 2 Percent of seeds of *E. esula* reaching recruitment and establishment stages (mean \pm SE) on *Formica* mound edges and 3 m away from mounds. Seeds were monitored the growing season following planting in **a** 2008 ($n = 6$) and **b** 2009 ($n = 10$). Significance at $P < 0.05$ indicated by asterisks

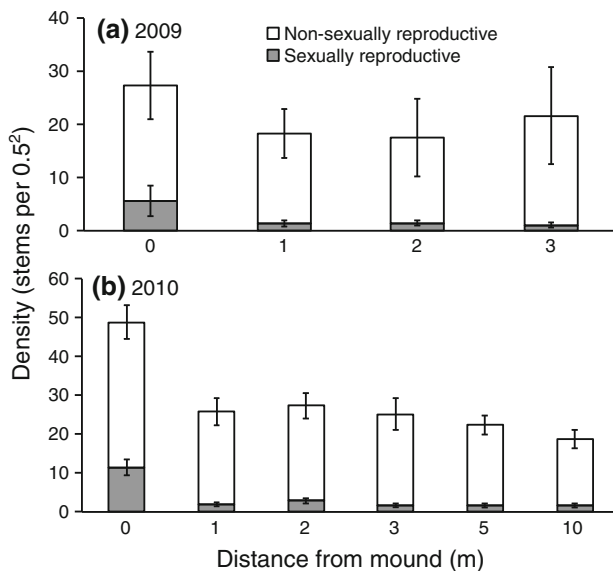


Fig. 3 Mean density of naturally growing *E. esula* in relation to distance from *Formica* mounds in **a** 2009 ($n = 11$ mounds) and **b** 2010 ($n = 34$ mounds). Bars represent the mean total stem density (number per $0.5 \text{ m}^2 \pm \text{SE}$), for two reproductive states

in 2010 (2009: $\chi^2 = 2.55$, $df = 3$, $P = 0.47$; 2010: $\chi^2 = 24.54$, $df = 5$, $P = 0.00017$) (Fig. 3). The proportion of sexually reproductive stem density relative to total stem density was also greater on-mounds in 2010 only (2009: $\chi^2 = 4.77$, $df = 3$, $P = 0.19$; 2010: $\chi^2 = 49.75$, $df = 5$, $P < 0.0001$). Average above-ground biomass was over five times greater on-mounds than off-mounds ($V = 36$, $P = 0.0078$) (Fig. 4). Density of reproductive and total *E. esula* did not increase with mound size ($S = 6,339.7$, $P = 0.86$ and $S = 6,746.59$, $P = 0.86$, respectively). Thatch diameter of *F. obscuripes* mounds averaged 0.45 m (range $0.2\text{--}0.7 \text{ m}$) ($n = 34$).

Soil characteristics

Soil collected on-mounds had greater available phosphorus (soluble P) and available nitrogen (ammonium— NH_4N and nitrate— NO_3N) than soil off-mounds (Table 1). However, organic matter and total nitrogen were not different on-mounds and off-mounds (Table 1).

Discussion

Removal of *E. esula* seeds by native ants occurred quickly with most seeds being removed within 24 h. Several ant species acted as secondary dispersers and deposited seeds in their nests. *Formica* mounds had higher levels of available nitrogen and available phosphorus than locations off mounds. These nutrients likely resulted in mounds being

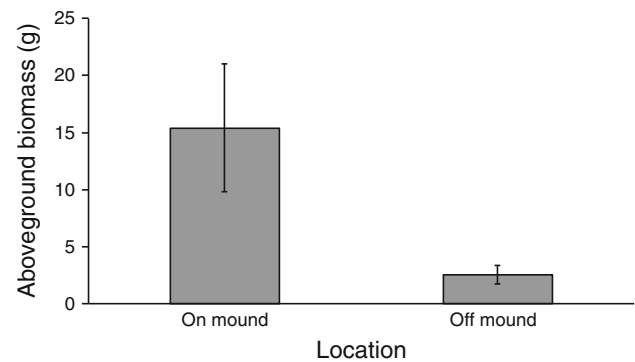


Fig. 4 Mean above-ground biomass (g per $0.5 \text{ m}^2 \pm \text{SE}$) of naturally growing *E. esula* on and off *F. obscuripes* mounds in 2010 ($n = 8$). Off-mound sites were 3 m from mound

favorable microhabitats for established *E. esula* plants, as evidenced by greater total and reproductive density and above-ground biomass on-mounds relative to off-mounds. However, greater nutrient availability on-mounds did not appear to enhance seedling recruitment or establishment.

Seed cache placement likely influences ant removal and deposition site of seeds. Several ant species removed just over half the seeds placed away from *Formica* foraging trails within 2 h, with 40% of the seeds observed delivered to a nest. In contrast, 100% of seeds placed on *Formica* foraging trails were removed within 2 h. The fate of these seeds was more difficult to determine, but most observed seeds were carried in a direction away from the nest. We hypothesize this behavior may be a consequence of ants following their trail pheromone to a foraging location where ant recruitment was already occurring (Culver and Beattie 1978) or in the interest of keeping the high traffic foraging trails clear of obstacles. Differences in seed fate between caches on and off foraging trails are consistent with previous results (Beattie and Culver 1977; Pemberton 1988). In addition to seed placement, ant identity may also influence dispersal distance, location and treatment of the seed (Culver and Beattie 1978; Horvitz and Schemske 1986b; Hughes and Westoby 1992; Servigne and Detrain 2008). During the removal observations, several ant species, including *F. obscuripes*, showed interest in seeds through antennation even if seed removal was not observed. Given this variability in seed fate due to seed placement location and ant behavior, future studies on myrmecochory should carefully consider both these factors in field experiment design (Vander Wall et al. 2005; Culver and Beattie 1978) and model development (Russo et al. 2006; Heinken and Winkler 2009).

Formica mounds had a positive effect on naturally growing *E. esula* density and total quadrat above-ground biomass, but not on seedling recruitment or establishment from experimentally planted seeds. These findings are

Table 1 Comparisons of soil characteristics (mean \pm SE, $n = 9$) on mound edges and a distance of 3 m from *F. obscuripes* mounds in 2009

Soil characteristic	On mound	Off mound	Test statistic	df	P value
Total nitrogen (%)	0.114 \pm 0.011	0.108 \pm 0.014	$V = 27$	n/a	0.65
Ammonium-nitrogen (NH ₄ N, ppm)	7.9 \pm 2.7	2.7 \pm 0.2	$V = 28$	n/a	0.021
Nitrate-nitrogen (NO ₃ N, ppm)	12.0 \pm 3.2	5.2 \pm 0.7	$V = 40$	n/a	0.044
Phosphorus (soluble P, ppm)	43.7 \pm 3.0	29.9 \pm 2.8	$V = 43.5$	n/a	0.015
Organic matter (%)	2.43 \pm 0.23	2.29 \pm 0.28	$t = 0.50$	8	0.63

consistent with other studies demonstrating positive effects of ant nests at later plant life stages (e.g., Hanzawa et al. 1988; Gibson 1993), and serve as a reminder to consider multiple stages of plant development when testing the directed dispersal hypothesis or other adaptive advantages of myrmecochory. The positive effect of mounds on *E. esula* growth is likely influenced by the greater nutrient availability on *Formica* mounds. Sand soils are typically deficient in nitrogen and other nutrients (Grigal et al. 1974), and *E. esula* growth, including clonal spread, is reduced under low levels of nitrogen (McIntyre and Raju 1967; McIntyre 1972). The fluctuating resource hypothesis predicts invading species will be more successful in habitats where competition for resources with native species is reduced (Davis et al. 2000; Mitchell et al. 2006). In this system, the greater above-ground biomass and density of *E. esula* associated with increased nutrient availability along ant mound edges are consistent with the predictions of this hypothesis. The observation that mounds did not increase seedling recruitment or establishment suggests emerging seedlings may not be nutrient-limited in this system, a likely consequence of ample seed resource provisioning by the parent plant (Kitajima 2002).

It is possible that other mechanisms may also influence the pattern of greater *E. esula* density and above-ground biomass associated with *Formica* mounds. *Euphorbia esula* growing on mounds may benefit from *Formica* individuals foraging on plant nectar (Selleck et al. 1962; M. Berg-Binder, personal observations) and defending the plant from damaging herbivores, including biological control agents (Gassmann et al. 1996). However, this hypothesis may be unlikely as no difference in herbivore damage was found between ant-excluded and control stems at this same field site in another study (M. Berg-Binder, unpublished data). Several factors, in addition to increased soil nutrient availability, have been associated with ant nests that may provide favorable conditions for plants (i.e., different light availability, Gibson 1993; soil porosity, McCahon and Lockwood 1990; or water availability, Cammeraat et al. 2002). Additionally, *Formica* ant activity and mound excavation may provide disturbance that is favorable for *E. esula* vegetative spread and/or growth. Many plant invaders respond favorably to disturbance, especially when nutrient enrichment coincides with site disturbance (Lake

and Leishman 2004). While these remain untested possibilities that were beyond the scope of this study, these mechanisms are not mutually exclusive with increased nutrient availability providing favorable conditions. The case for increased soil nutrient availability at *Formica* mounds being, at least in part, responsible for this pattern in *E. esula* abundance is reasonable, especially since soil nutrient abundance is quite low in sand soils (Grigal et al. 1974).

Because *Formica* ant mounds provided a benefit to *E. esula* during life stages following seedling establishment, it seems possible that non-myrmecochorous plants growing within the vicinity of ant mounds could also benefit from favorable mound conditions, regardless of the mechanism responsible. Graminoids and other plants, in addition to *E. esula*, commonly grow along *Formica* mound edges (Weber 1935; M. Berg-Binder, personal observations). In addition, it is possible that the nutrient enrichment of soil from *F. obscuripes* mounds may increase the quality of seeds produced by non-myrmecochores that can have ramifications for subsequent seedling development near the mounds (Parrish and Bazzaz 1985). Future studies should consider the effect of ant nests on non-myrmecochorous plant demography and population structure, as well as the quality of seeds produced.

While we focused on the demography of *E. esula* in this study, the ant community may also benefit from *E. esula* presence. Elaiosomes are a nutrient-rich resource (Fischer et al. 2008) that provides fat and protein to ants (Pember-ton 1988) and may ultimately influence ant colony growth and development (Morales and Heithaus 1998; Bono and Heithaus 2002). Ants also abundantly visit nectar glands located near the flowers of *E. esula* (M. Berg-Binder, personal observations). Given the high abundance of *E. esula* in areas it invades, this addition of new resources may influence the density and growth of native ants, as well as the insect community generally. Future research aimed at examining the effects by *E. esula* on the trophic ecology of ants and the insect communities is warranted.

Introduced species are among the leading threats to biodiversity (Wilcove et al. 1998; Pimentel et al. 2000), and the role of positive interactions between invasive and native species are likely under-appreciated (Simberloff and Von Holle 1999; Richardson et al. 2000). Seed dispersal relationships are good candidates for facilitative

interactions between introduced and native species worldwide (Richardson et al. 2000; Pemberton and Irving 1990; Alba-Lynn and Henk 2010), in part due to their lack of specialization and widespread distribution (Howe 1984; Garrido et al. 2002). The successful spread of *E. esula* in its introduced range is often attributed to long-distance dispersal events (Selleck et al. 1962; Dunn 1979), with its persistence in local areas due to its highly noxious latex sap, that provides defense from herbivores, and its extensive root system, that allows for re-growth following above-ground damage, and aggressive clonal spread (Selleck et al. 1962; Dunn 1979). Our findings demonstrate that the native ant community has the potential to influence local *E. esula* patterns of spread through short-distance dispersal events and provisioning of favorable microhabitats that may serve as important foci for invasive plant persistence and spread (Moody and Mack 1988). It has been shown that the rate that new foci are created may be more important in determining the overall rate of spread for an invasive species than the rate at which existing foci spread through a diffusion-like process (e.g., clonal spread) (Moody and Mack 1988). Research considering native ant communities' influence on local patterns of invasive myrmecochore spread will increase our understanding of the role positive species interactions play in the invasion process.

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