

Impacts of the Invasive European Red Ant (*Myrmica rubra* (L.): Hymenoptera; Formicidae) on a Myrmecochorous System in the Northeastern United States

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Abstract

We investigated the impact of an invasive ant species from Europe, *Myrmica rubra* (L.), on a myrmecochorous system (seeds dispersed by ants) in its invaded range in North America. We assessed: 1) how *M. rubra* process the myrmecochorous diaspores (seeds and elaiosome as a single dispersal unit transported by ants) in comparison with native ants; 2) its preference for common native and invasive diaspore species relative to native ants; 3) how far they disperse diaspores in the field; and 4) the diaspore removal rate by invertebrates and vertebrates in infested areas compared to noninvaded sites. Field experiments demonstrated higher diaspore removal rates over a 10-min and 24-h period by *M. rubra* compared to native ants. *M. rubra*'s diaspore dispersal distance was 40% greater compared to native ants. In two of three laboratory studies and one field study, there was no significant difference between the seed species which *M. rubra* and native ants selected. Our data suggest no long-term deleterious effects of *M. rubra*'s invasion on diaspore dispersal in the Maine plant community that is comprised of both native and invasive species. This implies that *M. rubra* benefits from the myrmecochorous plant species' diaspores by increasing their dispersal range away from the parent plant and potentially reducing seed predation. However, it is not known whether the fact that the native ant fauna and *M. rubra* are attracted to the same plant species' diaspores creates a high level of competition between the ants with deleterious effects on the native ant community.

Key words: myrmecochory, *Myrmica rubra*, elaiosome, mutualism, invasive species

Worldwide many plant species produce seeds (diaspores) adapted for dispersal by ants, a mutualism termed myrmecochory (Gorb and Gorb 2003, Lach *et al.* 2010, Warren and Giladi 2014). Their diaspores have elaiosomes, external structures that are attractive to certain ant species and induce ants to disperse the diaspores. These nutrient-rich appendages induce some ant species to carry the diaspores back to their nest where the elaiosome is consumed and the germinable seed is discarded (Gammans *et al.* 2005). The number of plant species and families that are classified as myrmecochores ranges between 2100–3000 species and 60–90 families (Berg 1975, Beattie 1985, Hughes and Westoby 1992, Gorb and Gorb 2003, Majer *et al.* 2011). In eastern North American forests, 30% of native herbaceous plants are thought to be myrmecochores (Beattie and Culver 1981, Handel *et al.* 1981).

Diaspore dispersal by ants may offer a wide range of benefits to the plant. Ants may increase dispersal distance by moving diaspores away from the parent plant, move them from other competitors and predators, and transport them to sites favourable for germination (Andersen 1988, Bond *et al.* 1991, Espadaler and Gomes

1996, Canner 2012). Within the ant nest, seeds may be protected from fire and seed predators (Handel and Beattie 1990, Bond *et al.* 1991, Ohkawara *et al.* 1996, Gorb and Gorb 2003). The benefits for the ant include the presence of four essential fatty acids, amino acids, and sterols in the elaiosomes (Gammans *et al.* 2005, 2006; Reifenrath *et al.* 2012), especially the nitrogen rich amino acid histidine (Fischer *et al.* 2008). However, the composition of the amino acids and fatty acids can impact seed removal preference by different ant species (Reifenrath *et al.* 2012). Fischer *et al.* (2005) have shown that elaiosomes of *Corydalis cava* (L.) Schweigg. & Körte can be a major food source for larvae of *Myrmica rubra* (L.) providing workers with 39% of their nitrogen and larvae with 61% of their nitrogen. Fokuhl *et al.* (2007) showed that *M. rubra* colonies provided with elaiosomes of *Scilla bifolia* L. and *Corydalis cava* contained significantly more worker pupae than those colonies deprived of elaiosomes. It has also been demonstrated that *Myrmica ruginodis* Nylander ant colonies that feed on elaiosomes may increase larval production by 102% and larval weight by 48% (Gammans *et al.* 2005), and that *Aphaenogaster rudis* (Enzmann) colonies increase

gyne production to 62.5% of brood when fed elaiosomes compared to 25.9% in controls (Morales and Heithaus 1998). The proposed benefits to the ant and plant from this interaction have been well studied, yet the potential impact of an invasive ant species on a myrmecochorous system in the invaded range has received little attention, though hypotheses abound (Lach 2003).

Social insects are some of the most successful invasive species and ants are among one of the most damaging invaders of terrestrial habitats (Holway *et al.* 2002, Ness *et al.* 2004, Pimentel *et al.* 2005). The most studied invasive ants include the southern imported fire ant, *Solenopsis invicta* Buren, and the Argentine ant, *Linepithema humile* Mayr. Both are native to South and Central America and have been introduced into Europe, Australia, Africa, and the United States (Holway *et al.* 2002, Silverman and Brightwell 2008, Ascunce *et al.* 2011). These introduced species have considerable negative impacts on their invaded communities through direct competition and predation on native species. This has led to the disruption of ecological processes including ant–plant mutualisms (Bond and Slingsby 1984, Zettler *et al.* 2001, Ness *et al.* 2004, Rodriguez-Cabal *et al.* 2012).

Bond & Slingsby (1984) found that Argentine ants, *L. humilis*, in Cape Fynbos, South Africa, were better competitors than native ants. The native ants act as mutualists, whereas the Argentine ant does not. They remove the elaiosome, but leave the seed on the soil surface so it is available to seed predators and not protected from fire. The authors suggest that the Argentine ant invasion could lead to a species composition shift of the plant community and that loss of plant species may occur over time. Christian (2001) found similar results with regards to the Argentine ant invading other South African shrublands. Zettler *et al.* (2001) conducted experiments on diaspore dispersal by the invasive ant, *S. invicta*, with several different myrmecochorous plant species. These invasive ants rapidly collected the diaspores and dispersed them, but a high percentage of seeds were destroyed in the process, therefore having a negative effect on the plant species. Other studies have shown that different species of ants will differentially disperse plants diaspores (Boulay *et al.* 2007, Gove *et al.* 2007, Ohnishi *et al.* 2008, Aran *et al.* 2010, Castro *et al.* 2010), so a shift in the ant community could result in a shift in diaspore dispersal and possibly shifts in the plant community. The invasive ant *Brachyponera chinensis* (Emery) resulted in a reduction in the native seed-dispersing ant *Aphaenogaster rudis*, but did not take over the diaspore-dispersing role in its invaded range that ultimately led to a shift in the plant assemblage (Rodriguez-Cabal *et al.* 2012, Warren *et al.* 2015).

Introduced populations of the European red ant, *M. rubra*, have become invasive in parts of the northeastern United States and eastern Canada (Grodén *et al.* 2005, Wetterer and Radchenko 2010). This ant species was first reported in North America by Wheeler (1908), who described an established population in Forest Hills, Massachusetts. Nest and foraging densities of *M. rubra* have been observed to be much greater in its introduced range than in their native European range (Grodén *et al.* 2005). *M. rubra* has dramatically decreased the native ant populations in invaded habitats (Grodén *et al.* 2005, Garnas 2005, Garnas *et al.* 2014, Naumann and Higgins 2015), increased abundance of Homoptera sap feeding herbivores (McPhee *et al.* 2012), and negatively impacted other insect abundance (Naumann and Higgins 2015, Verble-Pearson and Person 2016). Previous studies have shown that *M. rubra* has different diaspore handling techniques than other competing ant species. Seeds taken by *M. rubra* spend less time in nests compared to nests of their native counterparts, *Lasius niger* (L.) and *Aphaenogaster rudis* (Servigne 2010, Prior *et al.* 2014).

As a large proportion of the native herbaceous flora in the northeastern United States is myrmecochorous, the invasion by a European ant that results in displacement and elimination of the native ant fauna, begs the question: Does *M. rubra* have the potential to disrupt native plant population growth? The objective of this study was to assess the dynamics of ant mediated diaspore dispersal when native myrmecochorous ant species are suppressed or replaced by an invasive ant species. We addressed three of the following questions: 1) Is *M. rubra* attracted to native diaspores in the invaded range; 2) How do they process and discard the seeds compared to native ant species; and 3) Will seed predation by rodents, birds and other invertebrates be different in areas invaded by *M. rubra* compared to noninvaded sites occupied by native ants?

Materials and Methods

Diaspore Collection and Storage

All of the diaspores (seeds with associated elaiosomes) used in our laboratory and field assays were collected when fruits ripened and they became available for ant foraging in Orono (Penobscot County) and Mt. Desert Island (Hancock County), Maine. Diaspores were stored in sealed plastic bags in the refrigerator at 4°C for 2–8 mo until needed for experiments. Diaspores were allowed to acclimate to experimental conditions prior to set up. In addition, 15–25 diaspores for each test species were measured individually (length, accuracy = 0.1 mm; under a dissecting microscope at 40× with a reticle objective lens) and weighed in replicate sets of 20–50 diaspores on a balance and then individual mass was calculated (mg, accuracy = 0.1) Then diaspores were separated into seeds and their associated elaiosomes and individual parts were measured and weighed to quantify potential likelihood of detection (length) and resource (elaiosome wgt) for ant foragers (balance manufacturer and model: Ohaus, Explorer).

Laboratory Choice Assays

We designed three laboratory choice behavioral assays to determine the preference of *M. rubra* and native ant species for locally common seeds that possess elaiosomes. Diaspores of six native and one invasive plant species were collected from multiple sites throughout central Maine during the spring and summer of 2005. The diaspores collected were: sessile bellwort (*Uvularia sessilifolia* L.), rock harlequin (*Corydalis sempervirens* (L.)), common blue violet (*Viola sororia* Willd.), wood rush or luzula (*Luzula multiflora* (Ehrh.) Lejeune), bloodroot (*Sanguinaria canadensis* L.), longstalk sedge (*Carex pedunculata* Muhl.), and Canada thistle (*Cirsium arvense* (L.)). All diaspores were stored in paper bags at 4°C until used in experiments.

Experiments were conducted using *M. rubra* and five native ant species that are common in the Acadian forest ecosystem of Maine and known to forage for diaspores (Frank Drummond, personal observation). The Acadian forest ecosystem is a temperate ecoregion comprised of a combination of northern hardwood and boreal forest tree species that includes a variety of habitats on the hilly and mountainous terrain of New England in the Northeastern United States and Quebec and the Maritime provinces of Canada (Simpson 2015). Ant colonies were collected with an aspirator during the summer of 2005, either on Mt. Desert Island (Hancock Co., Maine) or at the University of Maine (Orono, Penobscot Co., Maine, 44°54'14"N, 68° 40'21"W). Six artificial nests of each ant species derived from individual colonies separated in distance by a minimum of 50–100 m, were constructed containing 50 workers and 20

larvae. These colony fragments were placed into plastic nest boxes, 22 × 22 × 10 cm. Twelve to fifteen additional larvae were added to the nests to stimulate workers to forage for food at the beginning of each trial. Each nest box was attached to a smaller 'foraging arena', 7 × 7 × 4 cm, by a 20 cm long Tygon tube. All colony fragments were fed and watered each week with a standard diet of white sugar and *Drosophila melanogaster* L. larvae placed into the foraging box (Brian and Abbot 1977, Elmes *et al.* 2004, Gammans *et al.* 2005, 2006). All colony fragments experienced high brood and worker survival during the experiment such that only a few individual cadavers (<10) were observed in the middens.

Colony fragments were allowed to habituate for 1 wk prior to the start of laboratory assays. Nests were selected randomly for each assay trial. When assays were not being conducted, the ants were allowed to roam freely between the nest box and foraging arenas. Prior to each assay, food was removed from the foraging arena, and the arena was cleaned with water to remove food residue and residual pheromone trails. Ants within the foraging arena were also removed except for a randomly chosen 'focus' ant that was allowed to settle while the foraging tube was closed. If the focus ant attempted to leave the foraging arena the foraging tube was opened and remained so until a new ant entered under its own volition. If no ant was present in the foraging box at the start of the experiment, the tube was left open until an ant entered. This procedure minimized disturbance and ensured that the workers observed were active foragers. Assays were conducted for 30 min.

Three different replicated experiments were conducted between August and September of 2005. The reason for three experiments is that the availability of diaspores and ant species varied over the duration of our study. Either three diaspores of *sessile bellwort*, *Canada thistle*, *common blue violet*, and *woodrush* (experiment 1) or three diaspores of *bloodroot*, *woodrush*, *longstalk sedge* and *Canada thistle* (experiment 2) were placed into the foraging arena and presented to the native ants: *Myrmica detritinodis* Emery, *Crematogaster cerasi* Emery, or *M. rubra*. A third experiment was conducted with the same diaspore species as experiment 1 (*sessile bellwort*, *Canada thistle*, *common blue violet*, and *woodrush*), but with three different species of native ant: *Formica argentea* Wheeler (*fusca* group), *Lasius alienus* (Foerster), and *Leptothorax longispinosus* Mayr, and *M. rubra* for comparison. For all experiments, all diaspores assayed were placed in the foraging arenas simultaneously at the start of each experiment in a randomized order using sterile soft-forceps (sterilized with flame from an alcohol lamp). During the assays, the duration (seconds) and frequency (number of events) that the ants antennated diaspores, moved diaspores to the nest, and chewed the elaiosome was recorded by stopwatch or counted. Chewing/biting the elaiosome was defined as the ant chewing/biting the seed/elaiosome with its mandibles. This was recorded as a measure of attraction towards the diaspores by the different ant species. Observations were made for 30 min after diaspores were deployed in the foraging arenas and then were left in the ant foraging and nest arenas for 2 d to determine diaspore fate.

In all of the laboratory assays, after each trial was completed, diaspore condition was visually inspected under magnification with a dissecting microscope for cracks in the seed coat and other signs of damage. Each experiment was analyzed separately. Data from the laboratory assays were analyzed by ANOVA using a split-plot design (JMP 2007). The unit of replication (a block factor) for all experiments was a 30-min period of observation in which a different colony fragment from a unique colony of each species was observed simultaneously by a laboratory researcher. Each colony was observed only once. There were six replicate blocks in experiments 1 and 2

and three replicate blocks in experiment 3. Main treatment effects were ant species. Ant species crossed with bioassay period was the random effect used to test 'ant species'. The 'diaspore species' effect was the split-plot factor. The interaction between ant species and diaspore species was tested by the residual mean square. Data were square root transformed to meet the assumptions of analysis of variance. Ordinal logistic regression was used to test the number of seed moves for each diaspore species by ant species since the data could not be normalized (JMP 2007).

The frequency of ant attraction, antennation and chewing, to diaspore and elaiosome length and mass was assessed with a linear model (attraction = experiment + diaspore or elaiosome length or mass). This was conducted for both native ants together and *M. rubra*.

Field Diaspore Removal Assays

Field experiments designed to assess diaspore removal rates and the distances that diaspores are dispersed by *M. rubra* and several common native ant species in the Maine Acadian Forest habitat were conducted at sites with native ants and *M. rubra* invaded sites on Mount Desert Island in August and September, 2005 and 2006.

Field experiments were conducted to determine the rate at which seven diaspore species: sessile bellwort, common blue violet, Canada thistle, longstalk sedge, rock harlequin, wood rush, and bloodroot were removed by *M. rubra* and two common native ant species of similar size: *M. detritinodis* and *C. cerasi*. Additional objectives were to determine the distance and the final location of the seed once the elaiosome had been removed. Each experiment was conducted at three native ant sites and three *M. rubra* invaded sites. These sites were embedded in an Acadian mixed hardwood/softwood forest (see following paragraph for representative plant communities). The native sites were: Bear Brook (44°21' 44"N, 68°11'49"W), Thompson Island (44°25'32"N, 68°21'75"W) and Sierr de Mont (44°21'47"N, 68°12'34"W) and three *M. rubra* invaded sites: Wood Chip (44°22'33"N, 68°15'31"W), Old Farm Road (44°22'36"N, 68°11'44"W) and Bear Brook (44°21'42"N, 68°11'52"W).

To determine diaspore removal rates by *M. rubra* and native ants, glass plates were placed onto shaded areas of high ant activity. Two experiments were conducted with seven diaspore species in total. Each plate had either three diaspores of *Uvularia sessilifolia*, *Viola sororia*, and *Cirsium arvense* (experiment 1) or three diaspores of *Carex pedunculata*, *Corydalis sempervirens*, *Luzula multiflora*, and *Sanguinaria Canadensis* (experiment 2). A stopwatch was started once all diaspores were added and the plate watched for 10 min. Ant species removing diaspores over this time were noted. Experiments were conducted during 20–31 August and 1–7 September 2005, respectively; for experiments 1 and 2. Ordinal logistic regression was used to test for differences in removal rates by ants for various offered diaspore species and the interaction between ant and diaspore species (Hosmer and Lemeshow 2000, JMP 2007).

To compare diaspore dispersal distances by *M. rubra* with native ant species, diaspore of *Viola sororia* were placed into areas of high ant activity. When a diaspore was picked up and carried by an ant it was followed until the diaspore was brought into the nest. The distance carried was then measured with a metric measuring tape. Ant tracking was conducted at various times of the day, but during normal hours of activity. Experiments were conducted during 25–30 September 2006. A *t*-test (Zar 1984, JMP 2007) was used to compare square root transformed diaspore dispersal distances observed in native ant habitats and invaded habitats.

To determine the final position of diaspores once ants had taken them to the nest and removed the elaiosomes, three *M. rubra*,

M. detritinodis, and *C. cerasi* field colonies were located by baiting with cookie crumbs. To do this, we laid down trails of crumbs (3–4 m in length) starting from the locations that diaspores were set out on the glass plates along cardinal direction vectors. Then we watched foraging ants pick up the crumbs and followed them back to their nests. After nests were located, ten *Viola sororia* diaspores painted with yellow acrylic paint were placed adjacent to the nest entrance. The midden piles and surroundings of the nests were searched after 1, 7, and 10 d for any marked diaspores or seeds. Experiments were conducted during 25–30 September 2006.

Comparative Predation Experiments

Field experiments were carried out in September 2005 and September 2006 to investigate diaspore removal by invertebrates and vertebrates in *M. rubra* and native ant sites. Field studies were conducted on Mount Desert Island, Hancock Co. (44°22'11"N, 68°18'52"W). This geographic region represents one of the earlier invasions in Maine, with documented presence of *M. rubra* in the 1960's (Grodén *et al.* 2005). Three sites were used in this study. The sites were the same for both years. In 2005, at each site only invaded areas were used in the experiment. In 2006, at each site one locale with only native ants present was paired with one invaded locale with *M. rubra* present and associated with an absence of native ant fauna. The paired locales within sites were matched such that soils, elevation, and plant communities were similar. One site (Old Farm Road) was adjacent to the NE coast of the island (44°22'36"N, 68°11'44"W). Both paired locales (invaded and native) had a predominately deciduous forest landscape of oak (*Quercus rubra* L.), cherry (*Prunus serotina* Ehrh.), alder (*Alnus rugosa* (Du Roi) Spreng.), white pine (*Pinus strobes* L.), and black spruce (*Picea mariana* (Mill.) B.S.P.). A second site chosen was Bear Brook, in the interior of the island (44°21'42"N, 68°11'52"W). In the *M. rubra* invaded area, the predominant vegetation is alder (*Alnus rugosa* (Du Roi) Spreng.), viburnum (*Viburnum spp.*), paper (*Betula papyrifera* Marsh) and gray birch (*Betula populifera* Marsh), and black spruce (*Picea mariana* (Mill.) B.S.P.), and white pine (*Pinus strobes* L.). In the native ant locale within the second site, there is alder (*Alnus rugosa* (Du Roi) Spreng.), viburnum (*Viburnum spp.*), pin cherry (*Prunus pensylvanica* L. f.), meadow sweet (*Spiraea spp.*), and gray birch (*Betula populifera* Marsh). The third site was on the N side of the island, near the Acadia National Park Visitor's Center (44°24'37"N, 68°15' 17"W). This native ant habitat was characterized by alder (*Alnus rugosa* (Du Roi) Spreng.), gray birch (*Betula populifera* Marsh), and meadowsweet (*Spiraea alba* Du Roi). The invaded habitat was predominately alder (*Alnus rugosa* (Du Roi) Spreng.), meadowsweet (*Spiraea alba* Du Roi), white ash (*Fraxinus americana* L.), and Japanese barberry (*Berberis thunbergii* DC).

The three diaspore species in each year were deployed in each of four (2005) or three (2006) enclosure treatments. In 2005, one enclosure, referred to as the 'vertebrate' treatment, excluded ants and other invertebrates, but allowed access to birds and rodents. A plant pot saucer 8 cm wide and 1 cm deep was fixed to the top of wooden stake of 1 cm diameter. The bottom of the saucer and the wooden stake were coated in a nondrying sticky coating (Tanglefoot), greatly reducing access to ants and other invertebrates. We acknowledge that a few insects could blow into the saucer, but we assumed that this number of insects would not appreciably affect our results. A stake was hammered into the ground so the saucer rested 4 cm above the soil surface. A second enclosure, referred to as the 'small invertebrate' treatment, excluded birds and rodents, allowed access to ants and other small invertebrates, but excluded large invertebrates such as *Harpalus rufipes* (Degeer), a common

seed predating carabid beetle in Maine (Zhang *et al.* 1997). Wire mesh size of 0.32 cm² was formed into cylinders with a diameter of 11 cm and 18 cm in height, and a mesh lid was placed on top. The open bottoms of the mesh cylinders were dug into the ground such that they covered an 8 cm diameter plant pot saucer. A third enclosure treatment, referred to as the 'large invertebrate' treatment, excluded birds and rodents, but allowed access to ants and all other invertebrates. Wire mesh of 1.27 cm square width was formed into cylinders with a diameter of 11 cm and 18 cm in height and a mesh lid was placed on top. The cylinder was similarly dug into the ground over an 8 cm diameter plant pot saucer as for the 'small invertebrate' treatment. The fourth treatment, referred to as the 'all' treatment or positive 'control', allowed free access to all seed predator species and comprised a plant pot saucer 8 cm in diameter and 1 cm deep placed directly onto the ground. In 2005, 20 diaspores each of bloodroot, wood rush, and blue violet were placed into each enclosure type. In 2006, three enclosure treatments were deployed: 1) control or all, 2) small invertebrate, and 3) vertebrate, and 15 diaspores each of bellwort, blue violet and wood rush were placed into each enclosure type. In both years, the numbers removed were recorded after 24 h.

A split-plot ANOVA (Milliken and Johnson 1984, JMP 2007) for was used in the 2005 experiment (three blocks, but four replicates of treatments within blocks) to test for the significance of the following effects within invaded habitats: enclosure treatment (main plot treatment) and diaspore species type (split-plot treatment); and the two-way interaction. In 2006, a split-split-plot ANOVA (Milliken and Johnson 1984, JMP 2007) was used to test for the significance of the following effects (three blocks, but four replicate treatments in each block): invaded versus noninvaded habitats (main plot treatments), enclosure treatment (split-plot treatments), and diaspore species type (split-split-plot treatments); and all two-way and three-way interactions. In both analyses, the dependent variable, proportion of diaspores taken from the feeding platforms, was transformed with the arcsine square root of the proportion (Zar 1984). A generalized linear mixed model was not used because of the complex nested design and the simpler approach with fixed effects ANOVA, as suggested by Murtaugh (2007).

Results

Diaspore Resource

The size and mass of diaspores and elaiosomes that we used in our experiments are listed in Table 1. Diaspore length has a range of almost 3.5× (1.1 to 3.8 mm), while diaspore and elaiosome masses have ranges of 5.5 and 23 times, respectively (0.3–15.0 and 0.1–2.3 mg).

Laboratory Choice Assays

Antennation of diaspores and subsequent chewing of elaiosomes were highly correlated in experiment 1 ($r = +0.738$, $P < 0.0001$) and experiment 2 ($r = +0.938$, $P < 0.0001$) for the three ant species. Therefore, the frequencies and durations of antennation and chewing were summed into a single index of preference for analysis.

In experiment 1, where four diaspore species (sessile bellwort, wood rush, Canada thistle, and blue violet) were presented to three ant species (*M. rubra*, *M. detritinodis*, and *C. cerasi*), differences in diaspore handling were observed between plant species, but not between ant species. There were no significant differences in the frequencies or durations of the combined measure of antennation and chewing by the three ant species ($P > 0.05$). However, there was a significant difference (Fig. 1) in diaspore antennation and chewing frequency ($F_{(3,45)} = 15.453$ $P < 0.0001$) and chewing duration

Table 1. Length and mass of diaspores and elaiosomes in study

Species	Diaspore	Diaspore	Elaiosome	Elaiosome
	Mass (mg)	Length (mm) ^a	Mass (mg)	Length (mm) ^a
Bellwort ^b <i>Uvularia sessilifolia</i>	15.0 ± 1.51	3.2 ± 0.07	1.6 ± 0.13	4.9 ± 0.11
Bloodroot <i>Sanguinaria canadensis</i>	11.6 ± 0.41	2.3 ± 0.36	1.5 ± 0.02	3.8 ± 0.94
Canada thistle ^c <i>Cirsium arvense</i>	2.9 ± 0.07	3.8 ± 0.03	0.1 ± 0.07	0.5 ± 0.01
Common blue violet <i>Viola sororia</i>	0.9 ± 0.11	1.9 ± 0.03	0.2 ± 0.06	1.1 ± 0.04
Longstalk sedge <i>Carex pedunculata</i>	0.3 ± 0.02	1.2 ± 0.03	0.1 ± 0.03	0.2 ± 0.08
Rock harlequin ^b <i>Corydalis sempervirens</i>	12.1 ± 0.21	1.6 ± 0.1	2.3 ± 0.32	1.4 ± 0.10
Wood rush <i>Luzula multiflora</i>	0.6 ± 0.08	1.1 ± 0.02	0.2 ± 0.04	0.4 ± 0.01

^aDiaspores and elaiosomes varied in shape, length represents longest dimension.

^bSpherical diaspores, length represents diameter.

^cExotic species.

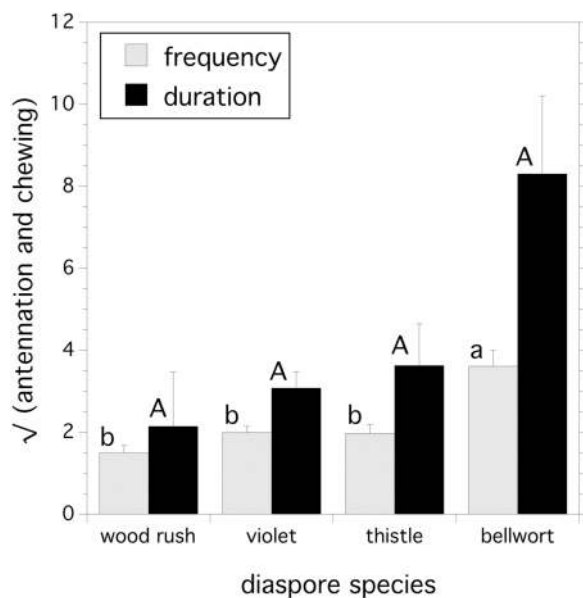


Fig. 1. The square root transformed frequency (shaded bars) and duration (black bars) of diaspore investigation (antennation) and chewing by three ant species for four plant species in the laboratory. Small letters denote differences in frequency and the capital letters denote differences in duration among the diaspore species when pooled over the three ant species (both ant and ant × diaspore species interaction, $P > 0.05$). Different letters associated with bars in each figure are significantly different (Tukey multiple comparisons, $P < 0.05$), lowercase letters for frequency and uppercase letters for duration. Diaspore species are ordered in the graph from smallest (left) to largest (right) in mass (mg). Error bars are standard errors of the mean.

($F_{(3,45)} = 129.879$, $P < 0.0001$) due to diaspore species. Ants exhibited a higher rate of behavioral response (frequency and duration) to bellwort than the other three diaspore species (Tukey multiple comparison test, $P < 0.05$). There was no significant interaction in antennation and chewing frequency or duration measures between ant species and diaspore species ($P = 0.101$, $P = 0.123$, respectively). There was also no significant relationship between ant species, diaspore species or their interaction on attempts to move diaspores to the nest by ants ($P > 0.05$).

In experiment 2, where four diaspore species (bloodroot, wood rush, longstem sedge, and Canada thistle) were tested with the same three ant species included in experiment 1, similar responses were observed. There were no significant differences in the frequency

of antennation and chewing of diaspores by the three ant species ($P = 0.07$), however, in this experiment, there was a significant difference in ant species for duration ($F_{(2,10)} = 4.573$, $P = 0.038$). There were significant differences in antennation and chewing frequency and duration due to diaspore species ($F_{(3,45)} = 40.212$, $P < 0.0001$, $F_{(3,45)} = 47.522$, $P < 0.0001$; frequency and duration, respectively) and the interaction of ant species and diaspore species ($F_{(6,45)} = 4.004$, $P = 0.003$, $F_{(6,45)} = 6.821$, $P < 0.0001$; frequency and duration, respectively). Figure 2A and B demonstrates that the interaction between ant and diaspore species for both frequency and duration of antennation and chewing can be explained by a more pronounced preference for bloodroot diaspores over the other three diaspore species by *M. rubra* (ca. 2.75× and 6×, frequency and duration, respectively) compared to the native ant species (1.7–2× and 3×, frequency and duration, respectively).

There was also a significant difference in attempts to move diaspores into the nest due to diaspore species ($\chi^2 = 12.234$, $df = 3$, $P = 0.007$), but not due to ant species ($P > 0.05$) or the interaction between ant species and diaspore species ($P > 0.05$). The proportion of diaspores that ants attempted to move to the nest were 50% for bloodroot, 16.7% for wood rush, 11.1% for thistle, and 5.6% for longstem sedge. Therefore, the attempt to move a diaspore to the nest appeared to be correlated with preference as reflected by antennation and chewing and diaspore size.

In experiment 3, with four diaspore species and four ant species, there was a significant difference in antennation and chewing frequency (square root transformed) between ant species ($F_{(3,6)} = 5.253$, $P = 0.041$), and a significant interaction between ant species and diaspore species ($F_{(9,24)} = 3.628$, $P = 0.006$). *Formica argentea* and *L. alienus* exhibited little preference for any of the diaspore species, whereas, *L. longispinosus* tended to prefer bellwort and *M. rubra* preferred violet (Fig. 3A). This preference of *M. rubra* in experiment 3 is in contrast to experiment 2 where *M. rubra* preferred bloodroot, although violet was not a diaspore species choice in that experiment. When duration (square root transformed) of antennation and chewing is considered, diaspore species was significant ($F_{(3,24)} = 2.949$, $P = 0.053$). Bellwort appeared the most preferred diaspore species and wood rush appeared to be the least preferred, independent of ant species (Fig. 3B). There was no significant relationship between movement of diaspores to the nest and ant species or diaspore species ($P > 0.05$).

Inspection of seeds for damage after each experiment showed no signs of cracked or damaged seed coats in any of the diaspore species, except for wood rush (the smallest of the diaspores species, Fig. 3A and B). We found five wood rush seeds with elaiosomes

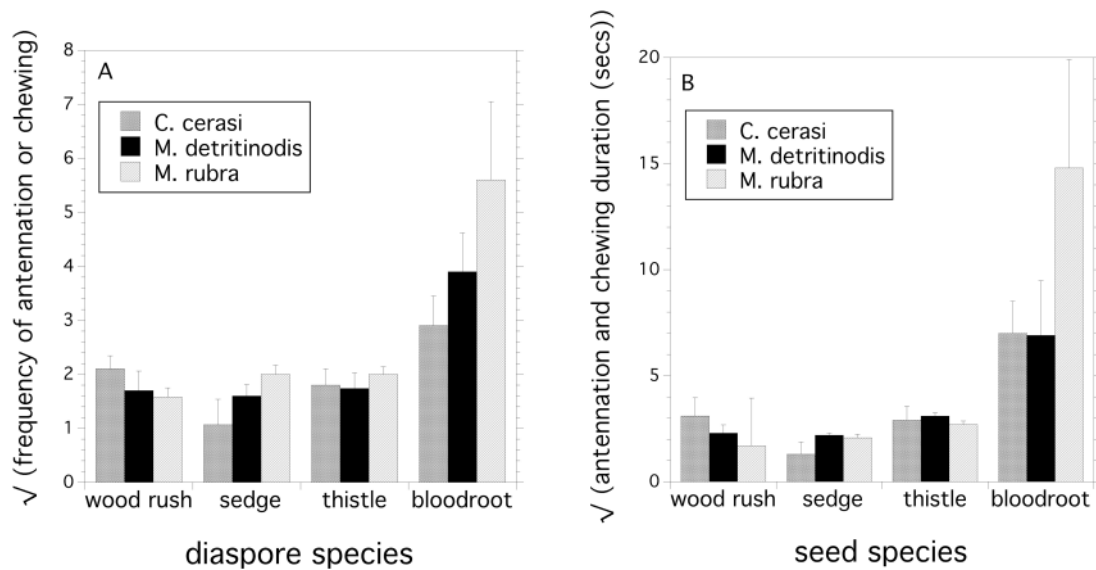


Fig. 2. The square root transformed frequency (A) and duration (B) of antennation and chewing of diaspore species by three species of ants. The interaction between diaspore species and ant species is $P = 0.003$ and $P < 0.0001$, for frequency and duration, respectively. Diaspore species are ordered in the graph from smallest (left) to largest (right) in mass (mg). Error bars are standard errors of the mean.

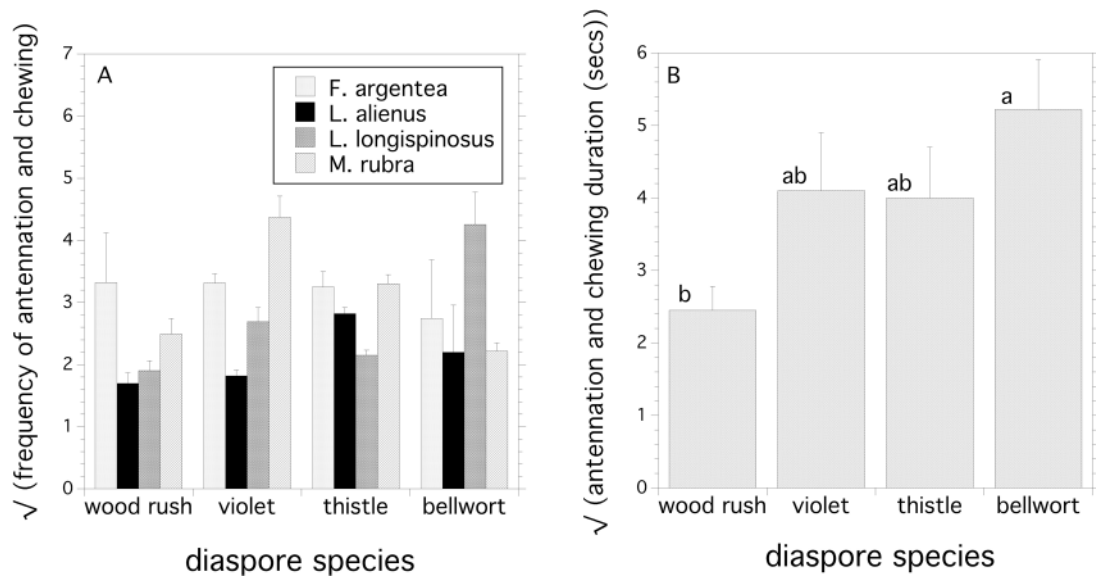


Fig. 3. The square root transformed frequency (A) and duration (B) of antennation and chewing of diaspore species by four species of ants. The interaction between diaspore species and ant species is $P = 0.006$ for frequency and the diaspore species effect is $P = 0.053$ for duration. Different letters associated with bars in Fig. 3B are significantly different (Tukey multiple comparisons, $P < 0.05$). Diaspore species are ordered in the graph from smallest (left) to largest (right) in mass (mg).

removed that were partially eaten and cracked. However, this damage constituted less than 3.1% of the total diaspores handled by the ants. Based upon our laboratory assays, we investigated if antennation frequency by native ants and *M. rubra* were determined by diaspore or elaiosome length or mass. For native ants, only elaiosome length was related to the frequency of antennation ($F_{(1,8)} = 9.539$, $P = 0.015$, $r^2 = 0.406$). Chewing frequency, but not antennation, in *M. rubra* was related to elaiosome length ($F_{(1,8)} = 5.621$, $P = 0.045$, $r^2 = 0.251$). Elaiosome length and mass were highly correlated ($r = + 0.739$, $P = 0.0006$) and diaspore mass was highly correlated to elaiosome length and mass ($r = + 0.681$, $P < 0.0001$; $r = + 0.764$, $P < 0.0001$, respectively). Thus, the larger the mass of the diaspore

and/or elaiosome mass or length, the greater the handling (antennation/chewing) was of a diaspore. This appeared to be the case for both native ant species and *M. rubra* in our laboratory studies.

Field Seed Removal Assays

Diaspore removal during the 10 min observation interval was much greater by *M. rubra* compared to the native ant species (*C. cerasi* and *M. detritinodis*) for both experiments ($\chi^2 = 11.839$, $df = 2$, $P = 0.003$; $\chi^2 = 27.401$, $df = 2$, $P < 0.0001$) (Fig. 4A and B). *M. rubra* consistently removed all diaspore species at a greater rate than native ant species in both experiments. A diaspore species effect was also seen in the field experiment 1 ($\chi^2 = 7.875$, $df = 3$, $P = 0.048$) showing that

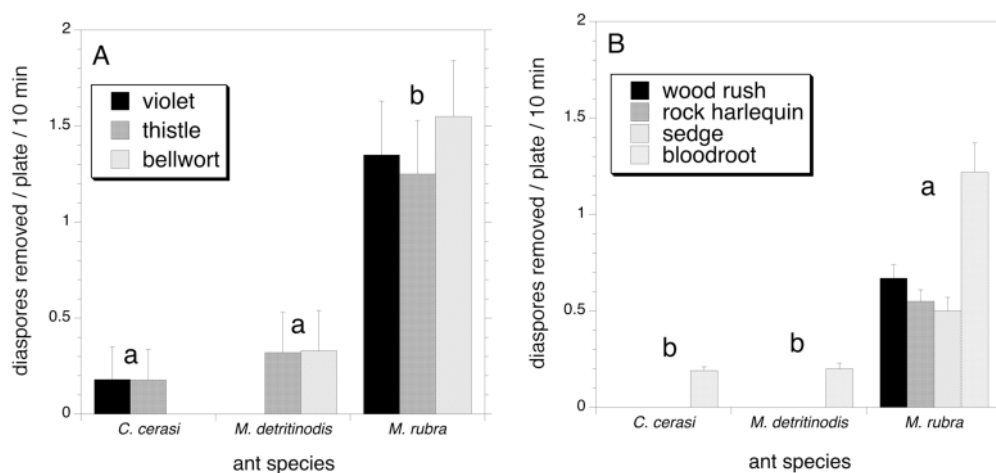


Fig. 4. The number of diaspores removed over a 10-min period by an invasive ant *M. rubra* and two native ants *M. detritinodis* and *C. cerasi* on Mt Desert Island, ME in 2005 for experiment 1 (A) and experiment 2 (B). Different letters associated with bars in each figure are significantly different (ordinal logistic regression multiple comparisons, $P < 0.05$). Diaspore species are ordered in Fig. 4A and B, within groups of bars, from smallest (left) to largest (right) in mass (mg). Error bars are standard errors of the mean.

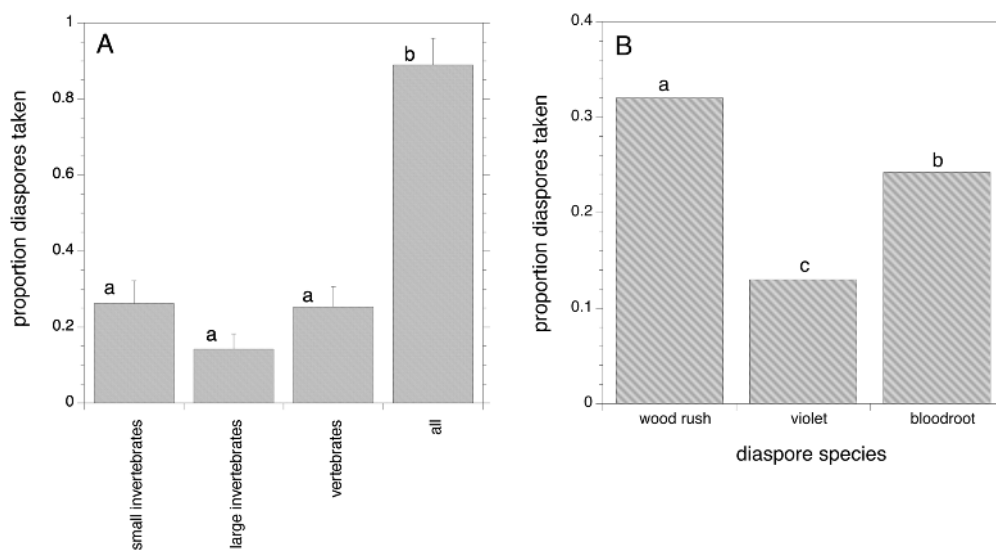


Fig. 5. The proportion of diaspores (arcsin transformed) removed over 24 h from *M. rubra* invaded sites in 2005. Different exclusions represent seed access to only small invertebrates (up to 0.32 cm in width), large invertebrates (up to 1.27 cm in width), vertebrates, or all fauna (A) and the proportion of seeds taken of the three seed species pooled across all exclusion treatments (B). Different letters associated with bars in each figure are significantly different (Tukey multiple comparisons, $P < 0.05$). Diaspore species is ordered in the Fig. 5B from smallest (left) to largest (right) in mass (mg). Error bars are standard errors of the mean.

removal of bloodroot was greater than the other three diaspore species, by all three species of ants. There was no ant species by diaspore species interaction ($P > 0.05$) for either field experiment.

M. rubra were found to take violet diaspores longer distances than native ants ($31.4 \text{ cm} \pm 4.2$ (SE) compared to $12.2 \text{ cm} \pm 2.2$; $t_{(0.05, 21)} = 3.66$, $P < 0.001$). The median distance for *M. rubra* dispersal of all diaspores was 24 cm, the mean 31.4 cm, the minimum recorded distance was 5 cm, and the maximum distance was 110 cm. The median diaspore dispersal distance for native ants was 7 cm, the mean 12.2 cm, and the minimum and maximum distances were 3 and 43 cm. The native ants recorded collecting diaspores were *Formica subsericea*, *C. cerasi*, and *M. detritinotus*. Marked (painted) violet diaspores placed adjacent to the nest entrances of these three ant species were not found as seeds in the middens or in the surrounding 1

m neighborhood of the nests. Therefore, it is assumed that the seeds remained within the nest, although we could have missed them.

Comparative Predation Experiments

A split-plot ANOVA for the 2005 field experiment revealed that exclusion treatment ($F_{(3,6)} = 27.333$, $P = 0.0007$) and diaspore species ($F_{(2,124)} = 15.670$, $P < 0.0001$) were significant, but the interaction between exclusion and diaspore treatment was not ($P > 0.05$). More diaspores were taken from the control (no exclusion) than the other exclusion treatments (Fig. 5A). Wood rush diaspores were removed at a higher rate than violet diaspores (Fig. 5B).

A split-split-plot ANOVA for the 2006 experiment revealed the following factors to be significant: ant invasion ($F_{(1,2)} = 20.222$, $P = 0.046$), exclusion type ($F_{(2,8)} = 28.159$, $P = 0.0002$), and seed

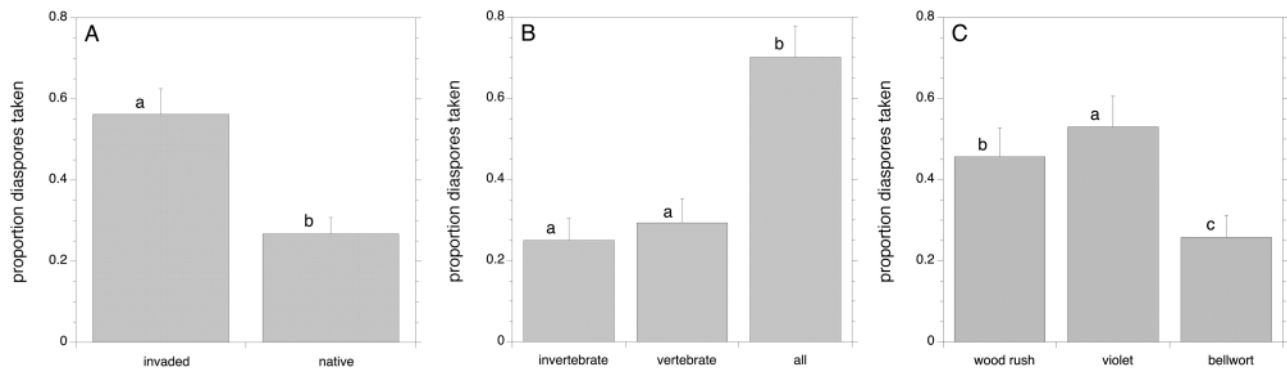


Fig. 6. The proportion of seeds (arcsin transformed) removed over 24 h from both *M. rubra* invaded and noninvaded sites in 2006 (A). Different enclosures represent seed access to only small invertebrates (up to 0.32 cm in width), vertebrates, or all fauna (B) and the proportion of seeds taken of the three seed species pooled across both invasion habitats and all enclosure treatments (C). Different letters associated with bars in each figure are significantly different (Tukey multiple comparisons, $P < 0.05$). Diaspore species is ordered in Fig. 6C from smallest (left) to largest (right) in mass (mg). Error bars are standard errors of the mean.

species ($F_{(2,24)} = 5.062$, $P = 0.015$). No two-way or three-way interactions were significant, although the enclosure \times seed species interaction suggested a potential effect if the experiment had increased power ($P = 0.061$). More seeds were removed in invaded areas than noninvaded areas (Fig. 6A). The control (no enclosure or 'all') treatment had more seeds removed than either the invertebrate or large animal enclosures (Fig. 6B) and violet seeds were removed at a higher rate than sessile bellwort seeds (Fig. 6C).

Discussion

What are the native plant community reproduction ramifications of an ant invasion in north temperate North America? In the field, we found that diaspore dispersal distance of violet was significantly greater when dispersed by *M. rubra* in comparison with the native ant species. Also again, in the field, *M. rubra* consistently removed a greater number of diaspores over an average 10-min period and over 24 h. Diaspores also appeared to be cached in nests by both *M. rubra* and the native ant species. There was also no consistent difference in diaspore preference between *M. rubra* and the native ant species in both the laboratory and field assays. Laboratory experiment 2 was the one exception to this, but the interaction between ant species and diaspore species appeared to be due to an exceptional attraction of bloodroot to *M. rubra*. The lack of a consistent difference in ant preference for diaspore species implies that there may be no long-term effect on plant community composition due to *M. rubra*'s invasion. *M. rubra*'s higher removal rate of the diaspores in comparison to the native ant species that we observed in the field is most likely due to the high nest density and increased demand for food from crowding of *M. rubra* colonies (Grodén *et al.* 2005, Garnas *et al.* 2007).

Previous studies have found invasive ants such as *L. humile* and *S. invicta* to behave in different manners to the native ant species which results in a detrimental effect on native plant fitness (Bond and Slingsby 1984, Christian 2001, Zettler *et al.* 2001, Rowles 2009; although see Stuble *et al.* 2010), however the results shown in this paper suggest that some invasive ants may potentially have greater benefits for native plant species, as was also shown by Rowles (2009), for an Argentine ant invasion in southeastern Australia. Although not extensively tested, from our laboratory observations on seed coat appearance after encounters, we believe *M. rubra* does not significantly affect seed germination. Seeds were consistently found with only the elaiosome removed and no visible damage to the seed in laboratory assays, except in one case with wood sedge.

Dispersal distance was greater by 40% when diaspores were dispersed by *M. rubra* compared to native ant-diaspore dispersal. This increases the likelihood of a given diaspore to be dispersed away from the parent plant and other competitors, and numerically responding seed predators. Although diaspores are taken only short distances by ants in comparison with wind or vertebrate dispersal (Hölldobler and Wilson 1990, He 2009), this may be enough to reduce competition with the parent plant and siblings (Andersen 1988, Espadaler and Gómez 1996). Higher dispersal distances also increase the ability to colonize new habitats (Cain *et al.* 2000) and ant dispersal distances can be increased by habitat change such as fire (Parr 2007).

M. rubra removed the diaspores at a significantly greater rate when compared with native ant species in the field. This may potentially reduce the risk of predation by seed eating large size invertebrates, rodents and birds. We found that on Mt. Desert Island, these seed predating taxa appear to contribute at least half of the seed removal, the other half by ants and other small invertebrates. A possible benefit to the plant of diaspore dispersal by ants is suggested by the predator avoidance hypothesis; diaspores that are taken by mutualistic ants are removed to nests or midden piles where seed predators do not readily encounter them (Beattie 1985, Hanzawa *et al.* 1985, Fedriani *et al.* 2004). Seed predation is a key process for many plant species and can affect population size, persistence and spatial distribution, and the diversity and structure of plant communities (Da Silva and Tabarelli 2001, Fedriani *et al.* 2004). If diaspores are taken into an ant's nest or buried under the soil surface, they may gain protection from fire, as well as, protection from predators as shown by Ness (2008). Both the observed native ants and *M. rubra* did not take seeds to midden piles during the length of this study. Therefore, it is assumed that all species tested left seeds without the elaiosome within the nest, as seen in other ant-diaspore mutualisms (Bond *et al.* 1991, Westoby *et al.* 1992). Servigne and Detrain (2008), however, found that after 24 h, *M. rubra* pulled all seeds brought into the nest out to middens and at least half of those rejected seeds still had the elaiosome attached. It is possible given the high rate of diaspore removal in the 24 h comparative predation study, that some diaspores were removed from the nest as these authors suggest, but picked up and consumed by another seed predator. However, no evidence of this was observed. Servigne and Detrain (2008) also reported a 'seed-size effect'. They found greater removal of smaller compared to larger diaspores, although they only tested two diaspore sizes. In the laboratory, we found that larger diaspores that possessed larger elaiosomes in length or mass were more highly

attractive as measured by antennation and chewing. This is a similar pattern reported by Mark and Olesen (1995). In fact, some believe that this relationship is consistent enough that it is ants that have created selection for large elaiosome size in diaspores (Edwards *et al.* 2006). However, in the field, similar to Servigne and Detrain (2008), we found that a higher proportion of wood sedge (the smallest diaspore) was removed compared to bloodroot or bellwort (both large diaspores), but the intermediate size violet diaspore was taken at the lowest rate in the first experiment and the highest rate in the second experiment. Our data suggest that while size may play a role, elaiosome quality might be more just as or more important, as suggested by Turner and Frederickson (2013).

Our results strongly suggest that the current *M. rubra* invasion of Maine Acadian Forest landscapes may be resulting in a positive interaction with native and invasive (Canada thistle) myrmecochorous plants. However, see McPhee *et al.* (2012), in regards to potential plant stress due to *M. rubra*-aphid mutualism and resulting enhanced herbivory. Most importantly our laboratory and field bioassays demonstrated that *M. rubra* was not selecting different seed species from the native ant species, implying that this ant's invasion will have little long-term effect on the native plant community through differential seed dispersal. However, Bologna and Detrain (2015) have shown that *M. rubra* sharply decrease and then completely stop seed retrieval after only a few weeks. It is possible our study did not run long enough to capture this behavioral shift. If *M. rubra* collects seeds for shorter periods of time compared to native species, this could result in a shift in the plant community. This shift could also be food resource dependent and might be determined by the availability and competition with other high-quality foods (Mayer *et al.* 2005). Ness (2009) showed that in eastern deciduous North American forests, *Aphaenogaster* spp. ants collect $74 \pm 26\%$ of the native myrmecochorous seeds. In those areas where *M. rubra* has invaded, native ant fauna have been almost eliminated (Grodén *et al.* 2005, Garnas 2005, Garnas *et al.* 2014). Possibly, the competition for myrmecochorous seeds might play a role in the outcome of the negative consequence of such an invasion that results in the decline of native ant biodiversity (Garnas *et al.* 2007).

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