Impact of an exotic earthworm on seed dispersal of an indigenous US weed

E. Regnier1*, S. K. Harrison1, J. Liu1, J. T. Schmoll1, C. A. Edwards2, N. Arancon2 and C. Holloman3

1Department of Horticulture and Crop Science, Ohio State University, 2021 Coffey Road, Columbus, OH 43210, USA; 2Department of Entomology, Ohio State University, 318 W 12th Ave., Columbus, OH 43210, USA; 3Department of Statistics, Ohio State University, 1958 Neil Ave., Columbus, OH 43210, USA

Summary

1. Ambrosia trifida L. (giant ragweed), an aggressive weed of US grain crops and indigenous to North America, colonizes no-tillage crop fields and undisturbed soils despite its large seed size and susceptibility to seed predation. Secondary seed dispersal is critical to seed survival and seedling establishment, yet mechanisms of secondary dispersal are poorly understood for such large-seeded weedy species.

2. Field experiments were conducted to determine how seed foraging by the European exotic burrowing earthworm Lumbricus terrestris L., affected A. trifida seed burial and seedling recruitment, and to determine seed selectivity by L. terrestris.

3. Earthworms collected and buried over 90% of A. trifida seeds placed on the soil surface at a rate eightfold faster than abiotic seed burial. There was a sixfold higher concentration of seeds in burrows than in surrounding soil and a mean of 127 A. trifida seeds per burrow after a single season of A. trifida seed dispersal.

4. Earthworms buried A. trifida seeds from 0·5 to 22 cm deep and reduced recruitment by 37% compared to seeds buried abiotically and protected from predators, due to burial of some seeds below emergence depth limits. However, seedling biomass was increased by 30%.

5. Earthworms foraged selectively among seeds of 11 large-seeded species and collected more seeds of A. trifida than of other species. The earthworms buried small (8·5 mm) A. trifida seeds more deeply and reduced their emergence more than large (11·5 mm) A. trifida seeds.

6. Synthesis and applications. The novel interaction of L. terrestris and A. trifida increases seed bank formation of A. trifida. Burrows of L. terrestris provide safe sites for seeds of A. trifida that may increase seedling establishment in environments with a high risk of seed predation. Control measures to prevent reproduction by A. trifida should be increased where L. terrestris is present due to the earthworms’ ability to bury and protect weed seeds. Selective seed caching by this widely distributed earthworm species may change plant community composition in agricultural and natural areas in North America and influence the evolution of seed traits. Land managers should consider the effects of L. terrestris on seedling regeneration of native and exotic plant species in areas undergoing colonization by L. terrestris.

Key-words: diplochory, giant ragweed, invasive earthworm, seed bank, seed burial, seed predation, secondary seed dispersal, weed management

Introduction

Postdispersal seed predation is a major source of weed seed mortality in agroecosystems (Mittelbach & Gross 1984; Harrison, Regnier & Schmoll 2003; Westerman et al. 2003; Heggenstaller et al. 2006). Seeds exposed on the soil surface are easily detected by predators and vulnerable to extremes of moisture and temperature that compromise seed viability. Secondary dispersal, the movement of seeds across and into the soil, is a critical factor influencing seed survival and thus plant population dynamics, plant species composition, and seed evolution (Vander Wall et al. 2005). Here we investigate...

Despite having numerous natural enemies (Bassett & Crompton 1982; Harrison et al. 2001, 2003), *A. trifida* has become a weed of major importance in tilled and no-tillage crop fields over the last two decades and is among the most competitive annual weeds in the US Corn Belt (Webster et al. 1994; Harrison et al. 2001; Gibson, Johnson & Hillger 2005). *Ambrosia trifida* also persists in successional habitats, where it often appears before colonizing crop fields. It dominates most associated species due to its early and prolonged emergence period, rapid growth, and large size (Abul-Fatih & Bazzaz 1979; Bassett & Crompton 1982). Seeds of *A. trifida* are dispersed singly within a hardened involucre (Brown & Brown 1984) and are among the largest and most variable in size of common weeds, ranging from 3 to 14 mm in length (Sako et al. 2001). Rodents, birds and ground beetles feed heavily on the seeds, causing near-total losses after dispersal (Harrison et al. 2003). The ability of *A. trifida* to persist in no-tillage crop fields and successional fields is unusual for a large-seeded annual given the lack of tillage to bury seeds and the suitability of these habitats for seed predators (Mittelbach & Gross 1984).

Large seeds often require animal-assisted burial to survive due to their inability to achieve burial through abiotic forces alone. Rodents, some birds, and seed-harvesting ants bury seeds in caches that are incompletely consumed, facilitating seedling establishment (Vander Wall et al. 2005). *Lumbricus terrestris* disperses small seeds by ingesting and depositing them in its casts (Grant 1983; Pearse, Roggero & Tipping 1994; Thompson, Green & Jewels 1994; Willems & Huismans 1994; Zaller & Saxler 2007) and also, for seeds too large to ingest, by dragging them below the surface (Milcu, Schumacher & Scheu 2006). The earthworm forages for surface litter that it stores in its vertical burrows and middens (mounds of castings and debris over the burrows) and feeds on the decomposing litter and associated microorganisms (Edwards & Bohlen 1996; Cortez & Bouché 1998). *Lumbricus terrestris* has been introduced by human activity throughout temperate agricultural, grassland, and urban soils. Its populations in agricultural fields increase with decreasing tillage and can reach 160 individuals m\(^{-2}\) (Edwards & Bohlen 1996). Due to its high consumption rate and burrowing habit, *L. terrestris* has a disproportionate effect on the soil habitat and biota relative to its abundance, and has been characterized as an ecosystem engineer (Butt & Nuutinen 2005). Although considered a desirable species in agroecosystems for its beneficial effects on soil fertility and physical properties, *L. terrestris* is viewed as an invasive species in northern US forests where its removal of litterfall threatens seedling regeneration and biodiversity of the forest understory (Hendrix & Bohlen 2002; Lawrence et al. 2003; Hale, Frelich & Reich 2006).

Disruption of animal seed dispersal pathways by exotic species has reduced establishment of some indigenous plant species (Christian 2001; Carney, Byerley & Holway 2003), but the potential for exotic species to increase rather than decrease secondary seed dispersal is not well established (Traveset & Richardson 2006). Elucidation of *A. trifida’s* secondary dispersal mechanisms is necessary to understand this plant’s colonization patterns and establishment across the landscape and the reasons for changes in its populations. Our goal was to determine if *L. terrestris* interacted with *A. trifida* seeds in agricultural fields and to evaluate the earthworm’s potential to increase secondary dispersal of *A. trifida*. Experiments were conducted to determine the effect of *L. terrestris* on *A. trifida* seed burial rate, seed bank formation, and seedling establishment, and to determine if *L. terrestris* gathers *A. trifida* selectively among other large-seeded species.

**Materials and methods**

Field studies were conducted from 2001 through 2007 at the Ohio State University Waterman Agriculture and Natural Resources Laboratory in Columbus, OH, USA (40°00’ N, 83°02’ W), on a Crosby silt loam soil (fine, mixed, active, mesic Aeric Eipaqualfs) with 2% organic matter and pH of 6.4. The same soil was used in laboratory experiments. Unwanted vegetation in field plots was controlled with 0.7 kg ha\(^{-1}\) glyphosate.

**SEED BURIAL RATE AND SEEDLING ESTABLISHMENT**

Experiments to determine the effects of *L. terrestris* on *A. trifida* seed burial rate and seedling establishment were established in October 2002 in two fields with the same long-term cropping and tillage conditions. In the 4 years preceding the experiment, one field had been cropped in soybean and chisel-tilled annually and the other had been fallowed and not tilled. Both fields had no history of *A. trifida* colonization.

Treatments of *L. terrestris* and *A. trifida* combinations comprised a 3 × 2 factorial arrangement with three earthworm–seed sowing depth treatments (hereafter referred to as earthworm treatments) and two seed size treatments as factors. The three earthworm treatments were: (i) *L. terrestris* absent and seeds buried 2.5 cm deep; (ii) *L. terrestris* absent and seeds deposited on the surface; and (iii) *L. terrestris* present and seeds deposited on the surface. The first treatment served as a control that was expected to produce maximum seedling populations due to seeds being sown at an optimum depth for emergence (Harrison et al. 2007). The two seed sizes were small (35 ± 1.4 mg, average dimension = 4.3 × 8.5 mm) and large (100 ± 4.7 mg, 7.4 × 11.5 mm). Seed viability, determined by tetrazolium assay (Peters 2000) was 73 ± 2.2% (soybean field) and 84 ± 1.2% (fallow field). Large pieces of surface litter were removed from within plots and 49 seeds were deposited on the soil surface 7 cm apart in a 7 × 7 square grid pattern. Treatments were arranged in a randomized complete block design and were replicated five (soybean field) or six (fallow field) times.

The treatments were applied to circular 0.6 m-diameter plots spaced 1.5 m apart. Rodents and birds were excluded from the plots by a 25-cm tall sheet-metal barrier that was erected around each plot, buried to a depth of 4 cm, and topped with bird netting. To establish plots without *L. terrestris*, carbaryl (385 g active ingredient ha\(^{-1}\)) was applied before depositing seeds (Edwards & Bohlen 1996). For treatments with *L. terrestris* present, earthworm populations were estimated by counting the number of middens in each plot (Edwards & Bohlen 1996). Midden numbers did not differ between fields and averaged 27 ± 1.8 middens (mean ± SE) m\(^{-2}\).
Seed burial rate was determined by recording the number of seeds remaining on the soil surface at frequent intervals through February 2002. Seeds collected by *L. terrestris* and placed in middens were noted separately. The time for 50% seed burial was calculated for each plot from a linear extrapolation between the two nearest measurement times.

Seedling establishment was characterized through emerged seedlings, shoot dry weight, and depth of emergence. Seedlings were counted periodically from first to last emergence (March through June) in 2002 and, again, in 2003, and the data were combined to obtain total cumulative emergence. In 2002, seedlings were removed on 15 May and their shoot dry weights were recorded (small seeds: \( n = 159 \); large seeds: \( n = 224 \)). Hypocotyl length below the soil surface was measured to determine emergence depth.

Data were analysed using a mixed effects linear model (PROC MIXED procedure in SAS software for Windows, Version 9.1.3, SAS Institute Inc., Cary, NC, USA). Treatments were considered fixed effects, and field and block were considered random effects. Model selection was performed using a backwards elimination procedure (Weisberg 1985). Initially, a full model including all main and interaction effects was considered. A criterion of \( P = 0.05 \) was used to assess variables in the model, and no variable was removed if it was included in a significant higher order interaction. Emergence depths were log-transformed before analysis to obtain a normal distribution of the residuals. The number of emerged seedlings was converted to the percentage of viable seeds sown.

### DISTRIBUTION OF SEEDS WITHIN EARTHWORM BURROWS

To determine the depth distribution of *A. trifida* seeds within *L. terrestris* burrows, an experiment was established in October 2002 and repeated in October 2003 in the fallow field described earlier. Nylon threads measuring 30 cm in length were attached to *A. trifida* seeds and three small and three large seeds were deposited on the soil surface near an earthworm burrow (randomly selected). Predator exclusion barriers, as previously described, were erected around each burrow. Four burrows were selected in 2002 and seven in 2003. Seed burial depth was determined from the length of thread pulled into the burrow. Data were analyzed using a mixed effects linear model as described previously. Seed size was treated as a fixed effect, and year and burrow as a random effect. Data were log-transformed before analysis.

### ASSOCIATION OF SEEDS AND SEEDLINGS WITH EARTHWORM BURROWS

The association of *A. trifida* with *L. terrestris* burrows was determined in 2003–2004 and 2006–2007 in a field (50 × 15 m) previously cropped in soybean and with no history of *A. trifida* colonization. *Ambrosia trifida* was introduced on the site in June 2003 at a density of 5 plants m\(^{-2}\). The plants were allowed to mature, disperse seeds, and establish naturally thereafter. The site was rotary-tilled and planted with soybean in 2004 and 2005, but was otherwise neither tilled nor cropped. The seedling population of *A. trifida* in 2007 was 52 plants m\(^{-2}\).

The site was sampled in November 2003 and December 2006 to determine the numbers of *A. trifida* seeds in *L. terrestris* burrows (burrow seed density) and outside of burrows (non-burrow seed density). A soil core (6 cm diameter × 15 cm deep) of either an *L. terrestris* burrow or non-burrow area (assigned randomly) was taken at 1.5 m intervals along four parallel transects spaced 10 m apart to obtain five cores each of burrow or non-burrow areas for each transect. Seeds of *A. trifida* were sieved from the soil samples, and the mean burrow seed density and non-burrow seed density was calculated for each transect.

Burrow numbers and surface area occupied by burrows (i.e. midden areas) were determined by counting middens and measuring their diameters in four 0.25-m\(^2\) quadrats placed at 1-m intervals along each transect (\( n = 16 \)). For each transect, the number of *A. trifida* seeds dispersed to burrows and non-burrow areas was estimated as the product of the appropriate mean seed density and surface area occupied.

The number of *A. trifida* seedlings emerging from *L. terrestris* burrows and from outside burrows was determined in April 2004 from 16 quadrats as described earlier, and in May 2007, in fifty 0.25-m\(^2\) quadrats placed at 1-m intervals along one transect placed diagonally through the site. Surface area occupied by burrows was calculated in 2004 as described above, and in 2007 by visual estimates of percentage midden area using a gridded quadrant (2007).

A one-sample, one-tailed z-test (Johnson & Kuby 2000) was used to test the null hypothesis that the proportion of seeds or seedlings distributed in burrows was equal to the proportion of the total surface area occupied by burrows. This would indicate that *L. terrestris* activity had no influence on their distribution.

### SEED SELECTION BY EARTHWORMS

Field and laboratory experiments were conducted to determine if *L. terrestris* foraged for seeds selectively among large weed and crop seeds. The field experiment was conducted in the fallow field, described earlier, in October 2003 and repeated in May 2004. Eight replicate plots of 30.5 cm diameter were established around single *L. terrestris* burrows, as described previously. Eighteen seeds each of *A. trifida*, *Xanthium strumarium* L., *Sicyos angulatus* L., *Helianthus annuus* L., *Glycine max* L., and *Zea mays* L. were placed on the soil surface in each plot. Seeds ranged in length from 7 to 18.6 mm. Seed species and number collected by *L. terrestris* were determined after 4 days.

In the laboratory experiment, seed selectivity by *L. terrestris* was tested among *A. trifida* and five other weeds with seeds ranging in length from 3.4 to 11 mm. PVC pipes (15 cm diameter × 30 cm long) filled with field soil served as experimental units. The soil was wetted to field capacity, and four large *L. terrestris* individuals were introduced onto the soil surface of each PVC pipe. The earthworms acclimatized at 18 °C for 1 week and established burrows during this period. Ten seeds each of *A. trifida*, *Convolvulus arvensis* L., *Pastinaca sativa* L., *Aristolochia versicolor* Benth., *Bidens frondosa* L., and *Ipomoea purpurea* (L.) Roth were deposited in each pipe. Seed species and number collected by *L. terrestris* were determined after 3 days. The experiment was conducted in May 2006 and repeated twice in June 2007, each time with eight replicates. The data from all experiments were subjected to ANOVA and Fisher’s protected LSD (\( P < 0.05 \)) was used to determine significant differences between treatment means.

### Results

#### SEED BURIAL RATE AND SEEDLING ESTABLISHMENT

The number of seeds remaining on the soil surface decreased rapidly in the presence of *L. terrestris* (Fig. 1). Mean time for 50% burial of seeds was 11 ± 4 days and did not differ between seed sizes. *Lumbricus terrestris* decreased the time for 50% burial by 77 ± 7 days compared to abiotic burial.
Abiotic burial of seeds by rainfall was minimal and burial occurred primarily by freeze–thaw cycles. By 20 days after seed deposition, *L. terrestris* had collected 94% of *A. trifida* seeds in its burrows and middens, and seed size did not affect the numbers collected (Fig. 2d). Nocturnal collection and burial of seeds by *L. terrestris* was confirmed by direct observation (Fig. 2a–c). When a foraging earthworm encountered an *A. trifida* seed, it gripped a ridge of the involucre and retracted quickly, pulling the seed toward its burrow. If the seed was too large to be pulled inside the burrow, it was left...
in the midden. Consistent with this observation, a greater percentage of large seeds, 35 ± 4%, were cached in middens compared to 2 ± 1% of small seeds (Fig. 2d).

Most of the total *A. trifida* emergence observed over the following 2 years occurred during the first year (Fig. 3). The greatest emergence occurred in the control treatment (*L. terrestris* absent, seeds sown 2·5 cm deep). There was no effect of seed size and, averaged over sizes, a cumulative total of 99% of seeds emerged. When seeds were sown on the soil surface without *L. terrestris*, emergence was reduced compared to the control (*P* < 0·0001), and there was no effect of seed size. Averaged over seed sizes, emergence was 86%. Reduced emergence from surface-sown seeds was probably due to losses in viability caused by desiccation and/or freezing. *Lumbricus terrestris* reduced emergence compared to either treatment without earthworms (*P* < 0·0001); emergence was 44% for small seeds and 64% for large seeds (*P* < 0·001). Compared to abiotic burial, *L. terrestris* reduced seedling recruitment by an average of 37%.

Shoot biomass of seedlings emerging from *L. terrestris* plots (0·69 ± 0·10 g plant⁻¹) was 30% greater than shoot biomass of seedlings from plots without *L. terrestris*, regardless of seed sowing depth (0·53 ± 0·05 g plant⁻¹, averaged over seed depths, *P* < 0·01). Shoot biomass was greater for large (0·61 ± 0·07 g plant⁻¹) compared with small seeds (0·56 ± 0·06 g plant⁻¹, *P* < 0·01), and there were no interactions of seed size with earthworm treatments.

**Distribution of Seeds within Earthworm Burrows**

Residuals for *A. trifida* seed burial depth data were not normally distributed and estimated medians are reported to describe the central tendency of the data. The seedling emergence depth of both large and small seeds was increased by the presence of *L. terrestris* (Fig. 4a; *n* = 224, *P* < 0·005 for large seeds; *n* = 159, *P* < 0·001 for small seeds). Median seedling emergence depth was 1·9 and 1·5 cm for large seeds, and 2·2 and 1·9 cm for small seeds when *L. terrestris* was present and absent, respectively. The presence of *L. terrestris* resulted in a slightly greater median emergence depth of 2·2 cm for small seeds compared to 1·9 cm for large seeds (*n* = 180, *P* < 0·05), but emergence depths did not differ between seed sizes when *L. terrestris* was absent.
ASSOCIATION OF SEEDS AND SEEDLINGS WITH EARTHWORM BURROWS

Mean *L. terrestris* midden numbers in the autumn of 2003 and 2006 were 38 ± 4 and 37 ± 4 middens m⁻², respectively. Average midden diameter was 5·9 ± 0·2 cm in 2003 and 7·2 ± 0·08 cm in 2006, and percentage surface area occupied by middens ranged from 4·8 to 14·5% over the two sampling times (Fig. 5). The proportion of total *A. trifida* seeds and seedlings that were in *L. terrestris* burrows ranged from 21 to 75% and was greater than the proportion of soil surface covered by middens at all sampling times (Fig. 5, *P* < 0·001 for all *z*-tests). Thus, the null hypothesis that *L. terrestris* activity had no influence on seed or seedling distribution was rejected. There were 5·7 and 6·4 times more seeds and seedlings in *L. terrestris* burrows than expected in 2003 and 2004, respectively, based on percentage cover by middens. In 2006 and 2007, there were 2·5 and 4·6 times more seeds and seedlings, respectively, in *L. terrestris* burrows than expected. A lower proportion of seeds and seedlings were aggregated in burrows at the second sampling (2005–2006) than at the first (2003–2004), probably due to spring tillage that disrupted burrows and redistributed some of the collected seeds. Mean *A. trifida* seed densities were 127 ± 18 and 67 ± 5 seeds soil core⁻¹ from burrows compared to 7 ± 1 and 6 ± 1 seeds soil core⁻¹ from areas without burrows in 2003 and 2006, respectively.

SEED SELECTION BY EARTHWORMS

*Lumbricus terrestris* collected seeds of all species tested, but seeds were collected selectively in both field (Fig. 6a) and laboratory (Fig. 6b) experiments. In field plots, *L. terrestris* collected similar numbers of *A. trifida*, *H. annuus*, and *S. angulatus* seeds, and more seeds of these species were collected than of *G. max*, *Z. mays*, or *X. strumarium* (*P* < 0·001). Seed collection ranged from 23% for *X. strumarium* to 86% for *A. trifida*. In the laboratory, *L. terrestris* collected similar numbers of *A. trifida*, *C. arvensis*, and *P. sativa* seeds, and collected more *A. trifida* seeds than *A. minus*, *B. frondosa* or

![Fig. 5.](image)

The proportion of *Ambrosia trifida* seeds and seedlings found in burrows and overlying middens of *Lumbricus terrestris* in relation to the proportion of total soil surface area covered by *L. terrestris* middens. Vertical bars represent one SE.

![Fig. 6.](image)

Percentages of seeds cached by *Lumbricus terrestris* in a field experiment after 4 days (a) and a laboratory experiment after 3 days (b). Means accompanied by the same letter are not significantly different (*P* < 0·05). Seeds are pictured below columns in relation to a 1-cm average diameter for an *L. terrestris* burrow. Seed dimensions (mm length × mm width, averages of measurements on 10 seeds) were: *A. trifida*, 9·4 × 4·8; *H. annuus*, 9·5 × 5·1; *S. angulatus*, 12·4 × 6·5; *G. max*, 7·0 × 6·1; *Z. mays*, 10·8 × 7·3; *X. strumarium*, 18·6 × 11·2; *C. arvensis*, 3·4 × 2·2; *P. sativa*, 5·8 × 4·5; *A. minus*, 5·9 × 2·2; *B. frondosa*, 11·0 × 3·3; and *I. purpurea*, 4·1 × 2·8.
Seed dispersal by earthworms

Discussion

The results of a series of experiments showed that the exotic European earthworm *L. terrestris* deliberately selected and buried the seeds of the North American native herb *A. trifida*, thus providing a means of secondary dispersal that dramatically reduced the time seeds were left exposed on the soil surface. *Ambrosia trifida* seeds on the soil surface are at high risk of predation by vertebrates (Harrison *et al.* 2003); thus, rapid burial by earthworms could have a protective effect and serve as an important survival mechanism for *A. trifida* and other large-seeded species that lack the fecundity and seed persistence traits typical of many small-seeded plants (Leishman *et al.* 2000).

Plants can acquire weedy characteristics due to increased availability of a suitable habitat, decreases in natural enemy populations or competing flora, and/or increased dispersal opportunities (Cousens & Mortimer 1995). Introduction of a new dispersal mechanism is possible through diplochory, in which dispersal occurs through two different stages and results in either a neutral or beneficial effect on seedling establishment (Vander Wall & Longland 2005). It appears that *A. trifida* has acquired a new form of diplochory as a result of the introduction and establishment of *L. terrestris* in North America, which may contribute to *A. trifida*’s weedy behaviour.

Earthworms buried some seeds below the 10-cm depth limit for *A. trifida* emergence (Harrison *et al.* 2007), reducing *A. trifida* seedling recruitment in spring compared to when earthworms were absent. Although deep seed burial can reduce *A. trifida* recruitment, it can also increase seed dormancy and persistence. Previous work showed that the percentage of *A. trifida* seeds remaining viable after 4 years of burial ranged from 1% for seeds buried 5 cm deep to 13% for seeds buried 20 cm deep (Harrison *et al.* 2007), thus indicating that seeds buried deeply by earthworms may produce a more persistent seed bank.

Milcu *et al.* (2006) reported that reductions in seedling recruitment from seeds buried by *L. terrestris* were greater for small-seeded compared to large-seeded species. We found that earthworms buried small *A. trifida* seeds deeper than large seeds, resulting in less seedling recruitment from small seeds. Small *A. trifida* seeds that *L. terrestris* buries more deeply than large seeds may be less vulnerable to predation, but large seeds are capable of emerging from greater depths (Harrison *et al.* 2007). These contrasting consequences of seed burial by *L. terrestris* could exert opposing selection pressures on seed size and contribute to the seed size variation in *A. trifida* reported by Sako *et al.* (2001).

Our surveys of an unmanaged population of *A. trifida* showed that *L. terrestris* buried the majority of *A. trifida* seeds dispersed to the soil surface. From our data, we estimated that a mean population of 38 *L. terrestris* individuals m$^{-2}$ buried 73% of *A. trifida* seed rain, or 4826 *A. trifida* seeds m$^{-2}$ after a single season of seed dispersal. The earthworms placed *A. trifida* seeds in middens and in vertical columns within burrow walls, resulting in aggregated clumps of *A. trifida* seedlings and the creation of small-scale spatial heterogeneity in seedling distribution. Others have reported a similar effect of *L. terrestris* on seedling aggregation for other species (Grant 1983, Milcu *et al.* 2006). Seedling aggregation probably increases intraspecific competition, but further study is needed to determine the net effects of spatial aggregation on *A. trifida* demography.

Results from our seed selection experiments and seed/seedling distribution surveys suggest that large seeds are common constituents of organic materials collected and stored by *L. terrestris*. The function(s) of seed burial by *L. terrestris* remain unclear because the earthworm has also been observed to collect small stones, beads, and bits of tile in its middens or burrows (Darwin 1881; Canti 2003). If seeds are collected as food, the earthworms may feed on dead, decaying seeds or seedlings that fail to emerge. In the case of *A. trifida*, the involucre surrounding the seed decomposes completely within 4 years but the pericarp surrounding the embryo remains intact (Harrison *et al.* 2007). It is possible that *L. terrestris* may feed on decaying involucres but leave the embryos intact, thus having relatively little impact on *A. trifida* seed viability.

In comparison to other secondary seed dispersers, *L. terrestris* may be among the first animals to encounter seeds in many temperate soils due to its high populations (Edwards & Bohlen 1996) and close proximity to freshly dispersed seeds. In our experiments, an average of more than 50% of *A. trifida* seeds cached in burrows emerged successfully, compared to less than 10% reported for various seeds cached by rodents or ants (Howard & Evans 1961; Levey & Byrne 1993; Hulme & Kollmann 2005; Ouden, Jansen & Smit 2005; Zhang, Xiao & Li 2005). Rodents and ants consume most cached seeds but *L. terrestris* cannot ingest particles larger than 2 mm (Shumway & Koide 1994). There was no evidence of *A. trifida* seedling herbivory by *L. terrestris*, and seedlings that emerged from *L. terrestris* burrows and middens produced greater shoot biomass than seedlings from other areas, probably due to higher soil moisture, organic matter and nutrient content (Edwards & Bohlen 1996).

In the selection experiments, *L. terrestris* preferred *A. trifida* seeds and others that were similar to *A. trifida* in size, shape, and texture. Selective seed collection was consistent with previous reports of selective ingestion of seeds and leaves (McRill & Sagar 1973; Shumway & Koide 1994; Edwards & Bohlen 1996; Zeller & Saxler 2007). Selection by *L. terrestris* among large seeds may depend both on their chemical composition (Curry & Schmidt 2007) and on morphological traits that influence pulling seeds into burrows, which range up to 12 mm in diameter (Edwards & Bohlen 1996). In addition to *L. terrestris* having a profound effect on secondary dispersal of a large-seeded plant species, its selectivity among seeds and
its propensity to bury them over a wide range of depths could ultimately influence plant community composition by affecting relative fitness among species.

The interactions between *L. terrestris* and *A. trifida* have implications for management of *A. trifida* as a weed problem. Our results clearly show the potential for *L. terrestris* to increase establishment of a weed by burying its seeds, whereby they are protected from vertebrate predation and placed in an environment favourable for growth. It seems plausible that *L. terrestris* may facilitate long-term establishment of *A. trifida* by increasing seed bank formation; thus, relative abundance of *L. terrestris* could be an important factor in management decisions for land where *A. trifida* or other large-seeded weedy species could potentially be introduced and become established.

Ecosystem managers should have an understanding of the landscape factors that may influence the interaction of *L. terrestris* and *A. trifida*, particularly regions characterized by a patchwork of row crop and forage production bordered by woodlands, successional areas, fence and hedge rows, and rail sidings. Such complex landscapes are common where *A. trifida* and *L. terrestris* populations overlap in the Midwestern USA and provide habitat for vertebrate and invertebrate seed predators; however, *L. terrestris* is likely to increase the potential for *A. trifida* to become established in such areas where seed burial opportunities are few and the risk of seed predation is high. There is recent interest in exploiting seed predation to help manage agricultural weeds (Westerman et al. 2003, 2006; Heggenstaller et al. 2006), but such efforts may have a lower probability of success where *L. terrestris* is present. Crop producers using management techniques to enhance weed seed predation (e.g. cover crops, refugia, reduced tillage) should be aware of the potential impact of *L. terrestris* on establishment of large-seeded weed species and devise alternative management strategies if necessary.

In successional areas or natural lands managed for conservation, the presence or absence of *L. terrestris* should be considered with regard to its potential effects on biodiversity and invasive plant species. *Lumbricus terrestris* is one of several exotic earthworms invading northern forests in North America (Hale, Frelich & Reich 2005; Suárez et al. 2006) and is also expanding its range in northeastern Europe (Pop & Pop 2006; Tiuonov et al. 2006). Invasive earthworms consume forest floor litter and reduce recruitment and diversity of native herbs and trees (Hale et al. 2006). Decreases in plant regeneration are thought to be due, in part, to increased exposure of seeds and seedlings to desiccation and predation, and lack of a suitable establishment and rooting medium (Frelich et al. 2006; Hale et al. 2006). Invasive earthworms are also thought to increase invasion by exotic plant species with small seeds that can establish more easily in thinner forest floors (Hale et al. 2006). The direct impacts of seed dispersal by earthworms on seed survival and seedling establishment in forest ecosystems are unknown. The potential for *L. terrestris* to cache large seeds in its burrows and middens and to provide favourable micro-sites for seedling establishment may contribute to the changing dynamics of native vs. invasive plant species in forest ecosystems by increasing recruitment of large-seeded species.

In conclusion, it appears likely that the *L. terrestris*–*A. trifida* interaction is a relatively new occurrence facilitated by human activity. We hypothesize that *A. trifida* seeds are attractive organic material for *L. terrestris*, eliciting seed burial by the earthworms and thereby acquiring the benefit of protection inside burrows at a low cost. Seed collection and burial could indirectly benefit earthworms through increased plant establishment that provides shelter and the return of plant litter near burrows (Milcu et al. 2006; Perrault, Eriksen-Hamel & Whalen 2007). Selective seed collection and differential burial depths inside burrows may result in differential seedling establishment among and within species, thereby influencing plant community composition and contributing to plant biotype selection and the evolution of seed or fruit traits. Our results corroborate earlier reports that *L. terrestris* collects and buries large seeds (Andrews 1907; Pearce et al. 1994; Milcu et al. 2006) and further demonstrates that *L. terrestris* should be considered an important dispersal agent for large-seeded plant species. The influence of *L. terrestris* seed burial on plant population dynamics warrants further investigation, particularly in environments where *L. terrestris* colonization is occurring outside of its native range.

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**References**


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