The patterns of harvester ant removal of wild radish seeds in the native range: the importance of generalist seed predators to weed management

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Summary

*Raphanus raphanistrum* L., wild radish, is a major weed of cropping systems in Australia, and is being targeted for biological control. Wild radish is a winter annual; consequently, biological control agents that significantly reduce the quantity or quality of seed production are likely to be effective. Surveys and ecological studies in the region of origin, the Mediterranean, included an assessment of the impact of phytophagous organisms on this weed and, in particular, on seed production. Seed production of wild radish in sown plots was also monitored in southern France. In previous surveys, we found that seed-harvesting ants caused the greatest seed losses of maturing seeds in southern France (11–91% seed loss per unit area). The ant species involved were *Messor* species, in particular *Messor sancta* Forel, a species native to the Mediterranean region, and *Messor rufitarsis*, common in Central Europe and throughout the Mediterranean south-east. In spring, the ants cut segments from green, nearly mature siliqua. The cut segments fall to the ground and some of them are then opened by the ants that carry the seeds to the nest. Remaining siliqua mature naturally, fall to the ground during summer and break into segments. Ants also harvested these mature segments on the ground prior to germination in autumn. We measured the impact of this seed predation by counting all the siliquae on each plant soon after ant harvesting started in late April and when wild radish plants were fully mature in late May. The results suggest ants are likely to have a significant impact on native wild radish populations. While these ants can not be considered as biological control agents, their overriding effect relative to other seed predators in the native range suggests associations between ants and other generalist seed predators on wild radish in Australia may also be providing some form of natural control.

Keywords: ant harvesters, *Messor* sp., *Raphanus raphanistrum*, seed predation, wild radish.

Introduction

Wild radish (*Raphanus raphanistrum* L.) (Brassicaceae) is distributed throughout the world and is a common weed of cultivation and disturbed areas (Piggin et al. 1978, Parsons & Cuthbertson 1992). *R. raphanistrum* occurs naturally in the Mediterranean region and occasionally forms dense populations. It is one of the most important weeds of grain crops in southern Australia and has developed herbicide resistance (Walsh et al. 2001). Biological control of wild radish is being investigated for its potential to provide a supplementary management option for this weed (Scott et al. 2002).

Surveys for potential biological control agents were undertaken in the Mediterranean region, considered as the native range of wild radish (J. Scott & J. Vitou, in preparation). Southern parts of Portugal, France, Greece and northern parts of Tunisia were surveyed. About 50 species of phytophagous insects were found associated with wild radish, but most of these have recorded host ranges that include other Brassicaceae,
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including economic species such as *Brassica napus* (canola), *Raphanus sativus* (garden radish), cabbage etc. (Scott et al. 2002). Genetic studies have shown wild radish is closely related to these important crops and may share a close ancestry with edible radish (J.F. Martin et al., unpublished). This makes this weed a very difficult target and essentially restricts the study of potential agents to those reducing seed production (Scott et al. 2002). *R. raphanistrum* produces seeds in siliquae that average between four and seven single-seeded segments per siliqua (Vitou & Scott 2002).

*Messor* species are the very large-headed ants of the seventeenth century fable “La Cigale et La Fourmi” by Jean de La Fontaine, where the ants build up a stock of seeds in their nest while the cicadas are singing! *Messor* species are typical harvester ants, feeding on seeds collected in the foraging area and storing seeds in nest chambers (Hahn & Maschwitz 1985). In arid countries such as Algeria, the ant-nest granaries can contain more than 100 L of seeds (Bernard 1968). Ant activity decreases, but does not stop, throughout the summer and early autumn, and ants also harvest pod segments on the ground before the seeds germinate in autumn. Probably originating in North Africa, the *Messor* genus consists of about 40 species living exclusively in the Old World, with eight species present in France. *Messor rufitarsis* is common in Central Europe and throughout the Mediterranean south-east, while *Messor sancta* is common in sunny and rocky Mediterranean fields (Bernard 1968, Hahn & Maschwitz 1985).

An insecticide and fungicide exclusion experiment was set up in southern France to study the impacts of natural enemies on wild radish throughout its growing season. This study identified a 7.5-fold increase in wild radish seed production per unit area in April 2002 across twenty 0.5 m² insecticide and fungicide treated quadrats (J. Vitou, unpublished data). The current study describes and tests the patterns of harvester-ant activity within this variable arena of resource availability.

**Materials and method**

An experiment was set up at Vendres, France (43°16'14"N, 03°13'30"E), in fallow land that had previously been a vineyard. The site was at 16 m altitude on a sandy-clay soil that had not been ploughed for at least 10 years. The plant community at this site was dominated by the evergreen shrub *Ditrichia viscosa* (L.) Gaertner (Asteraceae). Abundant wild radish plants were present in the neighbouring field.

A 30 m × 10 m plot was fenced to prevent disturbance from livestock or other large animals. Soil was professionally cultivated. Five blocks of six 0.5 m² quadrats were set up 3 m apart and 2.5 m from the fence. Quadrats were arranged in two rows of three with a separation of 1.5 m between quadrats (Fig. 1).

On 11 September 2001, 240 single-seeded segments of wild radish siliqua were sprinkled over four quadrats selected at random within each block and covered with soil to a depth of 2–3 cm. Each quadrat was treated with insecticide and/or fungicide or water, sprayed every three weeks, following a factorial experimental design. Every three weeks, weeds were removed by hand from the quadrats so that only *R. raphanistrum* plants remained. Twice a year, the plot was mown around the quadrats. By April, these treatments led to large spatial variation in seed production per quadrat that represented a 7.5-fold overall variation (Fig. 1). This study focuses on the responses of harvester ants to this variation. The results of the treatments are the subject of another paper (J. Vitou et al., in preparation).

Abundant ant activity was observed in early spring, when the siliquae were well developed but not yet lignified. Collections of ants were sent to specialists and identified as *Messor sp.*, *Messor sancta* Forel, and *Messor rufitarsis* Fabricius. The position of ant nest exit holes were recorded within the design (Fig. 1). All of the siliquae on each plant in each of the four quadrats per block were counted soon after ant harvesting started in late April. Siliquae, already harvested by the ants, were included in these counts through the persistence of the pedicels so this gave a total number of siliquae and the number left after an initial period of ant harvesting. In late May, when wild radish plants were fully mature, all of the remaining siliquae were collected in each quadrat of blocks 1, 3 and 5. The plants were uprooted and placed in a separate paper bag for each quadrat. In the laboratory, the number of siliquae in each quadrat was counted. Siliqua number was converted into estimated seed number based on an average number of 4.92 (± 0.04) seeds per siliqua obtained from a sample of 2185 siliqua collected and analyzed on this site the previous year (J. Vitou, unpublished data).

A simple experiment was set up on 11 September 2001 to measure segment collection from the ground by ants in late summer and early autumn, before the autumn rains. Twenty 10 × 10 × 5 cm high pots were prepared and filled with site-soil free of wild radish seeds. The pots were buried so that the pot rims were at ground level to allow the ants to enter the pots. In each block, four pots were laid down between the two rows of quadrats (Fig. 1). Ten pots selected at random received five segments and ten pots received 50 segments of *R. raphanistrum* collected at Vendres in May 2001. In each block, two pots of each density selected at random had the segments covered with soil from the site (0.5 cm depth), and in the other two pots the segments were placed on the surface. In November, when the new season seedlings were established, the pots were brought back to the laboratory where the soil of each pot was sieved, and the seedlings and remaining segments were counted.
**Statistical analysis**

Generalised linear models were used for analysis of seed numbers using the GLIM statistical package (McCullagh & Nelder 1983). The factors used were plant, quadrat and block. Dependent variables were total seeds per plant and per quadrat, seeds left per plant in late April and seeds left per quadrat in late May (Log(n+1) transformed). Seed loss per plant and per quadrat and per pot was analyzed using survival analysis with binomial errors, i.e. the initial total seed number as the binomial denominator and scaled for over-dispersion in the data (Crawley 1993).

**Results**

Ants were omnipresent during all our field surveys, and the impact of the harvester ants in the genus *Messor* was obvious. Ants harvested between 11% and 91% of available seeds per quadrat over a two-month period. Harvester ant activity on wild radish in the quadrats started in March 2002 and increased as the fruits started to mature. In early April, as fruits matured, the ants climbed the plant and removed only one or two segments of three to five siliquae per plant. Every week more were removed as they became suitable. When whole siliquae were suitable, the ants cut the pedicel causing the siliqua to fall to the ground. All seeds were removed on some plants. Ant activity was visible on the ground at the base of each plant where abundant pod fragments and empty siliqua segments were distributed. Ants cut large siliquae into segments and tended to open some segments to take only the seed or a fragment to their nest.

Seed production per quadrat available to the harvester ants ranged from 7410 to 56,974 seeds m⁻². The slope of the log–log plot of total number of seeds per individual plant and seeds left after the ant harvesting in April was one (Fig. 2). This slope indicates that the proportion of seeds harvested per plant was independent of the size of the plant. In contrast, the log–log plot of the number of seeds per quadrat over the same time period before and after ant harvesting (Fig. 3) had a slope significantly less than one, indicating that quadrats with greater numbers of seeds lost a higher proportion of seeds to the ants. By the end of May, this was even more noticeable, when the remaining numbers of seeds per quadrat was no longer a function of the initial seed production per quadrat (Fig. 4).

![Figure 1](image1.png)

**Figure 1.** Field experiment design with five blocks of six quadrats at Vendres, France. Number (multiples of 100) in each quadrat represents the seed production, grey quadrats represent the trap gardens (*R. sativus* and canola). Between the two rows of quadrats, four pots per block were buried at random. Two pots received five seeds (5), one was covered with soil (5c), two pots received 50 seeds (50), and one was covered with soil (50c). Ant nest holes are represented by the black rings.

![Figure 2](image2.png)

**Figure 2.** Seed survival by late April (Y) versus seed production (X) per plant. Y = 1.00X – 0.37. R² = 0.74.

![Figure 3](image3.png)

**Figure 3.** Seed survival by late April (Y) versus seed production (X) per quadrat. Y = 0.66X + 1.16. R² = 0.75.
With the tray experiment set up before germination, the percentage of the seed survival was significantly lower ($F_{1,19} = 34.11; P < 0.000$) than in soil covered seeds. Initial number of seeds added to the trays had no significant effect ($F_{1,19} = 3.98; P = 0.06$) on the seed survival (Fig. 5).

**Discussion**

Seed harvesting was density-dependent at the scale of the quadrat but plants of all sizes lost the same proportion of seeds to ants. The efficacy of the ants at harvesting seeds in relation to their density per quadrat levelled out the initial 7.5-fold difference in seed density between quadrats within 2 months of harvesting.

As a winter annual weed, wild radish produces abundant siliquae and seeds. Seed production from wild radish infestations ranged from 292 seeds m\(^{-2}\) from 1 plant m\(^{-2}\) to 17,275 seeds m\(^{-2}\) from 52 plants m\(^{-2}\) (Reeves et al. 1981). Total seed production prior to ant harvesting activity in our field plot where 960 seeds m\(^{-2}\) were planted and kept weed-free was between 698 to 26,508 seeds m\(^{-2}\) in 2001 (J. Vitou & J. Scott, unpublished), and between 7410 to 57,878 seeds m\(^{-2}\) and between 5 to 8226 seeds per plant in this study on the same site in 2002. When these 2002 seed densities were available to the harvester ants, the residual seed density dropped to between 4126 and 6892 seeds m\(^{-2}\). Ant activity therefore eliminated any differences in seed production resulting from the initial treatments. The number of viable seeds that the ants left behind may be a harvesting threshold below which the ants turn their attention to other resources. Nonetheless, this appeared to be more than enough for sufficient wild radish recruitment in the next germination period to ensure population replacement, particularly if the seeds became incorporated into a buried seed bank. The seed density and burial experiment showed that the ants harvested seeds in proportion to their density when on the surface or buried in the soil. For seeds on the surface, seed survival of 50 seeds per pot was lower than for 5 seeds per pot. Though this difference was not significant, it suggested a similar tendency to the density-dependent harvesting observed from the quadrats. Ants appear to be five times less successful at harvesting buried seeds (Fig. 5).
Harvester ants should only be able to maintain high abundance in plant communities that exhibit high seed production. Mediterranean herbaceous communities dominated by annuals exhibit this trait, and are a common habitat for *Messor* sp. (Wolff & Debussche 1999). Reyes-López & Fernández-Haeger (2002) observed that harvester ants tend to gather the most abundant and/or larger seeds within such communities, and that the superabundance of a given seed type in the environment prompts increased activity. Hahn & Maschwitz (1985) also found that ants were attracted to rich seed sources when they were available. The abundance of the large-seeded wild radish siliquae generated by this experimental design may have attracted higher than average harvester ant activity at this particular site. With reduced numbers of seeds available, ant activity decreased.

In Australia, McGeown (1999) suggested that ants (a total of 18 morphospecies) trapped within the study site, were the primary remover of the seeds of wild radish in north-eastern Victoria and southern New South Wales. Borger et al. (2002) point to the importance of ants for removal of wild radish seed in Western Australia, with 24 of 30 species known to consume such seeds (Minkey & Spafford Jacob 2002). Australia has a rich seed-harvester ant fauna. Eight harvester species were recorded from some Australia tropical study plots (Andersen et al. 2000), and harvester ants are capable of inflicting severe seed losses (Briese 1982). Recent studies in Australia suggest up to 90% of weed seeds are removed by ants in cropping systems and attracting ants for this purpose is now being considered (D. Minkey, pers. comm.).

This suggests wild radish seed harvesting by ants is important in both the native and exotic range of this weed. Any proposal to use a seed-removing biological control agent needs to be considered carefully. Unless the proposed agent could reduce seed density to levels below the harvesting threshold of the local ants, then such ants are likely to nullify any impacts of such agents. Further work on harvesting ants on wild radish seeds in Australia is clarifying this.

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References


