

Mercurialis annua L. (Euphorbiaceae) seed interactions with the ant *Messor structor* (Latr.), hymenoptera: Formicidae

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SUMMARY

Mercurialis annua seeds are shed all year round. In the period early April to mid October they are actively collected by *Messor structor*. This ant transports the seeds to the nest and removes a seed appendage containing lipid and protein reserves known as caruncle. After this 'manipulation' the seeds are able to germinate but they are deposited in small chambers inside the ant nest where they are prevented from germinating. They germinate if experimentally removed from the nest or if disturbed by man or animals. During the experiments the seeds germinated after being washed in running water for 24 h or if the caruncle was physically removed. The seeds of this plant species thus have two ways of terminating dormancy. Ants remove both the mechanical and the chemical inhibitor, water only removes the chemical one.

Key-words: ants, caruncle, *Mercurialis annua*, *Messor structor*, myrmecochory, seeds.

INTRODUCTION

Some of the seeds actively collected by ants have an appendage of a different colour, called elaiosome, caruncle or aril (Boesewinkel & Bouman 1984); this appendage contains food reserves, usually lipids but sometimes even proteins, starch and vitamins (van der Pijl 1972), which are an incentive to ants. When this structure is adapted to ant dispersal (myrmecochory) it is termed elaiosome if compact, caruncle if a protrusion of the micropylar area, and aril if the dispersing unit is the fruit (Fahn & Werker 1972). *Mercurialis annua* has a caruncle.

How seeds induce animals to collect them is not clear. Volatile unsaturated fatty acids have been postulated as attractants (Bresinsky 1963) but this is not universally accepted (Meeuse 1978). The attracting ability of these structures is not the same in all plant species. Elaiosomes and caruncles are responsible for seed dispersal and even germination as in *Mercurialis*. The seed does not germinate until the elaiosome or caruncle has been removed.

Interactions between seeds and ants are quite frequent in most parts of the world, but they are particularly abundant in Australia (Beattie 1983, 1985); in the northern hemisphere and temperate environments only a few examples are known (Beattie 1985). *M. annua* L. is a widely distributed herbaceous dioicous annual; it is known to have different levels of ploidy and with increasing ploidy monoicmism becomes frequent (Durand

& Durand 1985). New plants come up all year round but more frequently during the rainy months (September, October, March and April). The plants prefer cultivated sites such as orchards, vineyards, olive groves, and spring up spontaneously after the ground has been ploughed. It is unusual for them to germinate spontaneously, but this may be observed beside paths and in channels formed by rivulets during storms.

The seeds encounter different conditions according to the period of release. The mechanisms ensuring seed dispersal and germination must be quite efficient. Seed dispersal occurs in two steps ('diplochory' according to van de Pijl 1972). *Mercurialis* is at first autochorous (dispersal by the plant itself) and subsequently synzoochorous (dispersal by animals; ants in this case, attracted by specific signals from the seed) (Fahn & Werker 1972; Meeuse 1978). Diplochory is restricted to the period when ants are active; during the rest of the year the seeds are dispersed by rain if not eaten by other animals.

MATERIALS AND METHODS

Seed morphology

The seeds were divided transversally and only the upper part was fixed in glutaraldehyde (3%), dehydrated and embedded in glycol-methacrylate (O'Brien & McCully 1981), cut in 4- μ m thick sections and stained: (a) with PAS preceded by aldehyde blockade (O'Brien & McCully 1981) to detect total polysaccharides; (b) with BBF (Pearse 1972) to detect total proteins; (c) with Azur B (Jensen 1962) to detect lignin and suberin; and (d) with Sudan black B (O'Brien & McCully 1981) to detect lipids.

Seed permeability

Permeability was determined by placing the seeds in a solution of 0.05% basic fuchsin; water intake was followed by the progressive increase of the pink area.

Field observations

Field observations of spontaneous germination were performed in the Sienna district, central Italy. This district is in the centre of Tuscany at about 43°19'N, 11°20'E. Altitude ranges from 240 to 360 metres above sea level. Average annual rainfall is 824.3 mm, November is the wettest month with 109 mm and July the driest with 28 mm. The mean annual temperature is 13.6°C; January is the coldest month at 4.8°C and July the hottest at 23.3°C.

Germination and experiments with a *Messor structor* colony were performed at the edge of an uncultivated field 20 m from a house in the village of Cappuccini. The nests were situated near a slope and shaded by *Eryobotrya japonica* (Thunb.) Lindley and *Prunus avium* L. The interactions between *M. annua* seeds and the ants were observed during the period April–November 1987 and 1988. One nest was excavated in mid June of both years and the *M. annua* seeds and those of other plant species collected with pincers.

In order to understand the way in which ants discern *Mercurialis* seeds these were: (a) painted white (to verify chromatic attraction); (b) painted with transparent nail polish (to test olfactory attraction); (c) deprived of the caruncle (to determine whether the caruncle is the only attractant); (d) aged (seeds produced in December 1983); and (e) divided either longitudinally or transversally (to determine whether a part of the seed was attractive).

Seed germination

Germination of *M. annua* seeds was tested in: (a) seeds collected from ripe fruits allowed to dehisce in the laboratory: (i) with the caruncle; (ii) after removal of the caruncle with a

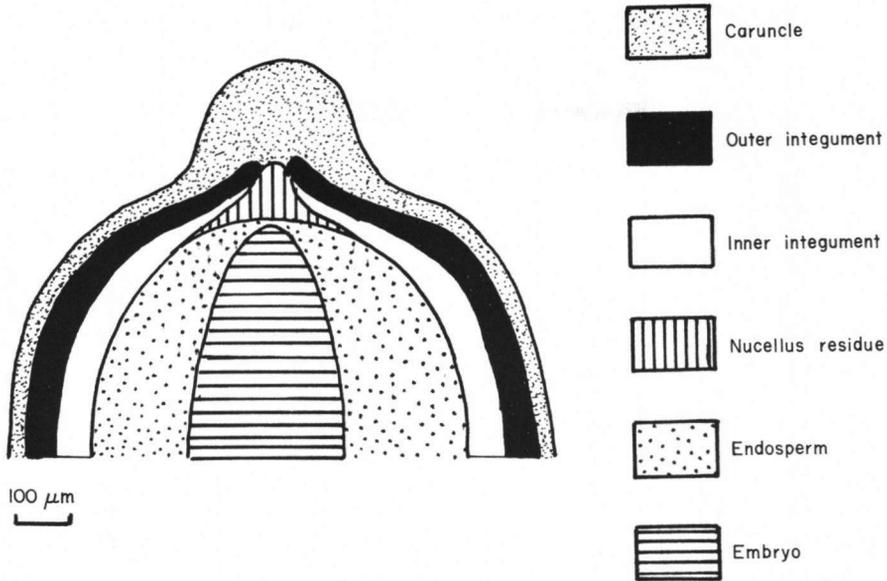


Fig. 1. Semi-diagrammatic representation of the micropylar part of a ripe *Mercurialis annua* seed.

razor blade; and (iii) after rinsing in running tap water for 12 h and 1, 2, 3 and 4 days; and (b) seeds collected inside the nest of *M. structor*: (i) after rinsing for 1 week in running tap water to remove any inhibitor deposited by the ants; (ii) without washing.

The seeds were sown at a depth of 20 mm in a 30-mm diameter, 35-mm long cylinder. The soil was from around *Mercurialis* plants. The cylinders were united in boxes of the same height and filled with soil. Care was taken to keep the seeds away from ants; the boxes were placed on a table whose legs stood in water. Two hundred seeds were sown in 200 cylinders for each germination test except for the seeds collected inside the ant nests: in this case, each lot was of 50.

RESULTS

Seed morphology

The seeds were 1.5–2 mm in length, their colour was brownish-green with a pale brown caruncle which continued with a 3-cell layer enveloping the seed (Fig. 1). Seed weight depended on season: during June 1988 it was 0.0025 ± 0.0004 g.

The seeds have two integuments, the external one is hard and has a palisade layer of Malpighian cells, the thick walls of which contain both lignin and suberin. The inner integument has thin pectocellulosic walls. The two integuments are pierced by a nucellus residue, the cells of which have thin suberized walls. Inner integument, caruncle, embryo and endosperm contain lipid and protein reserves (Fig. 1).

Seed dispersal

M. annua seeds are dispersed in two steps: first, by the explosive dehiscence of the fruit and then active collection by the ant *M. structor* which transports them to its nest. Diplochory

Table 1. List of seeds observed to be collected by *Messor structor*. All the species grow within a radius of about 30 m from the nests

Dicotyledonous	
Boraginaceae	
<i>Buglossoides purpureo-caeruleum</i> (L.) Johnston	
<i>Borago officinalis</i> L.	
<i>Myosotis arvensis</i> (L.) Hill	
Caryophyllaceae	
<i>Stellaria media</i> (L.) Vill.	
<i>Silene dioica</i> (L.) Clairv.	†
Euphorbiaceae	
<i>Euphorbia cyparissias</i> L.	*†
<i>Euphorbia characias</i> L.	*†
<i>Mercurialis annua</i> L.	*†
Fumariaceae	
<i>Fumaria capreolata</i> L.	*†
Labiatae	
<i>Salvia verbenaca</i> L.	
Scrophulariaceae	
<i>Pseudolysimachion spicatum</i> (L.) Opiz	
Violaceae	
<i>Viola odorata</i> L.	*†
Monocotyledonous	
Liliaceae	
<i>Muscari atlanticum</i> Boiss. et Reuter	

Species marked with * have seeds with caruncle, those with † were found in the seed chambers of ant nests.

only occurs from mid April to late October, when *M. structor* ants are active. During the rest of the year it is monochorous. The seeds are hurled up to 130 cm from the central stem of the plant but most fall between 20 and 30 cm away. The time of the day when the first seeds are shed depends on the time of year, and is a function of temperature and relative humidity (unpublished data). When a seed lands on the soil it is collected by the ants in a few seconds; seeds were seen to remain on the soil for no more than a minute. The ants also collect seeds, with and without caruncle, of other species growing near the nest (Table 1).

Seed permeability

From previous studies we knew that *M. annua* seeds do not germinate easily. The embryo and endosperm cells of seeds collected from the soil a few days after shedding did not hydrate. When the seeds were experimentally moistened the caruncle hydrated but even after a month the water had not penetrated the endosperm and embryo (Fig. 2). If the caruncle was removed with a blade the endosperm and embryo hydrated within a day (Fig. 2).

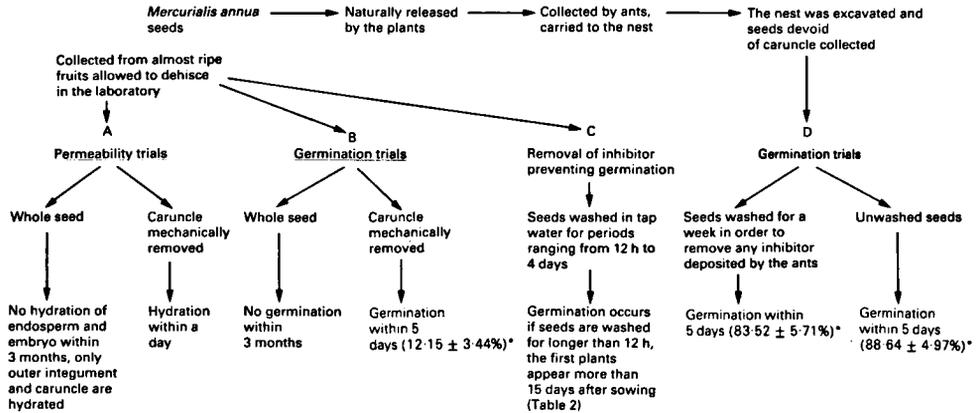


Fig. 2. Scheme showing natural and experimental trials of hydration and germination of *Mercurialis annua* seeds. *Values represent mean \pm SD of five replicates.

Seed germination

Seeds devoid of the caruncle germinated in 5 days whereas intact seeds failed to germinate in 3 months (Fig. 2). Seeds washed in tap water for different periods of time germinated only if washed for more than 12 h (Table 2). In the case of seeds washed for 2 days, the first seedlings appeared only 14 days after sowing. Washing with tap water for 2 days appeared more effective than 1, 3 and 4 days and gave a germination percentage of more than 25%, 21 days after sowing. Seeds washed for 1, 3 and 4 days always had a lower percentage germination.

No appreciable differences were observed in percentage germination of seeds collected during January 1987 and July 1988 (Table 2).

Seed-ant interactions

Seeds painted white or with transparent nail polish, seeds without caruncle, old seeds and seeds divided longitudinally or transversely were collected by *M. structor* ants and transported to the nest, as were stones not exceeding 1 mm in size. When the exposed seeds were cut in two, either longitudinally or transversely (i.e. when the endosperm and embryo, rich in lipids and proteins were accessible) the ants became very excited, agitating their heads and antennae. Even these divided seeds were transported to the nest.

In June of both years the nest was excavated and the seed chambers exposed. These seed deposits were situated at a depth of 10–30 cm; the 'centre' of the nest where the larvae are found was at 25–40 cm. Each chamber had one type of seed with very little other material. The seed species found were those growing around the nest and are listed in Table 1. *M. annua* seeds had been collected and were stored by the ants without the caruncle. Some of them were washed for a week to remove any germination inhibitor deposited by the ants. Washed and unwashed seeds were sown and started to germinate simultaneously within 5 days. No further germinations occurred after 10 days. Germination always exceeded 80% (Fig. 2).

DISCUSSION

In order to survive adverse conditions during dispersal, the seeds of many species become dormant and this may depend on internal and/or external factors (Bewley & Black 1985).

Table 2. Percentage of *Mercurialis annua* seeds germinating after washing for different periods of time

Times of appearance of cotyledons after sowing (days)	Seed washing time (days)											
	0.5		1		2		3		4		4	
	A	B	A	B	A	B	A	B	A	B	A	B
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	15.18 ± 2.21	14.73 ± 3.83	12.48 ± 1.97	13.51 ± 1.06	0.00	0.00	0.00	0.00
21	0.00	0.00	1.53 ± 0.08	2.16 ± 0.23	35.31 ± 4.33	29.05 ± 2.29	17.34 ± 2.00	14.66 ± 2.45	7.22 ± 0.88	3.45 ± 0.55	3.45 ± 0.55	3.45 ± 0.55
28	0.00	0.00	1.53 ± 0.08	2.16 ± 0.23	35.31 ± 4.33	30.60 ± 3.67	19.09 ± 4.96	16.73 ± 3.32	7.22 ± 0.88	3.45 ± 0.55	3.45 ± 0.55	3.45 ± 0.55
35	0.00	0.00	1.53 ± 0.88	2.16 ± 0.23	35.31 ± 4.33	30.60 ± 3.67	19.09 ± 4.96	16.73 ± 3.32	7.22 ± 0.88	3.45 ± 0.55	3.45 ± 0.55	3.45 ± 0.55

A: collected in January 1989; B: collected in July 1988.
 No appreciable differences were found between seeds collected in these two periods.
 Values represent means of five replicates ± SD.

From our experiments it is evident that *M. annua* seeds become dormant and this can be brought to an end by rain and/or ants. Ants can also induce secondary dormancy.

The fate of *M. annua* seeds depends on the season in which they are produced. Seeds shed in autumn, winter and early spring, when the ants stay in their nest, remain on the soil for a long time if not eaten by small mammals because germination is prevented by the caruncle. Rain removes the germination inhibitor. Seeds shed in the period late spring to early autumn are quickly collected and stored by ants. Seeds shed during July behave substantially in the same way as those shed during January, seed dormancy being terminated by water; percentage germination and time for seedling emergence are comparable. Seeds released in January certainly encounter water sooner than those shed in July. It is not easy to evaluate the amount of rain necessary to remove the inhibitor, i.e. to have the same effect as 24 h in running tap water. Seeds dispersed by small streams formed after heavy rain in autumn and early spring are very effective in removing the inhibitor and carrying the seeds far from the mother plant. Seeds dispersed by rain germinate sporadically and asynchronously compared to seeds dispersed by ants which germinate simultaneously when a nest is destroyed.

M. annua seed dormancy is dual, being both chemical and mechanical. It is chemical in the sense that the cells of the caruncle and/or nucellar residue have a substance which prevents the hydration of the inner part of the seed. It is also mechanical because seeds deprived of the caruncle (either naturally, by ants, or experimentally, with a blade) germinate in a few days. If the chemical inhibitor is removed by washing, the seeds take 14 days to germinate because the growing embryo must rupture the caruncle. The inhibition of intact seed germination in *Ricinus communis* L. and other species of Euphorbiaceae is chemical and the caruncle contains phenols. Extracts of this seed appendage inhibit the germination of caruncleless seeds which would normally germinate (Logoa & Pereira 1987).

From the experiments reported in Fig. 2 it is evident that *M. annua* seeds lose the germination inhibitor in two different ways. Seeds washed in tap water lose the chemical inhibitor only if washed for more than 12 h. Seeds whose caruncle is removed experimentally or by ants lose both inhibitors and germinate quickly. The low percentage germination in seeds whose caruncle was removed mechanically may be due to lack of precision with the blade. The seed is small and it is easy to damage the embryo; if too little caruncle is removed the seeds do not germinate.

Germination inhibitor was not found on caruncleless *Mercurialis* seeds collected in the ants' nest, however, they are prevented from germinating while in the nest. A possible explanation of this phenomenon could be that: (a) the seeds require light to germinate; (b) the microenvironment where the seeds are stored contains insufficient water for germination; and (c) the microenvironment where the seeds are stored contains a gas preventing germination. Low concentrations of oxygen or high concentrations of carbon dioxide are known to hinder seed germination (Pristely 1986).

Caruncles, elaiosomes and ant-plant mutualism may also be regarded from another point of view. Myrmecochory may increase seed survival because being buried in the nest allows the seed to escape predation (Beattie 1985). Hanzawa *et al.* (1985) found that seed predators such as deer mice (*Peromyscus maniculatus* Wagner) preferred seeds whose caruncle had been experimentally removed. This means that elaiosomes attract ants but repel predators. Caruncles containing repellent substances and reserves doubly aid the survival of a seed: (a) because the seed is not attractive to predators, and (b) because it is attractive to ants who bury it.

The seed buried inside the nest may be regarded as favoured because some of the plants having this device grow in very poor soil (from the mineral point of view) whereas the nest is rich in minerals (Beattie 1983, Beattie 1985). Seeds buried in the nest sprout vigorously at germination. This does not seem to be the case here because the stored seeds are prevented from germinating by the direct or indirect intervention of the ants. Even if the soil around the seed deposit is rich in minerals these are not available until the nest is destroyed and the soil turned.

M. structor collect *M. annua* seeds which have been painted white or with nail polish. This indicates that *Messor* do not recognize the seed by its colour or scent, as proposed by Bresinski (1963). Also, a seed with both endosperm and embryo exposed, i.e. with much easily accessible food, excites the ants. The fact that *Messor* even collects small stones suggests that it is an 'indiscriminate collector'. But how does it perceive the *Mercurialis* seeds that are ballistically launched? They are always launched from a height of 10–50 cm, seed weight is quite uniform and hence the shock produced by impact on the soil is also uniform. These shock waves are perceived and immediately recognized by *Messor* ant which run to collect the seeds. Ants perceive sound transmitted through the ground to their legs (Wilson 1971). The seeds of *Mercurialis* are launched by the fruit; seeds or stones dropped/deposited experimentally next to the nest are quickly collected by *M. structor*. This could explain why seeds of different species and any portable object are immediately collected. These objects may obstruct the vibrations of seed falling on the soil. If this is so for *Mercurialis*, the seeds of the other species which are not ballistically released must be recognized by *Messor* in a different way. Hurlled seeds are typical of a large number of species of the family Euphorbiaceae, e.g. species of the genera *Ricinus*, *Hevea*, *Hura*, *Sebastiania*, *Mercurialis* and *Euphorbia* which all have a caruncle and are dispersed by ants (Ridley 1930, Burkart 1951). *M. structor* was observed to collect the seeds of 13 species, four of which have carunculate seeds (Table 1) and three of which are hurlled.

Some ant species dispose of the seeds as soon as the edible part is consumed, but the seed may be left: (a) in an abandoned chamber of the nest (as with *Mercurialis*, see also Beattie 1983); (b) near the nest entrance with other organic waste (Beattie 1983); and (c) in the soil if the edible part is consumed outside the nest (Beattie & Lyons 1975; Lack & Kay 1987). The behaviour of ants towards seeds may depend on the insect species, type of seed or the co-evolution of both. In the case of *Mercurialis*, if the ants succeed in breaking the Malpighian layer, they have access to a great quantity of food. In time this hard layer may be removed or softened by bacteria and/or moulds inside the seed stores. This may be the reason for separating seeds according to species. The seeds of different species vary in morphology and in the cell types of the integument protecting embryo and endosperm; softening times depend on the composition of these layers. The genus *Messor* is known to have at least two kinds of workers: some collect the seeds and others clean and sort them inside the nest (Jolivet 1986). Not all the seeds observed to be collected by ants are found inside the seed deposits. The rest may have been consumed immediately or deposited in sites that were not excavated.

Ants may be seed dispersers or seed predators; the latter are also known as harvesters. *M. structor* can be regarded as a seed predator because: (a) it collects seeds with and without caruncle; the disperser collects only carunculate seeds and not those whose caruncles have recently been removed (Beattie 1985); and (b) it even collects small stones which is peculiar to seed predators (Jolivet 1986). All these features indicate that *Mercurialis* seeds, like the other seeds inside the nest, were gathered by ants with the intention of storing them and

eating them at a later date. It is true that *Messor* was only observed to eat the caruncle but any ant that can open a tough seed will certainly also eat the softer tissues.

Seeds collected by seed predators such as *M. structor* may be dispersed when a colony abandons its nest and seed stores. In this case some seeds may germinate and manage to come up. This is common in many of the plant's preferred sites, e.g. orchards, vineyards and orange groves. It raises the interesting possibility that the interaction between *Messor* ants and *Mercurialis* seeds was originally that of predator and prey, but in cultivated land it is not so easy for the predator. By making stored seeds safe from other enemies, *Messor* may have helped transform the plant into a pest. The predator ants routinely store the seeds, but the stores are disturbed by agricultural activity before the ants can eat them. Seeds deposited at a depth of 10–30 cm are greatly facilitated in their germination when the soil is ploughed or hoed.

M. annua is a pest species and prefers orchards, vineyards and orange groves where the ground is frequently ploughed. This allows the seeds to escape induced dormancy and to germinate as the agricultural operations occur. These operations make year-round germination possible. The development of agriculture has enormously increased the distribution of this species. Soil manipulation is intended to remove harassing plants, but this succeeds only if *Mercurialis* seeds are not buried inside ant nests. Before modern agriculture, only the tread of a large animal or underground animals like the mole were able to end the induced secondary dormancy of these seeds.

The interaction between *Mercurialis* and *Messor* is very useful for both. If the ant nest is near *M. annua* plants, the ants have a readily available source of food in the caruncle. The *Mercurialis* seeds have the possibility of germinating contemporaneously and abundantly when the seed deposits are destroyed by ploughing of the soil.

ACKNOWLEDGEMENTS

The author is greatly indebted to Prof. Ferry Bouman who instigated this research, Prof. Andrew Beattie for his invaluable suggestions, Marina Bassani, Lino Grieco and Caterina Tanda who helped to perform some of the experiments, and Dr Bruno Poldi who identified *Messor structor*.

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