Atti Soc. tosc. Sci. nat., Mem., Serie B, 111 (2004)
pagg. 143-146, figg. 2

D. CICCARELLI (*), A.C. ANDREUCCI (*), A.M. PAGNI (*), F. GARBARI (*)

THE ROLE OF THE ELAIOSOME IN THE GERMINATION OF SEEDS OF *MYRTUS COMMUNIS* L. (MYRTACEAE)

Abstract - Seeds of *Myrtus communis* L. (Myrtaceae), a common shrub occurring in the Mediterranean area, are characterized by the presence of an elaiosome. This structure is a fleshy and edible appendage responsible of seed dispersal by ants, a phenomenon known as myrmecochory. The involvement of the elaiosome in the germination of seeds of *M. communis* was studied. Germination tests were carried out on three different stocks of seeds for two different periods of harvesting (November 2002 and March 2003): control seeds, seeds without elaiosome and scarified seeds. During the first days of the experiment, it seems that the removal of the elaiosome or the abrasion of the seed coat could lead an early germination, probably due to a faster imbibition. However, at the end of the experiment the seeds of the two different periods of harvesting showed similar germination rates (final percentage germination) for all the three treatments. Further investigations are in progress to verify the presence of inhibitory substances in the elaiosome of *M. communis*.

Key words - Ant, elaiosome, germination, *Myrtus*, seed.

Riassunto - *Il ruolo dell'elaiosoma nella germinazione dei semi di* M. communis *L. (Myrtaceae).* I semi di *Myrtus communis* L. (Myrtaceae), arbusto comune nell'area del Mediterraneo, sono caratterizzati dalla presenza di un elaiosoma, ovvero di un'appendice fresca e commestibile responsabile della dispersione dei semi da parte delle formiche, fenomeno conosciuto come mirmecocoria. Nel presente lavoro si riporta lo studio del ruolo eventuale dell'elaiosoma nella germinazione dei semi di *M. communis.* I test di germinazione sono stati eseguiti su tre diversi stock di semi e per due diversi periodi di raccolta (Novembre 2002 e Marzo 2003): semi di controllo, semi senza elaiosoma e semi scarificati. Durante i primi giorni dell'esperimento, sembra che la rimozione dell'elaiosoma o l'abrasione dei tegumenti seminali porti ad una precoce germinazione, probabilmente dovuta ad una più veloce imbibizione. Tuttavia, alla fine dell'esperimento i semi di entrambi i periodi di raccolta hanno mostrato percentuali di germinazione simili per tutti e tre i trattamenti. Sono in corso ulteriori investigazioni per verificare la presenza eventuale di sostanze inibitorie all'interno dell'elaiosoma di *M. communis*.

Parole chiave - Formiche, elaiosoma, germinazione, *Myrtus*, seme.

INTRODUCTION

Elaiosome is an ecological term introduced by Sernander (1906) to indicate all fleshy and edible appendages of disseminules dispersed by ants. The presence of an elaiosome on the seeds of *Myrtus communis* L. (Myrtaceae), a fleshy-fruited shrub occurring in the Mediterranean area, was demonstrated for the first time by Aronne & Wilcock (1994). The Authors reported that the seeds of mirtle are dispersed by the ant *Messor minor* Andrè and the presence of elaiosomes represents an adaptation to this type of zoochory known as myrmecochory. In fact, elaiosomes contain reserves, usually lipids but sometimes also proteins or starch, that play any role in germination but seem designed to attract and reward ants (Van der Pijl, 1982).
Aronne & Russo (1997) showed the role of carnivorous mammals, such as red fox (*Vulpes vulpes* L.) and carnivores of the genus *Martes*, as seed dispersers of *M. communis*. In spite of the passage of the seeds through the digestive apparatus of the mammals studied, the elaiosome seems to remain intact. Therefore, dispersal by these carnivores may not prevent myrmecochory.
More recently, Ciccarelli *et al.* (in press) studied the anatomy and the ontogeny of the elaiosome of mirtle seeds, showing a low content of lipids, starch and proteins, an evidence that can be explained by the fact that myrtle is a plant with a multiple pattern of seed dispersal and myrmecochory could play a secondary role. The aim of this work is to predict the eventual functional role of the elaiosome of seeds of *M. communis* by germination experiments.

MATERIALS AND METHODS

Seeds of *Myrtus communis* were collected in November 2002 and March 2003 respectively from hills of Santa Maria in Castello, Vecchiano (Pisa, Italy; 43° 47' N, 10° 13' E) at 86 m above sea level. These two different periods of harvesting were chosen because fruits usually ripen in mid-November, but ripe fruits, if not dispersed, remain attached to the pedicel until the end of February and are therefore winter-persistent. Specimens of the investigated material are deposited in PI (Pisa Herbarium).

Seed germination experiments

Seed germination was tested on 100 seeds (25 seeds per dish) with their elaiosomes, 100 seeds without elaiosomes and 100 hand scarified seeds. These seeds were scarified using a razor to make a nick or slice in the seed coat. Germination test was carried out on both seeds collected in November 2002 and March 2003. The two germination tests were performed on seeds collected immediately after flesh-fruit removal.

(*) Dipartimento di Scienze Botaniche, Università di Pisa, via Luca Ghini 5, I-56126 Pisa, Italy. E-mail: garbari@dsb.unipi.it

Seeds, after elaiosome removal and scarification, were sterilized for 10 min in 10% sodium hypochloride and placed on filter paper saturated with distilled water in Petri dishes. The dishes were placed in chambers with a temperature of 25°C: 12 h in the dark and 12 h in the light (Mitrakos, 1981). The seeds were illuminated with white light. Every 24 h the Petri dishes were checked to see whether germination took place and to add water whenever it was necessary. Seeds were considered germinated when the radicle had emerged.

Data analyses

Seed germination data were analysed by performing analyses of variance with a one-way ANOVA and Tukey's test. Each Petri dish was considered as a replicate of 25 seeds, as all tested seeds were selected at random from the whole pool of seeds collected from many different individuals. Differences among different treatments were considered significant only for $P < 0,05$.

RESULTS

Seeds collected in November 2002

Seeds without elaiosome and scarified seeds started germinating already from the first day. On the other hand, control seeds germinated only after 8 days. Most seeds of all treatments germinated within 14 days. After 14 days both seeds (without elaiosome and scarified) had similar germination rates (see Fig. 1). After 21 days no new seeds had germinated for another one week.

Statistical analyses (Tukey's test) carried out on the three different treatments showed significant differences ($F = 14,05$, d.f. = 2, $P = 0.00$) between seeds without elaiosome and control seeds and between scarified seeds and control ones. There were no differences between seeds without elaiosome and scarified seeds.

Seeds collected in March 2003

Germination tests carried out on seeds collected in March gave similar results to those carried out on seeds collected in November. Seeds without elaiosome and scarified seeds began germinating on the first day. Most seeds of all treatments germinated within the first 11 days (see Fig. 2). After 21 days no new seeds had germinated for another one week.

Statistical analyses (Tukey's test) performed on the total germination data showed no significant differences ($F = 2,73$, d.f. = 2, $P = 0.07$) among the three different treatments. The same analyses performed on data relative to the first 10 days showed significant differences between seeds without elaiosome and control seeds and between seeds without elaiosome and scarified seeds. There were no differences between control seeds and scarified ones.

DISCUSSION

The presence of an elaiosome on the seeds of *Myrtus communis* suggests that this species, although fleshy-fruited, has a morphological adaptation to ant dispersal.

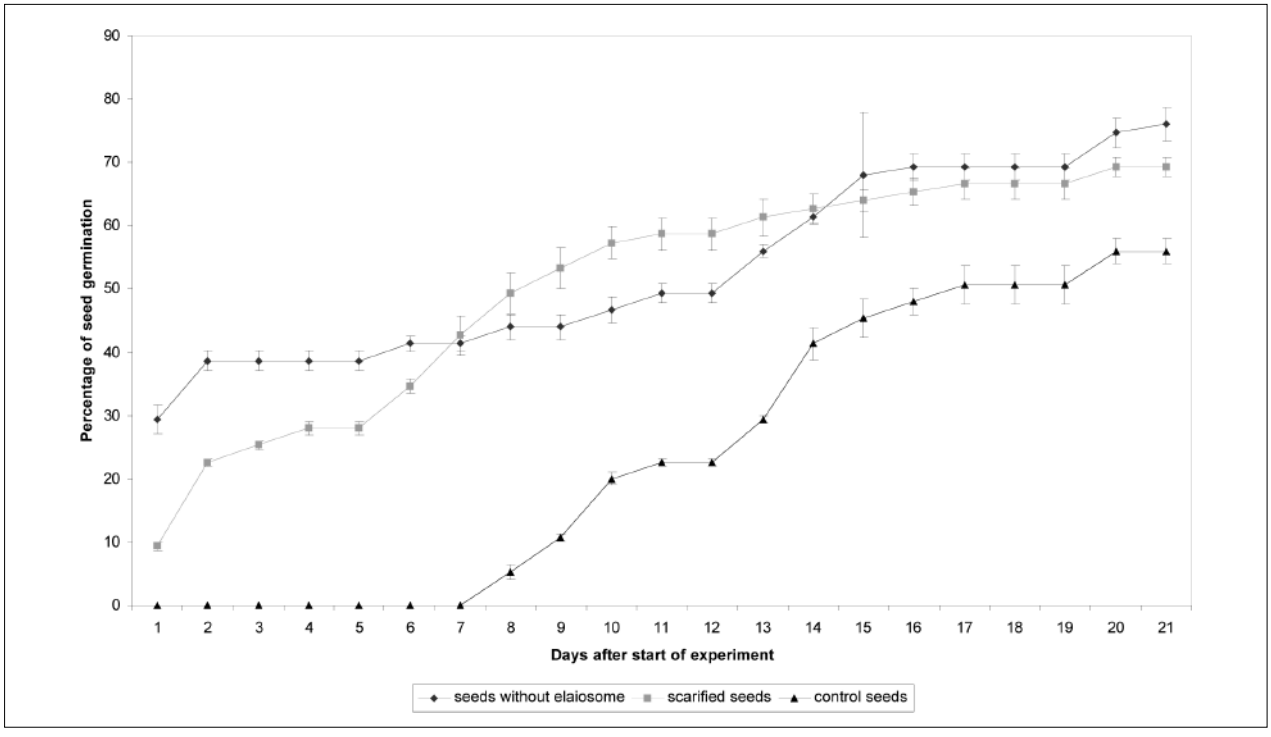

Fig. 1 - Percentage of germination (average ± standard deviation) for seeds collected in November 2002.

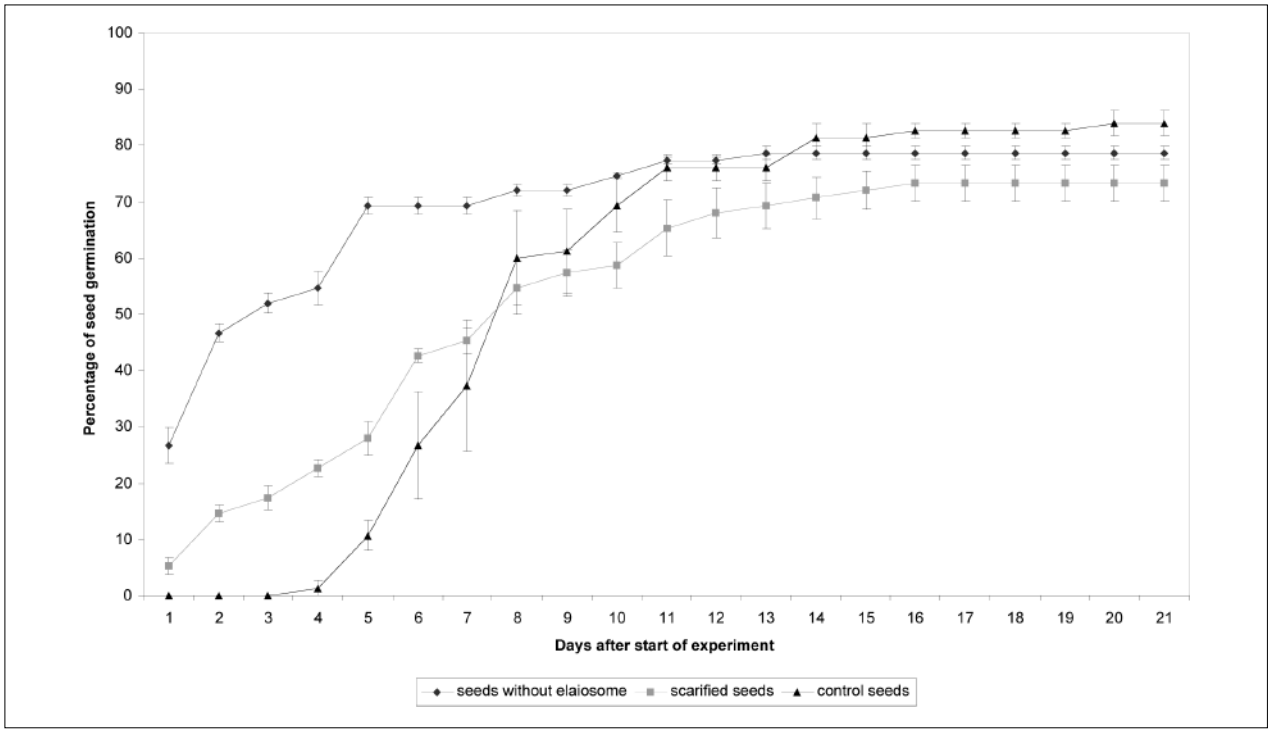

Fig. 2 - Percentage of germination (average ± standard deviation) for seeds collected in March 2003.

Transport to the ant nests may provide protection for seeds from predation by granivorous ants and vertebrates (Beattie, 1985; Espadaler & Gomez, 1996). Another service provided by ant dispersers is the removal of the elaiosome into the nest. Elaiosome removal, in relation with seedling emergence, has been shown to be important for Mediterranean myrmecochorous plants such as *Rhamnus alaternus* (Gomez *et al.*, 2003), *Knautia* (Mayer & Svoma, 1998), *Euphorbia characias* (Gomez & Espadaler, 1997) and *Mercurialis annua* (Lisci *et al.*, 1996; Lisci & Pacini, 1997).

Germination tests were carried out on seeds collected in two different periods of harvesting (November 2002 and March 2003) because *M. communis* fruits usually ripe in mid-November, but ripe fruits, if not dispersed, remain attached to the plant until the end of February, when they eventually fall on the ground.

The experiment was carried out on three different stocks of seeds for each period of harvesting: control seeds, seeds without elaiosome and scarified seeds. Actually, all these three situations could exist in the natural habitat: control seeds are seeds with elaiosome which have fallen on the ground and have not been collected by ants; seeds without elaiosome could be seeds collected by ants which have removed their elaiosome; scarified ones could represent seeds ingested by birds or mammals, which have passed through the guts of these animals so that seed coat was chemically or mechanically abraded.

Germination tests on seeds collected in November showed significant differences only between seeds without elaiosome and control seeds and between scarified ones and control seeds. Seeds without elaiosome and scarified seeds had faster germination rates and higher final germination percentage than control seeds. In fact, when control seeds began germinating (8 days after starting the experiment) the other two stocks had a germination percentage higher than 40%. It seems that the removal of the elaiosome or the abrasion of the seed coat could favour local absorption of water that diffuses into the outer integument more easily. It is worth considering that the micropylar area, which protects the root apex, lacks both integuments and is made of only a spongy tissue that is an extension of the elaiosome (see Ciccarelli *et al.*, in press). Therefore, when the elaiosome is removed this spongy tissue is usually cut away so that the root apex is exposed to air and can germinate more easily because no mechanical obstacle on its way is present. This could explain why seeds without elaiosome have the highest germination rate during the first days of the experiment.

As regards seeds collected in March, there are no significant differences among the three different treatments. Anyway, considering only the first 10 days of the experiment, significant differences were seen between seeds without elaiosome and control seeds and between seeds without elaiosome and scarified seeds. On the first day, for example, the percentage germination was 27% for seeds without elaiosome and 5% for scarified ones. Control seeds germinated only after 4 days. However, at the end of the experiment all three treatments had similar germination rates (see Fig.

2). It seems that the differences between control seeds and manipulated seeds could be ascribed to more efficient ventilation and imbibition due to excission of the elaiosome or to the mechanical scarification. It cannot be excluded the presence of substances that act as inhibitors inside the elaiosome. In fact, Gherardi & Valio (1976) reported that the aril contains phenolic substances which inhibited seed germination in *Carica papaya*; Lagoa & Pereira (1987) also showed the presence of inhibitory substances in the caruncle of *Ricinus communis*. These inhibitors could play a role in preventing germination seed during the unfavourable season in order to avoid exposing seedling to hard conditions. Further investigations are in progress to verify the presence of inhibitory substances in the elaiosome of *Myrtus communis*.

References

Aronne G., Wilcock C.C., 1994. First evidence of myrmecochory in fleshy-fruited shrubs of the Mediterranean region. *New Phytol.* 127: 781-788.

Aronne G., Russo D., 1997. Carnivorous mammals as seed dispersers of *Myrtus communis* (Myrtaceae) in the Mediterranean shrublands. *Plant Biosyst.* 131 (3): 189-195.

Beattie A.J., 1985. The evolutionary ecology of ant-plant mutualism. Cambridge University Press, Cambridge.

Ciccarelli D., Andreucci A.C., Pagni A.M., Garbari F., 2005. Structure and development of the elaiosome in *Myrtus communis* L. (Myrtaceae) seeds. *Flora* (in press).

Espadaler X., Gomez C., 1996. Seed production, predation and dispersal in the Mediterranean myrmecochore *Euphorbia characias* (Euphorbiaceae). *Ecography* 19: 7-15.

Gherardi E., Valio I.F.M., 1976. Occurrence of promoting and inhibitory substances in the seed arils of *Carica papaya* L. *J. Hort. Sci.* 51: 1-14.

Gomez C., Espadaler X., 1997. Manipulación por hormigas de semillas de *Euphorbia characias* (Euphorbiaceae) dentro del hormiguero. *Scientia gerundensis* 23: 53-61.

Gomez C., Pons P., Bas J.M., 2003. Effects of the Argentine ant *Linepithema humile* on seed dispersal and seedling emergence of *Rhamnus alaternus*. *Ecography* 26: 532-538.

Lagoa A.M., Pereira M.F.A., 1987. The role of the caruncle in the germination of seeds of *Ricinus communis*. *Plant Physiol. Biochem.* 25 (2): 125-128.

Lisci M., Bianchini M., Pacini E., 1996. Structure and function of the elaiosome in some angiosperms species. *Flora* 191: 131-141.

Lisci M., Pacini E., 1997. Fruit and seed structural characteristics and seed dispersal in *Mercurialis annua* L. (Euphorbiaceae). *Acta Soc. Bot. Pol.* 66 (3-4): 379-386.

Mayer V., Svoma E., 1998. Development and function of the elaiosome in *Knautia* (Dipsacaceae). *Bot. Acta* 111: 402-410.

Mitrakos K., 1981. Temperature germination responses in three Mediterranean evergreen sclerophylls. In: Margaris N.S., Mooney H.A. (eds.), Components of productivity of Mediterranean-climate regions. Basic and applied aspects: 277-279. Dr W. Junk Publishers, The Hague, Boston, London.

Pijl van Der L., 1982. Principles of dispersal in higher plants. Springer-Verlag, New York.

Sernander R., 1906. Entwurf einer Monographie der Europäischen Myrmekochoren. *Bihang till K. Sv. Vetensk. Akad. Handl.* 41: 1-410.

(ms. pres. il 12 dicembre 2004; ult. bozze il 7 luglio 2005)