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International *Ph. D.* Course in:
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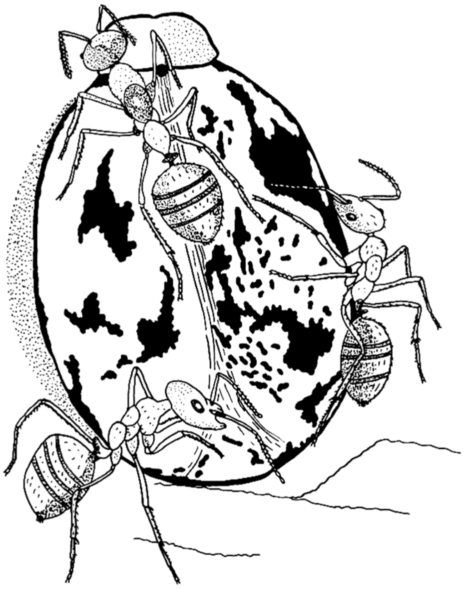
Philosophiae Doctor **Thesis**
Candidate for the award of the degree
Ignazio Li Vigni

Interactions between ants and diaspores of flowering plants (myrmecochory) in Sicily (Southern Italy): an important ecosystem function of the Mediterranean basin



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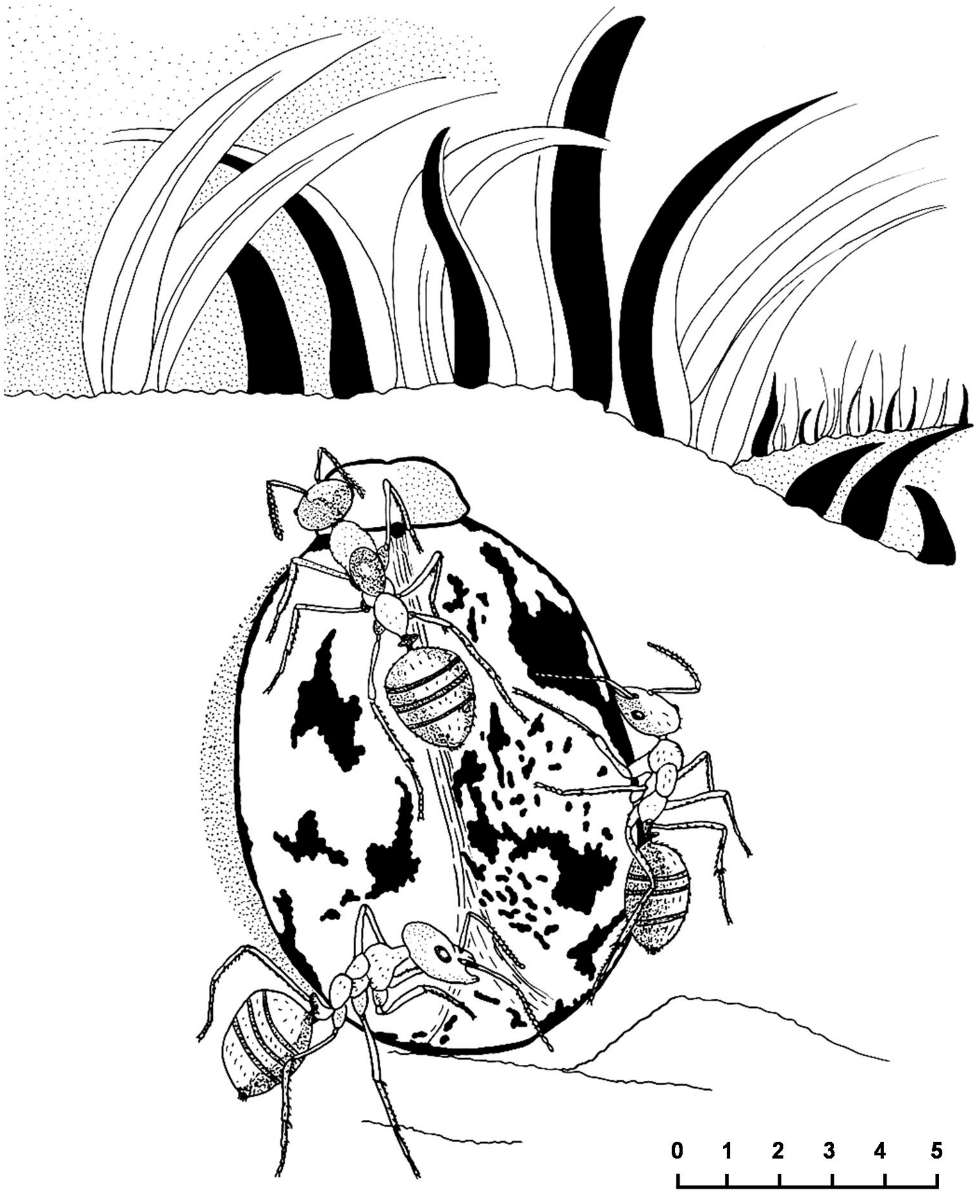
(**ON THE COVER**) Some ants carry a seed to their nest on the margins of the Ficuzza Wood (Western Sicily). It takes three *Formica (Serviformica) cunicularia* LATREILLE, 1798 (Formicinae: Formicini) workers [fresh weight (*n* = 50) 3.34–6.12 (4.97) mg; TL (*n* = 87) 4.13–6.72 (5.94) mm], to transport a seed of *Ricinus communis* L. (Euphorbiaceae) [fresh weight (*n* = 100) 328.62–463.75 (380.07) mg; TL (*n* = 100) 11.03–14.08 (13.14) mm]. Both species are natives of the Mediterranean basin, where they are very common, even in disturbed habitats. Scale bar in mm. (Original drawing © LI VIGNI from LI VIGNI & MELATI, 1999)



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> "Believe me, you will find more lessons in the woods than in books. Trees and stones will teach you what you cannot learn from masters."
>
> ST. BERNARD OF CLAIRVAUX (1090–1153)
> *Epistola, 106, 2.*

Preface

"After decades of studies on seed dispersal by animals, we are still ignorant about which seed–dispersing species are obligatory dispersers for the survival of many plants."

From "Why seed dispersers matter", an interview with P. M. FORGET (2010)
Chair of the 5th Frugivore and Seed Dispersal International Symposium.
http://news.mongabay.com/2010/0307–hance_forget.html (accessed 13 September 2014).

Since plants do not move from place to place, they have developed ways to make certain that their seeds are taken well away from them; this is called seed dispersal. Seed dispersal is important because if the seeds were to develop close to the parent plant, the young plants would not receive enough sunlight to grow. If plants are too close together they compete for light, water and minerals. It is much better if the seeds develop (i. e. germinate) in another area.

Seed dispersal has long been an object of fascination to biologists and the general public alike. Examples abound of structures that have clearly evolved to promote dispersal by wind, water or on the outside or inside of animals, but it is only recently that attention has turned to the question of just how well these structures work and what happens to the seeds of all those species (the majority) with no obvious adaptations for dispersal. Few things in seed ecology have changed more in recent years than our understanding of seed dispersal.

In the last decades the interest for plant–animal interactions increased dramatically and nowadays it is a firmly established discipline. The late development of this field was in part due to the historical progress of the knowledge on plants and animals in two separate lanes, only occasionally interacting with each other. Especially during the 80's, we witnessed to an explosion in the number of publications dealing with a variety of thematic on plant–animal interactions and this large amount of information allowed the development of many theories explaining ecological patterns and processes. Nowadays the study of plant–animal interactions encompasses various areas of knowledge (e. g. ecology, ethology, physiology, biochemistry) allowing a better comprehension of the phenomena under study.

The processes governing the evolution of plant–animal dispersal mutualisms have been the topic of important investigations. The observation that plants were generally dispersed by more than one animal species led authors to question the initial vision of pairwise tightly coevolved associations.

Myrmecochory (i. e. seed dispersal by ants) is a mutualistic interaction between ants and plants, where the elaiosome plays a critical role in ant attraction and subsequent seed removal. Over the World, thousands of species of herbs, shrubs, and trees are known to be principally ant–dispersed. In the myrmecochory rules, relationships, and bio–ecological characteristics surprisingly recur, albeit with local variations, in all biogeographic regions of the World. These analogies and these recurrences can be explained simply by the exceptional reappearance, in a multitude of plants and ants of various genera and species of similar ecological conditions that led to the phenomenon of convergent evolution in the plants.

Seed dispersal by ants although it is common in nature, is probably the least studied of the main seed dispersal syndromes. Biologists are just beginning to recognize the specialized mechanisms that make ants a major force in the spread of plants around the World. As the long list of known myrmecochores grows, biologists can expect to learn more about the importance of this seed–dispersal mechanism around the World. Further studies of the benefits of myrmecochory for plants and ants should also help to elucidate details of mutualistic relationships and their evolutionary consequences. Despite of the over 1,500 publications on myrmecochory and despite the large number of myrmecochores existing worldwide (possibly up to 23,000 species or 8.5% of all Angiospermae), there is still a tremendous lack of information.

Seed dispersal by ants is often studied empirically because it is a phenomenon difficult to observe in strictly natural conditions. The most effective method is to collect the seeds of a myrmecochorous plants, place them near the entrance of an ants nest, and wait that the workers will find them. This method involves the intervention of the observer and not a true natural observation.

In **chapter 1**, all these aspects are analyzed in relation to the myrmecochory.

The climate of a region will determine which plants will grow there, and which animals will inhabit it. The climate, also, indirectly controls the communities via changes in interspecific interactions. In the Mediterranean ecosystems of Southern Africa and Australia, the role of ants in the dispersal of seeds is well known and antagonistic interactions as well as myrmecochory occur. The high prevalence of seed dispersal by ants, compared to that by vertebrates, is viewed as evidence of adaptation to the soil nutrient deficiency typical of these environments. Instead, for a long time, ant dispersal has been considered unimportant in the Mediterranean basin and before 1994, no systematic study on ant–seed interaction had been undertaken. Only in the 1994 ARONNE & WILCOCK (see: First evidence of myrmecochory in fleshy–fruited shrubs of the Mediterranean region. *New Phytologist, 127 (4), 781–788*) have realized a systematic study on the myrmecochory in the Mediterranean shrubland of Southern Italy (in the Nature Reserve at Castelvolturno, in the Bay of Naples). Since then, few other studies have been made in Italy, Spain, and France.

In **chapter 2**, wanting to fill this gap, I have undertaken a systematic study on the myrmecochory in an extended Mediterranean area of Southern Italy. I focused in my work on the seeds fate of supposedly myrmecochores, i. e. flowering plants whose sexual diaspores have elaiosomes, in forest ecosystems of European–Mediterranean region, where these herbs are quite numerous. The coupling of the fruiting season and the peak foraging period of ants suggests that ants will disperse a majority of the seeds away from the parent plant. This study has been realized in two different areas of Sicily that altogether span a land area ofabout 50,000 hectares of Mediterranean flora: the Oriented Nature Reserve Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago (Western Sicily) and the Madonie Regional Natural Park (North–Western Sicily). The research conducted highlighted that in the Mediterranean area myrmecochory is a fundamental ecological interaction. In the study areas the myrmecochorous species were surveyed and they account for approximately 8% of the total number of plant species (over fifteen hundred). Myrmecochorous plant species never identified before were reported.

At current state–of–the–art a pitfall trap specifically effective in capturing epigaeic ants does not exist. In **chapter 3**, for the purposes of monitoring the Formicidae I conceived a new design of trap for ants, in order to meet the needs of maximizing capture of myrmecofauna reducing the presence of intruders, for filling a gap in the existing literature. The epigaeic myrmecofauna of the Ficuzza Wood reserve was collected with this protocol and twenty ant species have been identified.

In **chapter 4**, a complex study–type (cafeteria experiment, diaspore anatomy, seed germination in controlled conditions) conducted on a plant endemic to Sicily (*Corydalis densiflora* C. PRESL), for the first time as myrmecochores, is reported.

Since the topic of this *Ph. D.* thesis crosses different biological disciplines, it would have been impossible without the additional expertise of entomologists, myrmecologists, botanists, zoologists, and ecologists. I am thankful to C. RAPISARDA, G. MAZZEO, L. ZAPPALÀ (Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, Sezione di Entomologia applicata, University of Catania, Italy), and B. MASSA (Dipartimento di Scienze Agrarie e Forestali, Laboratorio di Zoologia applicata, University of Palermo, Italy) for their continuous support. G. DORIA, M. TAVANO, and R. POGGI (Museo Civico di Storia Naturale DORIA of Genoa, Italy) for making it possible to view the collections of the Italian ant species. F. RIGATO (Museo Civico di Storia Naturale of Milan, Italy) to have confirmed the ant species identification. D. PIRAINA (Museo Civico di Storia Naturale of Milan, Italy) for ant photography under a stereomicroscope. S. CAMBRIA (Banca di Germoplasma del Mediterraneo ONLUS of Palermo, Italy) for assistance in the plant species determination. B. L. PATERNOSTRO, M. DELIA, A. PIZZOLATO, and D. MAGGIORE (Banca di Germoplasma del Mediterraneo ONLUS of Palermo, Italy) for their constructive criticisms on an earlier draft of this thesis and for assistance in field work. A. WESTRICH and Z. LIEBERMAN (California Academy of Sciences, San Francisco, USA) for their helpful linguistic suggestions and corrections that improved the text.

Chapter 1

"– Everything wants to be everywhere – With this brief but significant phrase, the biologist Danish D. HILLENIUS (1927–1987), has characterized the irrepressible impulse with which all forms of life seek the smallest and most hidden corner of the Earth to preserve their species and to multiply. It is this imperious desire that is the basis of the competition, of natural selection, and at the end of evolution. – Everything wants to be everywhere – And everywhere results to be always another place."

BIJNSDORP R. (1993)

Myrmecochory: an ecologically significant ant–plant interaction with worldwide distribution

CONTENTS

1. THE ANTS

Insecta is the most abundant class of the phylum Arthropoda. One million species have been described and represent 70% of all known animal species (i. e. insects + others invertebrates + chordates: *n* = 1.4 million). Annually 8,000–10,000 new species of insects are discovered and 5–30 million yet to be found are estimated (CARWARDINE, 2007; CHAPMAN, 2009).

The ants are social insects classified in the suborder Apocrita GERSTAECKER, 1867 of order Hymenoptera which contains 115,000 species (HYMENOPTERA ONLINE, 2014) (estimate 300,000: CHAPMAN, 2009) and constitute a monophyletic group, the Formicidae family, divided into 20 subfamilies (***TABLE 1***) and 470 genera, within the Vespoidea superfamily (***TABLE 2***). Since the inception of modern taxonomy 253 years ago, from *Formica rufa rufa* LINNEAUS, 1761 (LINNAEUS, 1761) to *Mystrium eques* YOSHIMURA & FISHER, 2014 (YOSHIMURA & FISHER, 2014), almost 16,000 ant species and subspecies have been described (ANTCAT/BOLTON, 2014). Estimates suggest that the total number ranges between 25,000 and 30,000 species (LACH *ET AL.*, 2010), with the majority of the species confined to the tropics (HÖLLDOBLER & WILSON, 2009).

The head, alitrunk, and gaster are the three distinct body segments of the Formicidae. Ants are distinct in their morphology from other insects in having elbowed antennae, metapleural glands, and a strong constriction of their second abdominal segment (petiole). The third abdominal segment is often similarly constricted (postpetiole, characteristic of the largest subfamily Myrmicinae and of Pseudomyrmecinae, and most Ecitoninae). The presence of single–segment petiole or two–segment petiole is an easy way to visually classify major subfamilies of Formicidae. The elbowed antennae distinguish ants from other wingless wasps. Metapleural glands (situated in the metathorax) are secretory glands that are unique to ants. They are responsible for the production of an antibiotic fluid; this helps to prevent the growth of bacterial and fungal spores on the ants, and inside their nest. Ants are usually black, brown or reddish, and live in colonies with well–defined castes (typically a worker caste of sterile females and a reproductive caste of winged males and females). Virtually all ant keys are for workers only. Males and often queens can be radically different in appearance from workers. Males die shortly after mating and females tear off their wings after mating or just before entering a nesting site, and of course remain wingless for the rest of their lives of 9–10 weeks to 30 years, depending on the species. Ants are holometabolous, with the pupa in a cocoon or not, as determined by subfamily, subgenus or even species. In some species, worker pupae are naked or facultatively naked, while sexual pupae are in a cocoon. Nest–founding queens typically rear the first brood of small (nanitic or minim) workers alone, either sealed in a nest cell and feeding off stored fat, and lysing wing musculature (claustrally) or occasionally (semiclaustrally) or regularly foraging for food while rearing the first brood, but almost never after the first workers emerge (BORROR *ET AL.*, 1989; HÖLLDOBLER & WILSON, 1990) (**FIGURE 1** and **2**).

Interspecific body length is extremely variable. According to BEATTIE (1985), this difference is the same order of magnitude as that between a deer mouse and a mountain lion, a human and a blue whale or a sunflower and a spruce tree (**FIGURE 3**). A whole colony of Oceania *Carebara atoma* (EMERY, 1900) could live comfortably within the head capsule of a soldier of Borneo *Camponotus gigas* (LATREILLE, 1802) (HÖLLDOBLER & WILSON, 1994; MOFFETT, 2010). Besides the body size of the individual, colony size (number of individuals) also varies greatly.

The smallest known colonies of ants in the World belong to *Adelomyrmex biroi* EMERY, 1897 of New Guinea and contain just 10 adult individuals (WILSON, 1959). One among the largest polydomous “supercolonies”, constituting 45,000 nests which covered an area of 2.7 km^2, is that of *Formica yessensis* WHEELER, 1913 of Japan, with about 307 million of adults (HIGASHI & YAMAUCHI, 1979; HIGASHI, 1983).

Species richness encompasses a very wide range of morphological, physiological, and ecological diversity. Ants are among the most abundant terrestrial life forms (KAUTZ & MOREAU, 2011); they are predominant in terms of density among all arthropods, despite representing 1.6% of the total number of insect species. Ants often comprise 80% of the arthropods in tropical rain forests (DAVIDSON & PATRELL–KIM, 1996). For example, on the Ivory Coast savanna (Western Africa) the density of ants is 7,000 colonies and 20 million individuals per hectare, with one species alone, *Camponotus acvapimensis* MAYR, 1862, with 2 million of individuals (LÉVIEUX, 1966, 1982).

The ants are successful not only in terms of sheer numbers, but in their geographical coverage of Earth as well. They have a cosmopolitan geographical distribution and occupy a wide range of ecological niches: they live from the Sahara desert to the heart of Australia, from the forests North of the Arctic Polar Circle to the tundra (HÖLLDOBLER & WILSON, 1994).

It has been estimated that the population of ants on Earth amounts from one to ten million billion individuals and hence ants and humans have equivalent biomass (HÖLLDOBLER & WILSON, 2009). Within the environments where they occur, they have been estimated to contribute 15–25% of all the total terrestrial animal biomass, which well exceeds those of the vertebrates (SCHULTZ, 2000). All these features make them a very important taxon among terrestrial invertebrates, with fundamental ecological functions. It is for these reasons that ants have been used as a focal taxon to indicate biodiversity of an ecosystem (e. g. UNDERWOOD & FISHER, 2006). From a single leguminous tree in the Tambopata Reserve of Peru, 43 species of ants belonging to 26 genera have been recovered, about equal to the entire ant fauna of the British Isles (WILSON, 1987).

They perform important ecological roles as herbivores (the Neotropical leaf–cutting ants in their environments are also the main herbivores: CHERRETT, 1982) and they are major arthropod predators, and scavengers among invertebrates. Soil–nesting ants turn more soil than earthworms, thereby circulating large amounts of essential nutrients to the soil, favoring also the infiltration of water and the exchange of oxygen (FOLGARAIT, 1998). In addition, the Formicidae are a source of food for lizards, small predatory mammals, insectivorous birds, and arthropods including spiders, ant–lions, and beetles (COLLINGWOOD *ET AL*., 2011). One of the most important adaptations contributing to the success of ants was the development of "eusociality" (*sensu* HÖLLDOBLER & WILSON, 1994).

After a modest beginning in the Cretaceous, which took place in Africa 95 million years ago (MYA) (SCHMIDT *ET AL*., 2010), the Formicidae came to occupy key positions in the majority of terrestrial ecosystems, establishing relations with thousands of species of arthropods (KISTNER, 1982), with unknown number of fungi and microorganisms (MUELLER *ET AL*., 2001), and 470 species of myrmecophile plants belonging to 50 families (JOLIVET, 1998). In 20 species of plants belonging to several genera, pollination by ants has been demonstrated, but the true number may be closer to one hundred (RICO–GRAY & OLIVEIRA, 2007). At least 11,500 species of myrmecochorous flowering plants belonging to 77 families, and 334 genera have diaspores with elaiosomes, which serve as food reward, so that the ants enact dispersion (LENGYEL *ET AL*., 2010).

2. THE FLOWERING PLANTS

The division of Angiospermae LINDLEY, 1830 or Magnoliophyta CRONQUIST, TAKHT. & ZIMMERM., 1966 is the most diverse group of advanced land plants. Angiospermae are seed–producing plants like the Gymnospermae LINDLEY, 1830 and can be distinguished from the Gymnospermae by a series of synapomorphies (i. e. derived characteristics). These characteristics include flowers, endosperm within the seeds, and the production of fruits that contain the seeds.

The Angiospermae are the "higher" more (= seed plants or Spermatophyta LINNAEUS, 1753) evolved plants of the plant kingdom. The Angiospermae experienced a tremendous radiation in all geographic regions during the Mid–Cretaceous (90 MYA) (RICO–GRAY & OLIVEIRA, 2007). The total number of flowering plant species is estimated to be in the range of 270,000 to 400,000 (THORNE, 2002; GOVAERTS, 2003; CHAPMAN, 2009). The Angiospermae are in a ratio of about twenty to one against Pteridophyta LINNAEUS, 1753 (or seedless vascular plants, i. e. lycopods, selaginellas, horsetails, and ferns) and Gimnospermae (i. e. substantially conifers), in practice the rest of terrestrial plants or vascular plants. The flowering proved to be an unusually effective means of reproduction, spreading (whatever its origin) to become the dominant form of land plant life (***TABLE 5***).

The seed develops from the ovule. All mature seeds contain an embryo and a protective covering called a seed coat (testa). In early development all Angiospermae seeds also contain an endosperm, but in many seeds the endosperm is completely absorbed by the developing embryo. The embryo and endosperm are products of fertilization while the seed coat develops from the integuments of the ovule. Each ovule inside an ovary develops into a seed when fertilized. The stalk of the ovule and of the subsequent seed is termed the funicle and may play a part in the dispersal of the seed from the mature ovary (the fruit). If the seed is detached from the funicle it will leave a scar, the hilum. The distal end of an ovule is enveloped by one or two layers of tissue, the integuments, which usually do not meet completely at the top leaving a hole, the micropyle, through which the pollen tube may find entry to the ovule at fertilization. As the ovule enlarges into a seed, one or both integuments develop into the seed coat. The micropyle may remain visible on the seed. Some ovules are bent over on the funicle (anatropous as opposed to orthotropous) and the micropyle in the seed is therefore next to the hilum, the funicle appearing as a ridge fused down the side of the seed and then known as the raphe. The seed coat can be much elaborated and very hard (sclerotesta). If it develops with a soft layer it is termed a sarcotesta (***FIGURE 4***).

The function of the seed is to protect the embryo, to sense environmental conditions favorable to germination, to nourish the germinating seedling, and disperses the progeny to a new location. The seed coat contains a variety of adaptations related to protection and dispersal mechanisms; usually, it forms a dry layer that prevents destruction of the seed by dehydration or predation. It may contain waxes for water impermeability, mucilage to make seeds sticky, compounds resistant to digestion by animals, etc.

Fruit develops from organs of the flower and thus involve differentiation or re–differentiation of preexisting organs. Evolutionarily, floral organs represent modified leaves (carpels) and so the fruit is also a modified leaf. Fruits serve two functions: to protect the seeds during development and then to disperse the seeds following maturation. Most fruit develops from the ovary. In fact some schemes classify fruit derived from a single ovary as "true fruits" while "false fruits" are composed of tissues derived from flower parts other than the ovary or from more than one ovary. In true fruits the outside of the fruit is called the pericarp and develops from the ovary wall. The pericarp can be dry and papery, woody or fleshy. These pericarp differences reflect adaptations to different dispersal mechanisms. The fruit can contain a single seed or many seeds. The pericarp of some fruits is further differentiated into specialized layers called exocarp, meso– and endocarp (e. g. ESAU, 1977; FAHN, 1990; RAVEN *ET AL.*, 1999; RUDALL, 2007; BERG, 2008).

3. SEED DISPERSAL

The dispersal constitutes a determinant step in the biological cycle of most organisms (BOULAY *ET AL.*, 2006). In the flowering plants, the completion of their biological cycle goes through the dispersal of reproductive structures: the pollen for the cross–fertilization and the seeds for the colonization of new areas (HERRERA, 2002). Seed dispersal is central to plant reproduction, population genetics, and ecology because it determines the movement of plant genes in space and in time. Even subtle changes in traits that influence seed dispersal can therefore have far–reaching consequences on a multitude of other biological traits and, thus, dispersal mode is one of the most fundamental life history traits in plants (LENGYEL *ET AL.*, 2010).

Seed dispersal has attracted scientists since long ago, i. e. DARWIN (1859), KERNER (1896), SCHMIDT (1918), ULBRICH (1928), RIDLEY (1930), PIJL VAN DER (1982), MÜLLER–SCHNEIDER (1983).

Seed dispersal has long been an object of fascination to biologists and the general public alike. Examples abound of structures that have clearly evolved to promote dispersal by wind, water or on the outside or inside of animals, but it is only recently that attention has turned to the question of just how well these structures work and what happens to the seeds of all those species (the majority) with no obvious adaptations for dispersal. Few things in seed ecology have changed more in recent years than our understanding of seed dispersal (FENNER & THOMPSON, 2005).

Seed dispersal is one of the central topics in modern biology, with implications for ecology, ethology, plant and animal biogeography, speciation and evolution (MURRAY, 1987).

Seed dispersal is an important function provided by the facilities of an ecosystem and it has an impact also on plant species richness, abundance, and successions of vegetation (EHRLÉN & ERIKSSON, 2000; TÜRKE, 2011). Seed dispersal links the sessile plants to ecological processes operating at larger geographic scales such as long–distance dispersal, colonization, range expansion, isolation and speciation (BULLOCK & NATHAN, 2008).

Seed dispersal is also an ecological challenge for plants, that they frequently overcome using animal movement in exchange of food rewards (BOULAY *ET AL.*, 2006). Seed dispersal modes are usually classified into five broad types (syndromes): unaided (e. g. passive, ballistic), by wind (anemochory), by water (hydrochory), by vertebrates externally (e. g. on fur: exozoochory) or internally (passing through the gut: endozoochory), more specifically by insects (entomochory) and in particular by ants (myrmecochory) (PIJL VAN DER, 1982). Although it is recognized that these modes differ dramatically in their benefits (and costs) to plants, the number of origins and global significance of different modes of seed dispersal remain poorly understood (PIJL VAN DER, 1982; LENGYEL *ET AL.*, 2010; VITTOZ & ENGLER, 2007).

Fruit is frequently a seed dispersal tool that attracts animals to eat or otherwise disturb it, incidentally scattering the seeds it contains. Seed dispersal by animals (zoochory) is a critical plant–animal mutualism that plays an important role in the gene flow, demography, distribution and evolution of plants (HOWE & SMALLWOOD, 1982; PIJL VAN DER, 1982; RICHARDSON *ET AL.*, 2002). Theoretical developments considering spatial variations of interactions suggest that even such diffuse mutualisms may be driven by coevolutionary processes (THOMPSON, 1994). Hence, plant–disperser coadaptations likely vary with space and time depending on the species involved in the mutualism in conjunction with abiotic (e. g. climate) and biotic (e. g. presence of predators or parasites) constraints. The expected result is a geographic mosaic in which well–matched adaptations evolve locally and further extend to other populations while unfavorable associations tend to be eliminated (BOULAY *ET AL.*, 2006).

The interactions between animals and plants are diverse and omnipresent in nature. These organisms have been evolving together for millions of years, with varying types and degrees of interaction between them. In some cases, plants and animals share an extremely specialized interaction, being dependent on each other and exhibiting peculiar morphological, ethological and physiological adaptations. However, the large majority of interactions between plants and animals are not specialized and are usually facultative (HERRERA, 2002).

In the last decades the interest for plant–animal interactions increased dramatically and nowadays it is a firmly established discipline. The late development of this field was in part due to the historical progress of the knowledge on plants and animals in two separate lanes, only occasionally interacting with each other. Especially during the 80's, we witnessed to an explosion in the number of publications dealing with a variety of thematic on plant–animal interactions and this large amount of information allowed the development of many theories explaining ecological patterns and processes. Nowadays the study of plant–animal interactions encompasses various areas of knowledge (e. g. ecology, ethology, physiology, biochemistry) allowing a better comprehension of the phenomena under study (HERRERA & PELLMYR, 2002).

The processes governing the evolution of plant–animal dispersal mutualisms have been the topic of important investigations. The observation that plants were generally dispersed by more than one animal species led authors to question the initial vision of pairwise tightly coevolved associations (HOWE, 1984; BOULAY *ET AL.*, 2006).

4. ELAIOSOMES

The elaiosome, working as alimentary bounty urges ants to gather the diaspores (i. e. seeds or seeds and fruits together + elaiosome) and to scatter them about along their way unharmed soon after removing the elaiosome; this is partial carpophagy of the diaspore with consequent indirect seed dispersal by ants (LI VIGNI & MELATI, 1999).

These edible external appendages are shown in thousands of plant species worldwide (HANZAWA *ET AL.*, 1988), they may vary, e. g. in their shape, coloration, size (TAHTADZHJAN, 1985) and are different in their origin, but they all consist of parenchymatous lipid–rich cells (KOMAR, 1978). These structures are found both in seeds (e. g. *Chelidonium majus* L., *Corydalis aurea* WILLD., *Viola odorata* L.) or in seeds and fruits together (e. g. *Borago officinalis* L., *Lamium amplexicaule* L., *Pulmonaria officinalis* L.) and appear in plants systematically far from each other, so that in these cases we can talk about convergent evolution in flowering plants (LI VIGNI & MELATI, 1999).

Generally the elaiosome is: a caruncle (or micropylar aril: elaiosome originating in the region of micropyle); a strophiole (or raphal aril: elaiosome originating along raphe–antiraphe) or an aril (or true aril: elaiosome originating on a funicle) (TAHTADZHJAN, 1985; GORB & GORB, 2003) (***FIGURE 5***).

5. SEED DISPERSAL BY ANTS (MYRMECOCHORY)

Myrmecochory, i. e. diaspore dispersal by ants, is a mutualistic interaction between ants and plants, where the elaiosome plays a critical role in ant attraction and subsequent seed removal. Over the World, thousands of species of herbs, shrubs, and trees are known to be principally ant–dispersed (BEATTIE & HUGHES, 2002). In all cases studied to date, the primary function of elaiosomes is to attract ants and elicit the transport of the diaspore usually to the nest by the ants as destiny. In the nest, ants consume the elaiosome or, more often, feed it to their larvae (because provide various nutrients that are essential for insect reproduction and development). The intact seeds (i. e. still viable: LI VIGNI & PATERNOSTRO, 2005) are then abandoned in garbage piles in chambers in the nest or outside the nest. Elaiosomes thus function as rewards for ants in much the same way as fruits or berries serve as rewards for vertebrate dispersers (BRESINSKY, 1963; BEATTIE, 1985; BREW *ET AL.*, 1989; FISCHER *ET AL.*, 2005; BOULAY *ET AL.*, 2006;

EDWARDS *ET AL.*, 2006; FOKUHL *ET AL.*, 2007; FISCHER *ET AL.*, 2008).

In the myrmecochory rules, relationships, and bio–ecological characteristics surprisingly recur, albeit with local variations, in all biogeographic regions of the World. These analogies and these recurrences can be explained simply by the exceptional reappearance, in a multitude of plants and ants of various genera and species of similar ecological conditions that led to the phenomenon of convergent evolution in the plants (BRONSTEIN *ET AL.*, 2006).

Myrmecochory was first studied in depth by SERNANDER, a botanist at the University of Uppsala in Sweden. In 1906 he published a distinguished review on Central European myrmecochorous shrubs (he recorded approximately 120–150 species: MAYER *ET AL.*, 2000; WEISS, 1908). SERNANDER was the first to use the terms: diaspore, elaiosome, and myrmecochorous (WEISS, 1908).

There are ant species that carry seeds to their nests with the purpose of preying those (granivorous species). These ants, named harvester ants, collect a large number of seeds to their nests and usually destroy almost all of them (e. g. DETRAIN & TASSE, 2000). Several works have highlighted the impact of harvester ants as post–dispersal seed predators, and their role in shaping plant communities composition, and structure (BROWN & HUMAN, 1997; MACMAHON *ET AL.*, 2000). Seed–harvesting ants, ecologically significant seed predators which store in underground granaries, are dominant elements in terms of biomass, and/or colony numbers, in the desert, and drier grasslands in warm, temperate, and tropical regions around the World (HÖLLDOBLER & WILSON, 1990). They are primarily species of the Myrmicinae genera *Pheidole, Monornoriurn, Pogonornyvmex, and Messor,* which span the full spectrum from generalist omnivores to specialist granivores (ANDERSEN, 1991). Harvesting ants feed on seeds, but compensate, at least in part, for the damage caused by this predation by serving as important agents of seed dispersal (HÖLLDOBLER & WILSON, 1990). Usually, the species are highly polymorphic, with the larger individuals possessing powerful mandibles capable of cracking open seeds. Seed fragments are fed to larvae, but probably many harvested seeds escape destruction either by being abandoned in stores or by germinating quickly within the ant nests. Thus, seed harvesting by ants, which could be viewed as exclusively detrimental, actually may carry some benefits to the plant through dispersal and provision of local nutrients to the seedling (GULLAN & CRANSTON, 2009). Since it seed dispersal may occur when seeds are accidentally lost in transport or seed stores are abandoned, we can define seed dispersal by harvester ants "myrmecochory *sensu lato*".

Several ant species discard seeds or seeds and fruits together which are too hard, but which have an elaiosome. The dispersal by ants of elaiosome–seeds is defined "myrmecochory *sensu stricto*" (e. g. ULBRICH, 1928; BARROSO *ET AL.*, 2013; LIMA *ET AL.*, 2013).

Myrmecochory is a common syndrome (i. e. a complex of special morphological, anatomical, biochemical, and phenological adaptations of plants, especially of the herbs, that enhance ant attraction and thus increase the effectiveness of myrmecochory: GORB & GORB, 2003) especially in Southern Hemispheric shrublands of Southern Africa and Australia, and in the temperate woodlands of Northern Hemisphere, e. g. central Europe, and Northern America deciduous forests where myrmecochores can represent up to 30–50% of herbaceous biomass (SERNANDER, 1906; HANDEL *ET AL.*, 1981; WOLFF & DEBUSSCHE, 1999; GORB & GORB, 2003; SEIFERT, 2007; TÜRKE *ET AL.*, 2010). Throughout East–Northern America temperate deciduous forests, the ants disperse up to 70% of understory plant species (CANNER, 2010) (***FIGURE 7***).

In general, a single ant species complex (e. g. *Aphaenogaster rudis* ENZMANN, 1947 in East–Northern America temperate deciduous forests) disperses a majority of seeds. The coupling of the fruiting season and the peak foraging period of ants suggests that ants will disperse a majority of the seeds away from the parent plant (CANNER, 2010).

According to the studies of DUNN *ET AL.* (2007) and BRADSHAW *ET AL.* (2011):

a) Most origins of seed dispersal by ants are associated with transitions from passive dispersal (including both wind and gravity) to myrmecochory. Only a very few cases where myrmecochorous seeds evolved from vertebrate–dispersed fleshy fruits fruit have been observed –

b) Morphological features for myrmecochory have evolved at least twenty times within the monocots –

c) Origins of myrmecochory are not associated with the rise of forests during the Cretaceous or with subsequent transitions of plant lineages into closed canopy habitats, nor are they contemporaneous with the origins of fleshy fruits. Instead, the origins of myrmecochory are closely associated with the rise in relative abundance of ants (proportion of all individual insects in fossils) towards the end of the Eocene and more recently. The highest observed rate of origination of ant dispersal occurred substantially later (essentially the present) than that for fleshy fruits –

d) Elaiosomes evolved in response to changes in ant communities. An alternative to the hypothesis that ant communities have driven patterns of origination of elaiosomes is that changes in forest structure and climate have played a more important role. More specifically, it has been hypothesized that in closed canopy shaded habitats, dispersal by animals, including dispersal of seeds by ants, is more likely to evolve than in open canopy habitats due to the relative absence of wind.

Elaiosomes have independently evolved at least 100–150 times in flowering plants and their presence is estimated in up to 23,000 or 8.5% of all species of flowering plants (*n* = 270,000); in this way the elaiosomes are a dramatic example of convergent evolution in flowering plants (LENGYEL *ET AL.*, 2010). Myrmecochory provides one of the best examples to date for convergent evolution in general and for the repeated evolution of plant–animal mutualisms more specifically.

The families containing myrmecochorous genera are represented in all major lineages in the angiosperm phylogeny. Although myrmecochory is present in several families that diverged early (100 MYA) in angiosperm phylogeny, most families in which myrmecochory is found originated at or after 70–80 MYA (these time periods do not necessarily represent the dates of the evolutionary transitions to myrmecochory) (LENGYEL *ET AL.*, 2010). LENGYEL *ET AL.* (2010) increase the number of myrmecochorous species from 3,000 (BEATTIE, 1985), and 4,000 (LI VIGNI & MELATI, 1999) to 11,500, indicating that myrmecochorous plants are a globally significant portion of plant diversity.

The ants involved in myrmecochory are carnivorous or omnivorous species that generally prey upon terrestrial invertebrates (GÓMEZ & ESPADALER, 1995). These ants are attracted by the elaiosomes due to their overall chemical affinities with ant prey and also by sharing the same chemical cues that elicit removal behaviour in ants (e. g. HUGHES *ET AL.*, 1994). Most elaiosomes are particularly rich in lipids and evidence suggests that fatty acids are involved in ant attraction. Oleic acid is abundant in the lipid fractions of the elaiosomes of many species. This fatty acid, which is responsible for corpse–carrying behavior in some ants, probably induces ants to pick up diaspores (LANZA *ET AL.*, 1992).

The distance that the seed is moved depends on the distance of the source plant from the nest, and on the densities of food plants, and nests within habitat (COUSENS *ET AL.*, 2008). This distance varies with both ant species and environment (ANDERSEN, 1988; ANDERSEN & MORRISON, 1998). In a review of the World literature, GÓMEZ & ESPADALER (1998) found that the mean ant–dispersal distance was 0.96 m, with a range from 1 cm to 77 m. The ants scatter the diaspores a short distance away. Studies on the distribution of existing flora have demonstrated the remarkable productiveness of small repeated displacements of seeds. On the other hand, the review of cases of transference over large distances (due to water or air streams or migratory animals), has led to the affirmation that the effectiveness of this is all but illusory. There would, for example, be problems of acclimatization, contending with the pre–existing vegetation. It seems that dispersal over a limited distance, which assures a gradual diffusion, has been the course followed by the majority of plants in carpeting the Earth's surface, rather than transportation over large distances, which presents serious difficulties concerning environmental conditions. Dispersal over a short distance, therefore, in small stages, i. e. *propinquus*, without doubt prevails over *longinquus* dispersal, i. e. over an extensive area (CAPPELLETTI *ET AL.*, 1935).

The larger ant species disperse seeds further than do smaller ants. The mean and maximum distances that ants transported seeds increase with ant body length. The directly proportional relationship between the size of ants in terms of length and width of the body and the distance of dispersed seeds has been only recently recognized (e. g. NESS *ET AL.*, 2004) (***FIGURE 8***).

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TABLE 1 **– EXTANT AND EXTINCT SUBFAMILIES OF FORMICIDAE (ACCORDING TO BOLTON, 2014).** The number of taxonomic entities of the Formicidae family is continually subject to adjustments. The following numbers are updated at September 17, 2014 and are taken from ANTCAT/BOLTON. Extant: 16 subfamilies, 39 tribes, 324 genera, 12,984 species, 2,092 subspecies. Fossil: 4 subfamilies, 8 tribes, 146 genera, 688 species, 2 subspecies.

EUROPEAN FAMILIES	WORLD EXTANT	FOSSIL
	1. Agroecomyrmecinae	**17.** †Armaniinae
	2. Amblyoponinae	**18.** †Brownimeciinae
1. ▶	**3.** Aneuretinae	**19.** †Formiciinae
2. ▶	**4.** Dolichoderinae	**20.** †Sphecomyrminae
3. ▶	**5.** Dorylinae	
	6. Ectatomminae	
4. ▶	**7.** Formicinae	
	8. Heteroponerinae	
5. ▶	**9.** Leptanillinae	
	10. Martialinae	
6. ▶	**11.** Myrmeciinae	
	12. Myrmicinae	
	13. Paraponerinae	
7. ▶	**14.** Ponerinae	
	15. Proceratiinae	
	16. Pseudomyrmecinae	

***TABLE 2* – TAXONOMIC HIERARCHY OF FORMICIDAE (ACCORDING TO INTEGRATED TAXONOMIC INFORMATION SYSTEM, 2014 AND MITROIU *ET AL.*, 2014).** Ants are classified in a single family (Formicidae) in the Hymenoptera order. They are closely related to wasps and bees. LATREILLE (1809) erected the name Formicariae based on the type genus *Formica*. STEPHANS (1829) first spelled Formicidae. MAYR (1865) provided the name Formicinae as subfamily of Formicidae, while HEER (1867) emended the name Formicaria. ASHMEAD (1905) placed Formicidae under the Formicoidea superfamily. BROTHERS (1975) later on the Formicidae family was transferred to Vespoidea superfamily (PRESTY, 2014 and reference cited therein).

TAXONOMIC	HIERARCHY
– ***1.*** *Kingdom*	*Animalia*
— **2.** Subkingdom	Bilateria
—— **3.** Infrakingdom	Protostomia
——— **4.** Superphylum	Ecdysozoa
———— **5.** Phylum	Arthropoda
————— **6.** Subphylum	Hexapoda
—————— **7.** Class	Insecta
——————— **8.** Subclass	Pterygota
———————— **9.** Infraclass	Neoptera
————————— **10.** Superorder	Holometabola
—————————— **11.** Order	Hymenoptera
——————————— **12.** Suborder	Apocrita
———————————— **13.** Infraorder	Aculeata
————————————— **14.** Superfamily	Vespoidea
—————————————— **15.** Family	Formicidae
——————————————— **16.** Subfamily	Formicinae
———————————————— **17.** Tribe	Formicini
————————————————— **18.** Genus	*Formica* LINNAEUS, 1758
—————————————————— **19.** Subgenus	*Formica* LINNAEUS, 1758
——————————————————— **20.** Species	*Formica (Formica) rufa* LINNAEUS, 1761
———————————————————— **21.** Subspecies	*Formica (Formica) rufa rufa* LINNAEUS, 1761

***FIGURE 1* – WORKER ANT OF *FORMICA EXSECTOIDES* FOREL, 1886 (FORMICINAE: FORMICINI) IN DORSAL VIEW SHOW THE PRINCIPAL MORPHOLOGICAL FEATURE USED IN TAXONOMY.** The appearance of this species' is very striking: both its head and thorax are red–orange; its gaster is black–brown. Formicids are eusocial aculeates with a wingless worker caste forming perennial colonies. An antenna with 4–12 segments in female castes, male's posse's 9–13 segments. Antennae geniculate between the long basal segments (scape) and the remaining funicular segments. Second abdominal segment reduced, forming a node or scale, isolated from alitrunk in front and the remaining abdominal segment also reduced and isolated post petiole. Wings of alate queens deciduous shed after mating. Metapleural gland generally present on alitrunk, opening above the metacoxa (BOLTON, 1994). Scale bar in mm. (Original drawing © SMITH, 1965; modified redrawn © LI VIGNI, 2014)

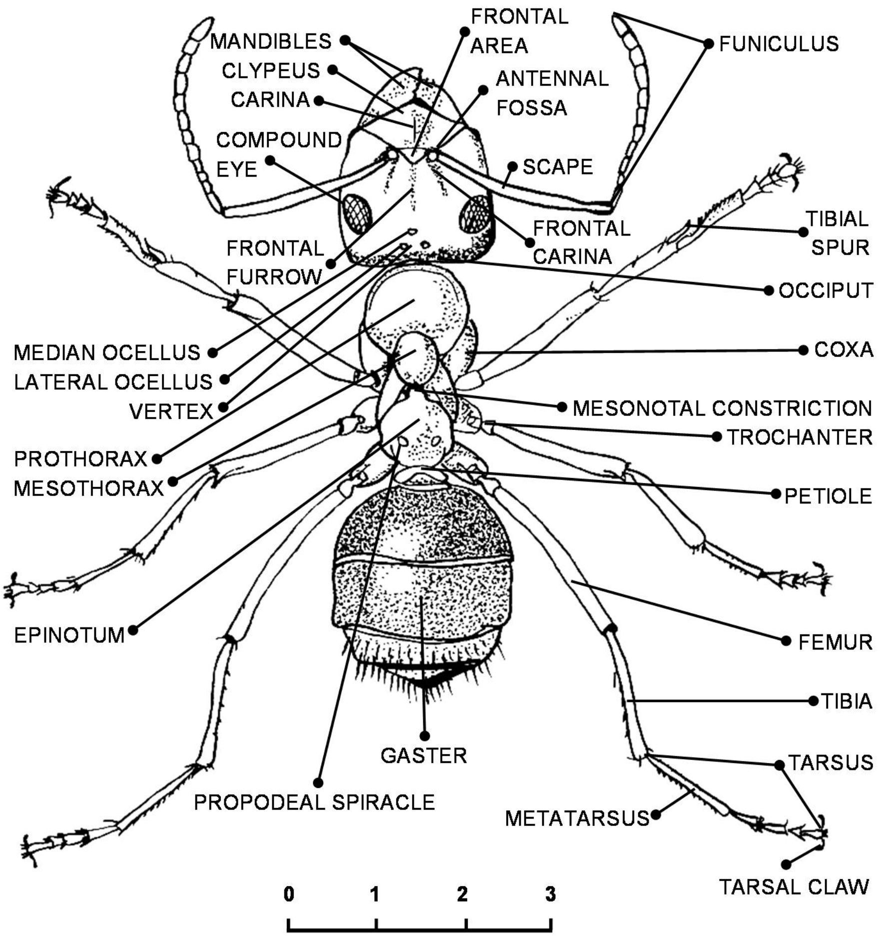


FIGURE 2 **– MORPHOLOGY OF A WORKER ANT (IN LATERAL VIEW).** Diagram showing the main morphology of a worker ant of the species *Pachycondyla verenae* (FOREL, 1922). Ant bodies are segmented into four sections, the head, the alitrunk or thorax, the petiole, and the gaster or metasoma. Ants are one of the most successful groups of insects. They are of particular interest because they are social insects and form highly organized colonies or nests which sometimes consist of millions of individuals. Ants are found on every continent except Antarctica. (Original drawing © RUIZ, 2006)

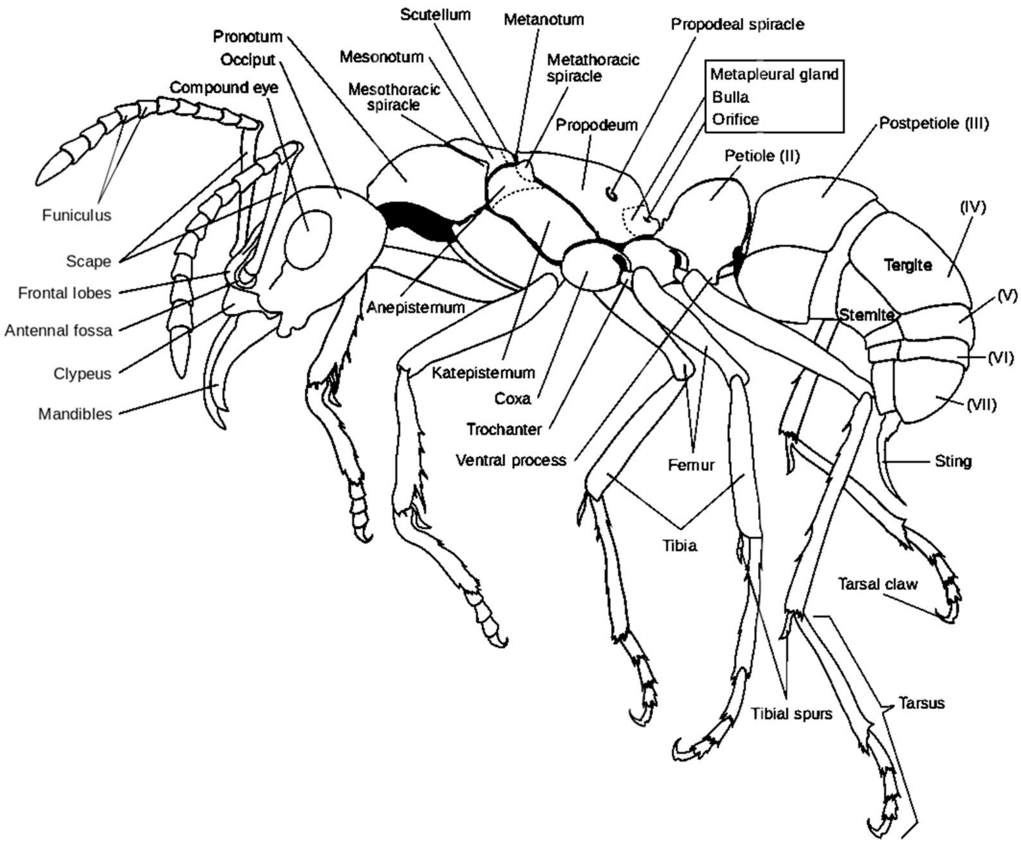


***FIGURE 3* – DIFFERENCE IN SIZE IN INTERSPECIFIC ☿ ANTS.** Head of *Myrmecia nigriscapa* ROGER, 1861 (Myrmeciinae: Myrmeciini) in full–face view bearing *Tapinoma melanocephalum* (FABRICIUS, 1793) (Dolichoderinae: Tapinomini) in dorsal view. Although two species don't naturally occur together, size differences of this magnitude can routinely be found among ants foraging on a plant, especially in tropical regions. Scale bar = 1 mm. (Original drawing © TURNBULL from BEATTIE, 1985)

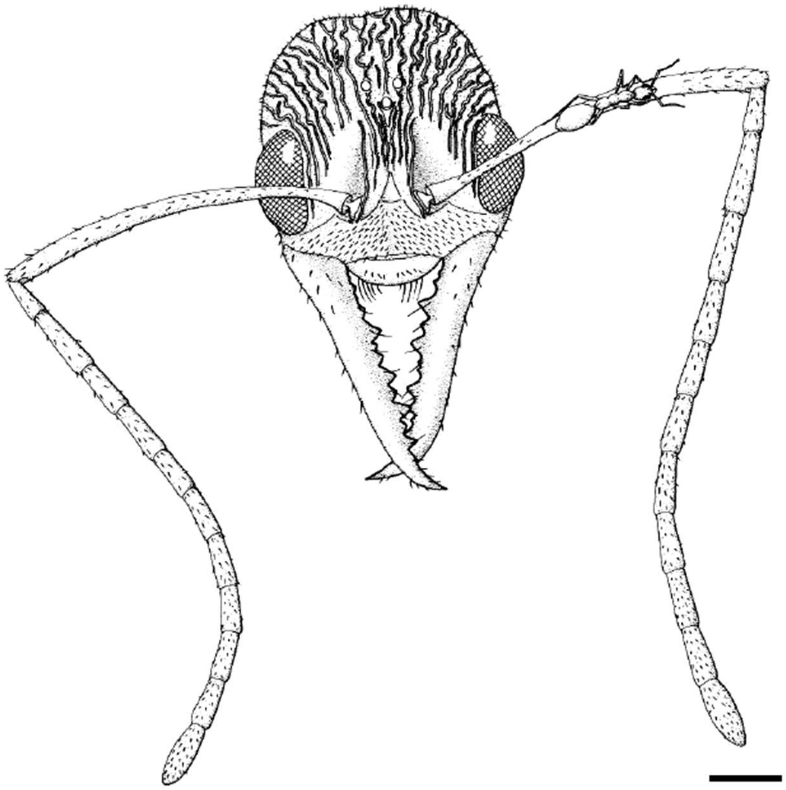


TABLE 3 **– TOTAL NUMBER OF LIVING PLANTS SPECIES IN THE WORLD (ACCORDING TO CHAPMAN, 2009).** The World's species encompass nearly four hundred thousand plants. Most plant species are flowering plants, classified as angiosperms.

TAXON	DESCRIBED/ACCEPTED	ESTIMATE
Bryophyta	16,236	22,750
Algae (Plant)	12,272	unknown
Ferns and allies	12,000	15,000
Gymnosperms	1,021	1,050
Magnoliophyta	268,600	352,000
TOTAL	**310,129**	**390,800**

***FIGURE 4* – SEED DEVELOPMENT.** Diagram of a generalized anatropous bitegmic dicotyledonous seed with perisperm: the testa is derived from the ovule outer integument and the inner integument of the ovule forms the tegmen. (Original drawing © RUDALL, 2007)

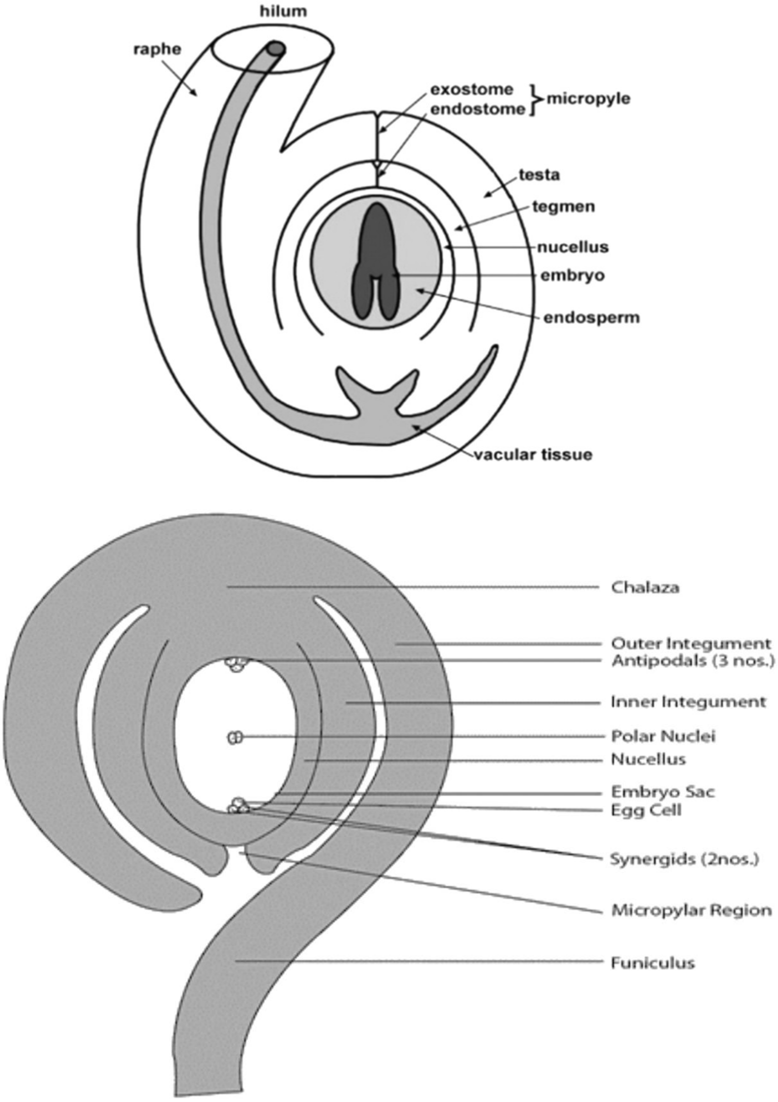


***FIGURE 5* – ARILLOIDS.** In many seeds dispersed by animals there is a hard testa with a conspicuous swollen fleshy addition (an arilloid). If this outgrowth is on the raphe it is specifically termed a strophiole, if next to the micropyle, a caruncle (especially if hard). More elaborate structures at the micropylar end of the seed are termed arillodes, which if detached can leave an additional scar referred to as a false hilum. A fleshy outgrowth of the funicle enveloping most of the seed is termed an aril (BELL, 1991). Below: seed (Lima bean) showing lateral, ventral, and dorsal views. (a) strophiole; (b) caruncle; (c) arillode; (d) aril (A: aril. Ar: arillode. C: caruncle. F: funicle. H: hilum. I: integument. M: micropyle. O: anatropous ovule. S: strophiole. T: testa). (Original drawing © BRYAN, from BELL, 1991)

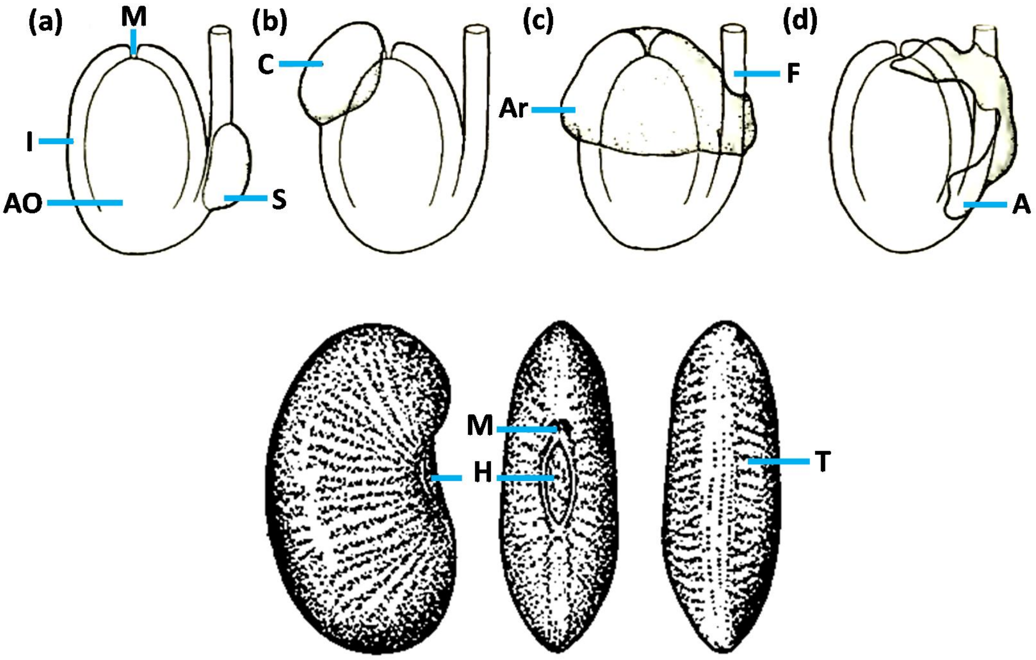
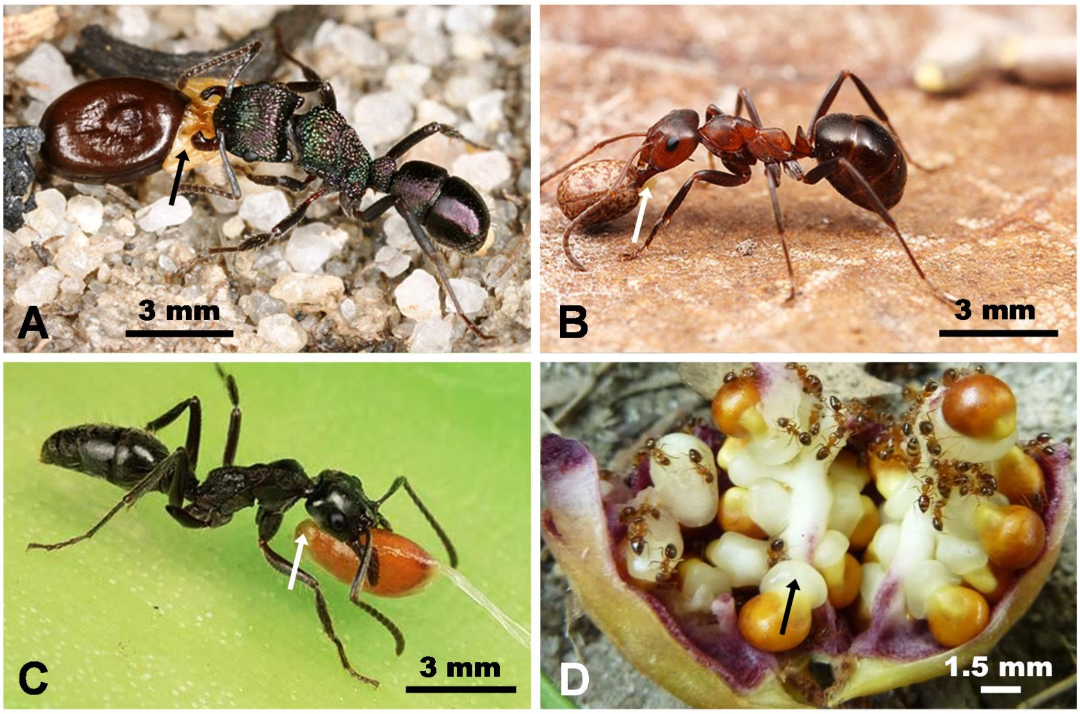


***FIGURE 6* – WORKER ANT *OF FORMICA RUFA* LINNAEUS, 1761 (FORMICINAE: FORMICINI) REMOVES AN ELAIOSOME–SEED OF *CORYDALIS CAVA* (L.) SCHWEIGG. & KÖRTE SUBSP. *CAVA* (PAPAVERACEAE: FUMARIOIDEAE).** *F. rufa* is a boreal member of the *Formica rufa* group (6 subspecies in Europe: *angusticeps*, *constricta*, *grouvellei*, *obscurata*, *rufa*, and *rufopratensoides*: MITROIU *ET AL.*, 2014) of ants and it is the type species for that group. It is native to Europe but it is also found in Northern America, in both coniferous, and broad–leaf broken woodland, and parkland. Workers are bicolored red and brownish–black, with a dorsal dark patch on the head and promesonotum, and are polymorphic, measuring 4.5–9.0 mm in length (LI VIGNI *ET AL.*, unpublished data). In Sweden SERNANDER (1906) during 19 hours observed *F. rufa* carrying 366 seeds and fruits whit elaiosomes. Assuming that these ants were at work on 80 favourable days in the year and were to work for twelve hours a day, the total number of seeds transported would be 36,480. Of course many if not most of these seeds would be carried to the nests, around which he often found the seeds thrown out with the elaiosomes bitten off, but many are left on the way, as is well shown by a diagrammatic figure of the occurrence of plants bearing myrmecochorous seeds in the vicinity of an ant hill (WEISS, 1908). (Photo © SEIFERT, 1996 see references)

***FIGURE 7* – ECOLOGICAL RELATIONSHIPS BETWEEN ANTS SIZE AND MYRMECOCHOROUS SEEDS SIZE. A** *Rhytidoponera metallica* (SMITH F., 1858) worker (TL 6.5–8.0: LI VIGNI *ET AL.*, unpublished data) transporting *Acacia neurophylla* W. FITZG. seed in sclerophyl forests of Western Australia. (Photo credit © GUENARD, 2007) **B** A *Formica exsectoides* FOREL, 1886 worker (TL 4.5–7.5: LI VIGNI *ET AL.*, unpublished data) transporting an *Euphorbia esula* L. seed in Atlantic areas of Northern America. (Photo credit © WILD, 2009) **C** *Pachycondyla goeldii* (FOREL, 1912) worker (TL 7.0–10.0: LI VIGNI *ET AL.*, unpublished data) transporting *Anthurium* sp. seed in tropical forests of Southern America. (Photo credit © MOFFETT, 1999) **A**, **B** and **C**: the moderate sizes of these ants allow conducting "large" seeds in their nests. **D**: workers of opportunistic ant *Nylanderia flavipes* (SMITH, 1874) (TL 1.5–1.7: LI VIGNI *ET AL.*, unpublished data) are stealing the elaiosome from seeds of *Trillium* sp. in hardwood forests of North–Eastern United States. The small sizes of these ants rarely allow conducting "large" seeds in their nest. (Photo credit © http://antsbeesbutterfliesnature.blogspot.it/, 2011). The arrows indicate the elaiosome.



Chapter 2

> "For millions of years, day and night, year after year, fruits and seeds
> have migrated across the globe, ensuring the survival of genera and species.
> And only in this way will future generations continue to do so. Plants which reproduce
> themselves with seeds are currently the most widespread on the planet."
>
> LI VIGNI & MELATI (1999)

(•)Myrmecochorous plants of Sicily (Southern Italy)

(•)This chapter was prepared for publication in *Annali di Botanica*.

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ABSTRACT

Myrmecochory is diaspore (i. e. seeds or seeds and fruits together + elaiosome) dispersal by ants. It is an ecologically significant ant–plant interaction with worldwide distribution, and a widespread and diverse mutualism between ants and flowering plants in which the elaiosome is provided to ants as a food reward. Myrmecochory is remarkably common among the Mediterranean species of Australia, Southern Africa, and Cape fynbos. Myrmecochory is considered to be very rare in the Californian chaparral and in the Mediterranean region proper.

The *status quaestionis* in Sicily (Southern Italy) and more extensively in Italy has shown that the topic of myrmecochory is still almost unexplored. We drew up the first list of the myrmecochorous plants of the Sicilian territory. We found 178 species of Angiospermae (135 Magnoliopsida and 43 Liliopsida) myrmecochores *sensu stricto*, among them 94 are confirmed myrmecochores (i. e. observed in the field) and 84 are potentially myrmecochores (i. e. bearing an elaiosome but never observed in the field).

KEYWORDS Myrmecochory. Elaiosome. Angiospermae. Ants. Mediterranean region. Sicily.

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ACKNOWLEDGEMENTS

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1. INTRODUCTION

Seed dispersal by ants, i. e. myrmecochory, although it is common in nature, is probably the least studied of the main seed dispersal syndromes (BRONSTEIN *ET AL.*, 2006). Biologists are just beginning to recognize the specialized mechanisms that make ants a major force in the spread of plants around the World. As the long list of known myrmecochores grows, biologists can expect to learn more about the importance of this seed–dispersal mechanism around the World. Further studies of the benefits of myrmecochory for plants and ants should also help to elucidate details of mutualistic relationships and their evolutionary consequences (HANDEL & BEATTIE, 1990). Despite of the over 1,500 publications on myrmecochory and despite the large number of myrmecochores existing worldwide (possibly up to 23,000 species or 8.5% of all Angiospermae: LENGYEL *ET AL.*, 2010), there is still a tremendous lack of information.
The elaiosome, working as alimentary bounty urges ants to gather the diaspores (i. e. seeds or seeds and fruits together + elaiosome) and to scatter them about along their way unharmed soon after removing the elaiosome; this is partial carpophagy of the diaspore with consequent indirect seed dispersal by ants (LI VIGNI & MELATI, 1999).

These edible external appendages are shown in thousands of plant species worldwide (HANZAWA *ET AL.*, 1988), they may vary, e. g. in their shape, coloration, size (TAHTADZHJAN, 1985) and are different in their origin, but they all consist of parenchymatous lipid–rich cells (KOMAR, 1978). These structures are found both in seeds (e. g. *Chelidonium majus* L., *Corydalis aurea* WILLD., *Viola odorata* L.) or in seeds and fruits together (e. g. *Borago officinalis* L., *Lamium amplexicaule* L., *Pulmonaria officinalis* L.) and appear in plants systematically far from each other, so that in these cases we can talk about convergent evolution in flowering plants (LI VIGNI & MELATI, 1999).

Seed dispersal by ants is usually considered as a mutualism: ants feed on elaiosomes before rejecting and dispersing seeds in their nest surroundings. Myrmecochory is remarkably common among the Mediterranean species of Australia, Southern Africa (BERG, 1975; DAVIDSON & MORTON, 1981; RICE & WESTOBY, 1981; BOND *ET AL.*, 1991), and Cape fynbos (MILEWSKI & BOND, 1982; BOND & SLMGSBY, 1983). It is considered to be very rare in the Californian chaparral (BERG, 1966; BULLOCK, 1989) and in the Mediterranean region proper (SERNANDER, 1906).

Both in forested regions and in more arid open areas, myrmecochory may play an important role; in particular many myrmecochores species can be found in the fynbos of the Cape Province in Southern Africa, sclerophyllous vegetation similar of the Mediterranean "maquis" (BEATTIE, 1985).

In the Mediterranean ecosystems of Southern Africa and Australia, the role of ants in the dispersal of seeds is well known and antagonistic interactions as well as myrmecochory occur. The high prevalence of seed dispersal by ants, compared to that by vertebrates, is viewed as evidence of adaptation to the soil nutrient deficiency typical of these environments. Instead, for a long time, ant dispersal has been considered unimportant in the Mediterranean basin and before 1994, no systematic study on ant–seed interaction had been undertaken. Only in the 1994 ARONNE & WILCOCK have realized a systematic study on the myrmecochory in the Mediterranean shrublands of Southern Italy (in the Nature Reserve at Castelvolturno, in the Bay of Naples). Since then, few other studies have been made in Italy, Spain, and France. This study aims to survey myrmecochorous plants of Sicily (Southern Italy).

2. MATERIALS AND METHODS

2.1. *Status quaestionis* in Italy

A comprehensive literature review was conducted on the research project and related topics, by consulting books and journals available at various Italian and foreign libraries, including through telematics services, as well as various international scientific databases available on line, such as: "antbase.org", "antcat.org", "antweb.org", "antwiki.org", "archive.org", "gap.entclub.org", and "hol.osu.edu". Overall, more than five hundred publications (between primary and secondary literature, both in digital and paper format) were consulted.

2.2. STUDY AREAS

This study has been realized in Sicily (Southern Italy, European–Mediterranean region), inside two different protected areas: the Oriented Nature Reserve (ONR) Bosco della

Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago and the Madonie Regional Natural Park (RNP).

The reserve territory of the ONR Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago includes five municipalities of the Province of Palermo: Corleone, Godrano, Marineo, Mezzojuso, and Monreale. This reserve, extended 7,397.49 ha, is the largest ONR of Southern Italy (FEDERICO, 2009). The altitude of the area ranges between 350 m ASL at Contrada Drago and 1,613 m at Rocca Busambra peak (GIANGUZZI *ET AL.*, 2004): a calcareous–dolomitic mountain that combined with Rocca Ramosa massif, makes up a massive ridge extending over 16 km from west to east and marks southern border of the reserve (***FIGURE 1***).

The Madonie RNP is a protected area of 39,679 ha, which includes the territory of fifteen municipalities of the Province of Palermo. The Natural Park is delimitated by the Tyrrhenian Sea on the North, the Pollina River on the East, by the Northern Imera River on the West and by the system of clayey hills of Gangi, Petralia, and Polizzi Valley on the South (ALAIMO, 1998) (***FIGURE 2***).

2.3. CLIMATIC ZONE

Climate is the characteristic condition of the atmosphere near the Earth's surface at a certain place on Earth. It is the long–term weather of an area (at least 30 years). Two of the most important factors determining an area's climate are air temperature and precipitation (REDDY, 2008).

According to RIVAS–MARTINEZ Worldwide Classification System (RMWBS) (RIVAS–MARTÍNEZ, 1981, 1996, 2007), the Euro–Mediterranean region is part of the Mediterranean macrobioclimate, in the Mediterranean Pluviseasonal Oceanic bioclimate (MPO), characterized by hot and dry summer and mild and rainy winters that denotes different thermotypes and ombrotypes according to the particular thermal characteristics and rainfall.

The average winter temperature in the Mediterranean region is +10 °C, the summer one +24 °C; these values increase from North to South and from West to East. The average annual precipitations is of 760 mm; under the geographical aspects, their distribution can be considered as opposite to that of temperature: decrease from North to South and from West to East so, as a great approximation, North and West area of the Mediterranean region are fresher and more humid areas while the Southern and Eastern areas are hotter and drier (FENAROLI, 1985). In the summer, the climate is influenced by the presence of high pressure (which determines the absence of rain) and in winter by low pressure systems, so it is characterized by clear days, clear sky, high temperatures and dry air during the warm months, variable sky, frequent rain and low temperatures, but myths, during the cold months. This association between dry summers and rainy winters is a true anomaly because normally in other climates, rainfall is more frequent in the summer.

The Mediterranean climate extends into five regions of our planet, located on the same latitudes, between about 30–45° both North and South from the equator: two in the Northern Hemisphere (Mediterranean basin and California), three in the Southern Hemisphere (central Chile, the Western Cape Province of Southern Africa, and parts of Western and Southern Australia), located on the Western sides, and South–West of the continents. The largest region with this climate is the Mediterranean basin with about 60% of the World's total Mediterranean climate area (South–Western and Southern Australia: 22%; California: 10%; Central Chile: 5%; Western Cape Province: 3%) (DI CASTRI *ET AL.*, 1981; DALLMAN, 1998) (***FIGURE 3***). Mediterranean climates and their unique ecosystems are only found on 5% of the land surface of the Earth and they have an outstanding floristic richness, with more than 20% of the plant species of the World (BRADSHAW *ET AL.*, 2011) and approximately 35% endemic species, thus representing one of the major centers of plant biodiversity on the planet (ROSSELLÓ, 2003).

The bioclimate of the Oriented Nature Reserve (ONR) Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago has been referred to as thermotypes: a) thermo–mediterranean (mean annual temperature [T] = 17 °C; mean maximum temperature of the coldest month [M] = 16 °C; mean minimum temperature of the coldest month [m] = 7 °C) lower subhumid ombrotype (mean annual precipitation [P] = 700 mm) – b) meso–mediterranean (T = 14.5 °C, M = 11.5 °C, m = 2 °C) from lower to upper (P = 900 mm) subhumid ombrotypes – c) supra–mediterranean (T = 10.5 °C, M = 6 °C, m = –2.5 °C) upper subhumid and lower humid (P ≥1000 mm) ombrotypes (GIANGUZZI *ET AL.*, 2004).

The bioclimate of the Madonie RNP thanks to its remarkable altitudinal range has a high climatic variability: thermotypes from thermo–mediterranean to supra–mediterranean and ombrotypes from dry to humid and subhumid (for values see above) (***FIGURE 4***).

2.4. ECOLOGY

World biomes are controlled by climate. The climate of a region will determine which plants will grow there, and which animals will inhabit it (REDDY, 2008). The climate, also, indirectly controls the communities via changes in interspecific interactions (STUBLE *ET AL.*, 2013). All three components, climate, plants and animals are interwoven to create the fabric of a biome.

The Mediterranean basin biome is represented in the cooler areas, from the hardwood forests of evergreen sclerophyllous. As soon as climate becomes warmer, the forest thin out and is replaced by “maquis”: wooded areas here and there, with wide open spaces of shrubs and herbaceous plants. Where most trees do not grow and the soil is calcareous, the maquis turns into a formation called “garrigue”, dominated by bushes.

The forests of the ONR Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago have a Mediterranean vegetation wich includes a large variety of plant types and lots of botanic endemisms; in 2004, GIANGUZZI, GIARDINA, and SCUDERI recorded 99 vegetal families with 1,173 specific and intraspecific entities (GIANGUZZI *ET AL.*, 2004). These mainly consist of extensive native forest formations of thermophilous evergreen oaks (*Quercus ilex* L. and *Q. suber* L.), deciduous oaks (*Q. amplifolia* GUSS., *Q. dalechampii* TEN., *Q. cerris* L., the endemism *Q. gussonei* (BORZÌ) BRULLO), riparian and rupicolous vegetation groups, shrub, bush, meadows, and non–native forest formations (woods of *Pinus pinea* L., *Eucalyptus camaldulensis* DEHNH., and *Castanea sativa* MILLER). There are also a number of other wild forest species such *Acer campestre* L., *A. pseudoplanatus* L., *Pyrus amygdaliformis* VILL., *Fraxinus ornus* L., *Malus sylvestris* MILLER. The shrubby vegetation is mainly represented by: *Crataegus monogyna* JACQ., *Ruscus aculeatus* L., *Asparagus acutifolius* L., *Rosa sempervirens* L., *Erica arborea* L.. Among the herbaceous species worthy of mention include: *Cyclamen repandum* SM., *Paeonia mascula* (L.) MILLER, *Iris pseudopumila* TINEO, the endemism *Viola tineorum* ERBEN

& RAIMONDO. The reserve is also home to a rich contingent of orchids including the endemic species such as: *Ophrys pallida* RAFIN. and *Ophrys lunulata* PARL.. Rocca Busambra is home to numerous rupestrian endemism as: *Centaurea busambarensis* GUSS., *Aster sorrentinii* (TOD.) LOJAC., *Armeria gussonei* BOISS., *Anthemis cupaniana* TOD. EX LOJAC., *Gagea busambarensis* (TIN.) PARL., and *Valantia deltoidea* BRULLO. At the foot of the Rocca Busambra there is also one of the few Sicilian stations of *Atropa belladonna* L. (GIANGUZZI *ET AL.*, 2004; FEDERICO, 2009). The reserve preserves a number of optimal habitats for many animal species, owing to the variety of ecological niches available, ranging from thick woods to open glades, from meadows to steppes and cliffs. Furthermore, the presence of small hill lakes and the nearby artificial lake of Scanzano offers shelter and protection to approximately 80% of the Sicilian wild fauna, particularly birds. There is no other place in Europe, covering the same surface area, with such a wide variety of animal species (ALAIMO, 2010).

The Madonie Mountains represent the richest area of Sicily in terms of vegetal biodiversity and number of endemic plants. Indeed on an area that is only the 1.56% of the entire surface of Sicily, there are about 1,500 taxa, the 57% of the entire regional flora and there are 170 endemic plants, the 46% of endemic plant present in the island (RAIMONDO *ET AL.*, 2004). This remarkable floristic richness is matched by an equally remarkable variety of vegetation, thanks to the presence of different aspects, including Mediterranean shrubland, evergreen forest, deciduous oak and beech forests. Beech forest, developing on calcareous ground, represents the climax forest vegetation. This cenosis is dominated by *Fagus sylvatica* L., while *Acer pseudoplatanus* L., and *A. campestre* L. are more sporadic. In the undergrowth *Ilex aquifolium* L. is frequent. The herbaceous layer includes several nemoral species such as *Allium ursinum* L. subsp. *ucrainicum* (KLEOPOW & OXNER), *Anemone apennina* L. subsp. *apennina*, *Cyclamen repandum* SM. subsp. *repandum*, *Cyclamen hederifolium* AITON subsp. *hederifolium*, *Gagea fragifera* (VILL.) EHR. BAYER & G. LÓPEZ, *Primula vulgaris* HUDS. subsp. *vulgaris*, *Scilla bifolia* L., and several endemic or rare plants, e. g. *Colchicum bivonae* GUSS., *Colchicum triphyllum* G. KUNZE, *Euphorbia myrsinites* L. subsp. *myrsinites*, *Gagea bohemica* (ZAUSCHN.) SCHULT. & SCHULT. F., *Knautia calycina* (C. PRESL) GUSS. and *Viola nebrodensis* C. PRESL, *Viola parvula* TINEO. However in the summit this forest is present only in small fragments with a tree layer that rarely reaches the height of a real tree, either due to soil and climatic reasons but mainly because of old logging and grazing. Another peculiar aspect of this area is the vegetation that occupied the bottom of the dolines, typical expression of karst in

Madonie Mountains.

2.5. SPECIES SELECTION

The list of the myrmecochorous plant species was established compiling three sources of data: 1. the plant species whose diaspores bear a nutritional appendage (i. e. an elaiosome) selected from the samples present in the Banca di Germoplasma del Mediterraneo, Monreale PA, Italy (out of a total of about 800 species retained in this gene bank in the form of seeds); 2. the plant species whose diaspores with elaiosome have been found near the anthills; 3. the plant species whose seeds have been observed transported by ants.

Life form refers to the vertical position of vegetative buds, classified following the RAUNKIÆR system.

2.6. SEM PHOTOS

Diaspores were observed and photographed with a SEM lSI 130 (Cambridge), operating at 20 kV. It was not necessary to use the standard critical point procedure; diaspores were observed with SEM after a 90–day drying period, cleaning with compressed air and metallization with palladium gold (Au Pd), carried out for 10 minutes using an electrical current of 15 milliamperes. Generally the elaiosome is: a caruncle (or micropylar aril: elaiosome originating in the region of micropyle of the ovule); a strophiole (or raphal aril: elaiosome originating along raphe–antiraphe of the ovule) or an aril (or true aril: elaiosome originating on a funicle of the ovule) (TAHTADZHJAN, 1985; LI VIGNI & MELATI, 1999; GORB & GORB, 2003) (***FIGURE 5***).

3. RESULTS AND DISCUSSION

A clear examination of the *status quaestionis* in Sicily and more extensively in Italy has shown that the topic of seed dispersal by ant is still almost unexplored. The research carried out in Italy on this important widespread phenomenon, key to many ecological systems both forest and agricultural, is very limited (about thirty in all) and relates to a small amount of bio–ecological information and equally to a small number of myrmecochorous species (***TABLE 1***). It appears, therefore, clear that much work remains to be done in a territory such as Italy that, despite its limited size, boasts of a great floristic richness, that puts it at the first place in Europe in absolute numbers of plant species. The number of vascular plant species currently censused (about 6,700: CONTI *ET AL.*, 2005) is, in fact, approximately half of the species estimated for the entire European continent. Only in Sicily there are more species than in the United Kingdom, the surface of which is approximately equivalent to that of Italy. The greatest plant diversity of Italy compared to countries of Central Europe and the Nordic is related to its low latitude in the European context and is explained by the known latitudinal gradient of species richness, according to which the biodiversity decreases with increasing latitude. On the other hand, if we compare the floristic richness of Italy with that of other countries in the Mediterranean, we see again that Italy shows the highest number of species per unit area: another supremacy linked in this case to the exceptional physical heterogeneity of the territory, which has generated an equally rich diversity of eco–systematic (BLASI *ET AL.*, 2005).

In Sicily 3,173 Angiospermae (2,463 Magnoliopsida and 710 Liliopsida) are present (RAIMONDO *ET AL.*, 2010). We found 178 specific and intraspecific taxa of Angiospermae both native and naturalized (135 Magnoliopsida and 43 Liliopsida) myrmecochores *sensu stricto*, among them 94 are confirmed myrmecochores – i. e. observed in the field – and 84 are potentially myrmecochores – i. e. bearing an elaiosome but never observed in the field – (this species list is arranged in alphabetical order; this list reports biologic forms and subforms and the chorotypes) (***FIGURE 6***):

ANGIOSPERMAE (MAGNOLIOPSIDA) (*n* = 2,463)

1. ***Acinos alpinus* subsp. *meridionalis* (NYMAN) P. W. BALL** Lamiaceae Ch suffr Orof. S–Europ.
2. ***Acinos alpinus* subsp. *nebrodensis* (KERNER & STROBL) C. BRULLO & BRULLO** Lamiaceae Ch suffr Orof. S–Europ.
3. ***Acinos minae* (LOJAC.) GIARDINA & RAIMONDO** Lamiaceae Ch suffr Endem. Sic.
4. ***Ajuga chamaepitys* (L.) SCHREB. subsp. *chamaepitys*** Lamiaceae T scap Eurimedit.
5. ***Ajuga chamaepitys* subsp. *chia* (SCHREB.) ARCANG.** Lamiaceae T scap Eurimedit.
6. ***Ajuga chamaepitys* subsp. *suffrutescens* (WILLK.) GREUTER & BURDET** Lamiaceae T scap Eurimedit.
7. ***Ajuga reptans* L.** Lamiaceae H rept Europ.–Caucas.
8. ***Ajuga tenorii* C. PRESL** Lamiaceae H ros Subendem.
9. ***Anchusa azurea* MILL.** Boraginaceae H scap Eurimedit.
10. ***Anchusa hybrida* TEN.** Boraginaceae H scap Stenomedit.

11. ***Anemone apennina*** **L.** Ranunculaceae G rhiz SE–Europ.
12. ***Anemone coronaria*** **L. var. *coronaria*** Ranunculaceae G bulb Stenomedit.
13. ***Anemone coronaria*** **var. *coccinea* (Jord.) Nyman** *Ranunculaceae* G bulb Stenomedit.
14. ***Anemone hortensis*** **L.** Ranunculaceae G bulb N–Eurimedit.
15. ***Anemone palmata*** **L.** Ranunculaceae G rhiz S–Stenomedit.
16. ***Aremonia agrimonoides*** **(L.) DC.** *Rosaceae* H ros NE–Stenomedit.
17. ***Borago officinalis*** **L.** Boraginaceae T scap Eurimedit.
18. ***Carduus nutans*** **L. subsp. *nutans*** Asteraceae H bienn Endem. Sic.
19. ***Carduus pycnocephalus*** **L. subsp. *pycnocephalus*** Asteraceae H bienn Eurimedit.–Turan.
20. ***Carduus pycnocephalus*** **subsp. *arabicus* (Murray) Nyman** Asteraceae H bienn Eurimedit.–Turan.
21. ***Carduus pycnocephalus*** **subsp. *intermedius* (Lojac.) Giardina & Raimondo** Asteraceae H bienn Eurimedit.?
22. ***Centaurea calcitrapa*** **L.** Asteraceae H bienn Eurimedit.
23. ***Centaurea jacea*** **L.** Asteraceae H scap Eurasiat.
24. ***Centaurea melitensis*** **L.** Asteraceae T scap S–Stenomedit.
25. ***Chelidonium majus*** **L.** Papaveraceae H scap Eurasiat.
26. ***Cirsium arvense*** **(L.) Scop.** Asteraceae G rad Eurasiat.
27. ***Cirsium vulgare*** **(Savi) Ten. subsp. *vulgare*** Asteraceae H bienn Paleotemp.
28. ***Cirsium vulgare*** **subsp. *crinitum* (DC.) Arènes** Asteraceae H bienn W–Medit.
29. ***Cirsium vulgare*** **subsp. *silvaticum* (Tausch) Dostál** Asteraceae H bienn Paleotemp.
30. ***Corydalis intermedia*** **(L.) Mérat** Papaveraceae G bulb C–Europ.
31. ***Corydalis densiflora*** **C. Presl** Papaveraceae G bulb Endem.
32. ***Cyclamen hederifolium*** **Aiton subsp. *hederifolium*** Primulaceae G bulb N–Stenomedit.
33. ***Cyclamen hederifolium*** **subsp. *confusum* (Grey–Wilson) Grey–Wilson** Primulaceae G bulb N–Stenomedit.
34. ***Cyclamen hederifolium*** **subsp. *hederifolium* var. *poli* (Delle Chiaje) Giardina & Raimondo** Primulaceae G bulb Endem.
35. ***Cyclamen repandum*** **Sm.** Primulaceae G bulb NW–Stenomedit.
36. ***Cynara cardunculus*** **L. subsp. *cardunculus*** Asteraceae H scap Stenomedit.
37. ***Cynara cardunculus*** **subsp. *flavescens* Wiklund** Asteraceae H scap W–Stenomedit.
38. ***Cynara cardunculus*** **subsp. *zingaroensis* (Raimondo & Domina) Raimondo & Domina Asteraceae** H scap Endem. Sic.
39. ***Cytisus scoparius*** **(L.) Link** Fabaceae P caesp Europ.
40. ***Euonymus europaeus*** **L.** Celastraceae P caesp Eurasiat.
41. ***Euphorbia akenocarpa*** **Guss.** Euphorbiaceae T scap SW–Stenomedit.
42. ***Euphorbia aleppica*** **L.** Euphorbiaceae T scap Eurimedit.–Turan.
43. ***Euphorbia biumbellata*** **Poir.** Euphorbiaceae Ch suffr W–Stenomedit.
44. ***Euphorbia bivonae*** **Steud.** Euphorbiaceae NP SW–Stenomedit.
45. ***Euphorbia ceratocarpa*** **Ten.** Euphorbiaceae Ch suffr Endem.
46. ***Euphorbia characias*** **L.** Euphorbiaceae NP Subendem.
47. ***Euphorbia corallioides*** **L.** Euphorbiaceae G rhiz Endem.
48. ***Euphorbia cuneifolia*** **Guss.** Euphorbiaceae T scap W–Stenomedit.
49. ***Euphorbia dendroides*** **L.** Euphorbiaceae NP Stenomedit.
50. ***Euphorbia exigua*** **L. var. *exigua*** Euphorbiaceae T scap Eurimedit.
51. ***Euphorbia exigua*** **var. *pycnophylla* K. U. Kramer & Westra** Euphorbiaceae T scap Endem. Sic.
52. ***Euphorbia exigua*** **var. *retusa* L.** Euphorbiaceae T scap Eurimedit.
53. ***Euphorbia falcata*** **L. var. *falcata*** Euphorbiaceae T scap Eurimedit.
54. ***Euphorbia falcata*** **var. *acuminata* (Lam.) St.–Amans** Euphorbiaceae T scap Eurimedit.

55. ***Euphorbia gasparrinii* Boiss.** Euphorbiaceae Ch suffr Endem. Sic.
56. ***Euphorbia helioscopia* L.** Euphorbiaceae T scap Cosmopol.
57. ***Euphorbia helioscopioides* Losc. & Pard.** Euphorbiaceae T scap Europa
58. ***Euphorbia heterophylla* L.** Euphorbiaceae T scap America
59. ***Euphorbia hirsuta* L.** Euphorbiaceae G rhiz Stenomedit.
60. ***Euphorbia lathyris* L.** Euphorbiaceae H bienn Eurimedit.–Turan.
61. ***Euphorbia linifolia* L.** Euphorbiaceae Ch suffr W–Stenomedit.
62. ***Euphorbia melapetala* Gasparr.** Euphorbiaceae NP Endem. Sic.
63. ***Euphorbia meuselii* Raimondo & Mazzola** Euphorbiaceae Ch suffr Endem.
64. ***Euphorbia myrsinites* L.** Euphorbiaceae Ch rept S–Europ.–Sudsib.
65. ***Euphorbia papillaris* (Boiss.) Raffaelli & Ricceri** Euphorbiaceae NP Endem. Sic.
66. ***Euphorbia paralias* L.** Euphorbiaceae Ch frut Eurimedit.
67. ***Euphorbia peploides* Gouan** Euphorbiaceae T scap Eurosib.
68. ***Euphorbia peplus* L.** Euphorbiaceae T scap Eurosib.
69. ***Euphorbia pithyusa* subsp. *cupanii* (Bertol.) A. R. Sm.** Euphorbiaceae Ch suffr W–Medit.
70. ***Euphorbia platyphyllos* L.** Euphorbiaceae T scap Eurimedit.
71. ***Euphorbia pterococca* Brot.** Euphorbiaceae T scap W–Stenomedit.
72. ***Euphorbia rigida* M. Bieb.** Euphorbiaceae Ch suffr S–Europ.–Sudsib.
73. ***Euphorbia segetalis* L.** Euphorbiaceae T scap W–Stenomedit.
74. ***Euphorbia serrata* L.** Euphorbiaceae G rhiz W–Stenomedit.
75. ***Euphorbia sulcata* Loisel.** Euphorbiaceae T scap W–Stenomedit.
76. ***Euphorbia terracina* L.** Euphorbiaceae T scap Stenomedit.
77. ***Fumaria capreolata* L. subsp. *capreolata*** Papaveraceae T scap Eurimedit.
78. ***Fumaria officinalis* var. *pycnantha* Loret & Barrandon** Papaveraceae T scap Paleotemp.
79. ***Fumaria parviflora* Lam.** Papaveraceae T scap Eurimedit.–Turan.
80. ***Glechoma hirsuta* Waldst. & Kit.** Lamiaceae H rept SE–Europ.
81. ***Helleborus bocconei* subsp. *intermedius* (Guss.) Greuter & Burdet** Ranunculaceae G rhiz Endem.
82. ***Knautia calycina* (C. Presl) Guss.** Dipsacaceae H scap Endem. Sic.
83. ***Knautia integrifolia* (L.) Bertol.** Dipsacaceae T scap Eurimedit.
84. ***Knautia timeroyi* subsp. *collina* (Schübler & G. Martens) Breistr.** Dipsacaceae H scap Eurasiat.
85. ***Lamium purpureum* L.** Lamiaceae T scap Eurasiat.
86. ***Lathraea squamaria* L.** Scrophulariaceae G rhiz Eurasiat.
87. ***Moehringia pentandra* J. Gay** Caryophyllaceae T scap Eurimedit.
88. ***Moehringia trinervia* (L.) Clairv.** Caryophyllaceae T scap Eurasiat.
89. ***Myrtus communis* L.** Myrtaceae P caesp Stenomedit.
90. ***Polygala monspeliaca* L.** Polygalaceae T scap Stenomedit.
91. ***Polygala preslii* Spreng.** Polygalaceae H scap Endem.
92. ***Portulaca cypria* Danin** Portulacaceae T scap Stenomedit.
93. ***Portulaca granulatostellulata* (Poelln.) Ricceri & Arrigoni** Portulacaceae T scap E–Europ., W–Asia
94. ***Portulaca nitida* (Danin & H. G. Baker) Ricceri & Arrigoni** Portulacaceae T scap Medit.
95. ***Portulaca oleracea* L. subsp. *oleracea*** Portulacaceae T scap Subcosmop.
96. ***Portulaca oleracea* subsp. *stellata* Danin & H. G. Baker** Portulacaceae T scap Regione Oloartica–Paleotrop. e Neotrop.
97. ***Portulaca papillatostellulata* (Danin & H. G. Baker) Danin** Portulacaceae T scap Medit.
98. ***Portulaca rausii* Danin** Portulacaceae T scap Stenomedit.
99. ***Portulaca sativa* Haw.** Portulacaceae T scap Subcosmop.

100. ***Portulaca sicula*** **DANIN, DOMINA & RAIMONDO** Portulacaceae T scap Stenomedit.
101. ***Portulaca trituberculata*** **DANIN, DOMINA & RAIMONDO** Portulacaceae T scap Stenomedit.
102. ***Portulaca zaffranii*** **DANIN** Portulacaceae T scap Stenomedit.
103. ***Primula acaulis*** **(L.) L.** Primulaceae H ros Europ.–Caucas.
104. ***Ranunculus ficaria*** **L. subsp.** ***ficaria*** Ranunculaceae G bulb Eurasiat.
105. ***Ranunculus ficaria*** **subsp.** ***bulbilifer*** **LAMBINON** Ranunculaceae G bulb
106. ***Ranunculus ficaria*** **subsp.** ***ficariiformis*** **(F. W. SCHULTZ) ROUY & FOUCAUD** Ranunculaceae G bulb
107. ***Ranunculus flammula*** **L.** Ranunculaceae H scap Eurasiat.
108. ***Ranunculus lanuginosus*** **var.** ***umbrosus*** **(TEN. & GUSS.) P. FOURN.** Ranunculaceae H scap Europ.
109. ***Reseda lutea*** **L. var.** ***lutea*** *Resedaceae* H scap Europ.
110. ***Reseda lutea*** **var.** ***mucronulata*** **(GUSS.) LOJAC.** Resedaceae H scap
111. ***Reseda luteola*** **L. var.** ***luteola*** Resedaceae H scap Eurasiat.
112. ***Reseda luteola*** **var.** ***gussonei*** **(BOISS. & REUT.) MÜLL.–ARG.** Resedaceae H scap
113. ***Rhamnus alaternus*** **L.** Rhamnaceae P caesp Eurimedit.
114. ***Rhamnus lojaconoi*** **RAIMONDO** Rhamnaceae P scap Endem. Sic.
115. ***Ricinus communis*** **L.** Euphorbiaceae P scap Tropical Region
116. ***Silybum marianum*** **(L.) GAERTN.** Asteraceae H bienn Eurimedit.–Turan.
117. ***Symphytum bulbosum*** **SCHIMP.** Boraginaceae G rhiz SE–Europ.
118. ***Symphytum gussonei*** **F. W. SCHULTZ** Boraginaceae G rhiz Endem. Sic.
119. ***Symphytum officinale*** **L.** Boraginaceae H scap Europ.–Caucas.
120. ***Thesium humile*** **VAHL** Santalaceae H scap Endem.
121. ***Veronica hederifolia*** **L. subsp.** ***hederifolia*** Scrophulariaceae T scap Eurasiat.
122. ***Veronica persica*** **POIR.** Scrophulariaceae T scap SW–Asia
123. ***Viola aetnensis*** **(DC.) STROBL subsp.** ***aetnensis*** Violaceae H scap Endem. Sic.
124. ***Viola aetnensis*** **subsp.** ***messanensis*** **(W. BEKER) MERXM. & LIPPERT** Violaceae H scap Endem.
125. ***Viola alba*** **subsp.** ***dehnhardtii*** **(TEN.) W. BECKER** Violaceae H ros Medit.
126. ***Viola arvensis*** **MURRAY** Violaceae T scap Eurasiat.
127. ***Viola hymettia*** **BOISS. & HELDR.** Violaceae T scap N–Eurimedit.
128. ***Viola kitaibeliana*** **SCHULT.** Violaceae T scap Europ.–Caucas.
129. ***Viola nebrodensis*** **C. PRESL** Violaceae H scap Endem. Sic.
130. ***Viola odorata*** **L.** Violaceae H ros Eurimedit.
131. ***Viola parvula*** **TINEO** Violaceae T scap W–Medit.–Mont.
132. ***Viola reichenbachiana*** **BOREAU** Violaceae H scap Eurosib.
133. ***Viola riviniana*** **RCHB.** Violaceae H scap Europ.
134. ***Viola tineorum*** **ERBEN & RAIMONDO** Violaceae H scap Endem. Sic.
135. ***Viola ucriana*** **ERBEN & RAIMONDO** Violaceae H scap Endem. Sic.

ANGIOSPERMAE (LILIOPSIDA) (*n* = 710)

136. ***Allium pendulinum*** **TEN.** Alliaceae G bulb W–Stenomedit.
137. ***Allium triquetrum*** **L.** Alliaceae G bulb W–Stenomedit.
138. ***Carex caryophyllea*** **LATOURR.** Cyperaceae H scap Eurasiat.
139. ***Carex flacca*** **subsp.** ***erythrostachys*** **(HOPPE) HOLUB** Cyperaceae G rhiz Europ.
140. ***Colchicum alpinum*** **subsp.** ***parvulum*** **(TEN.) NYMAN** Colchicaceae G bulb Endem.
141. ***Colchicum bivonae*** **GUSS.** Colchicaceae G bulb Subendem.
142. ***Colchicum cupanii*** **GUSS.** Colchicaceae G bulb Stenomedit.
143. ***Colchicum neapolitanum*** **(TEN.) TEN.** Colchicaceae G bulb W–Stenomedit.
144. ***Colchicum triphyllum*** **KUNZE** Colchicaceae G bulb Medit.

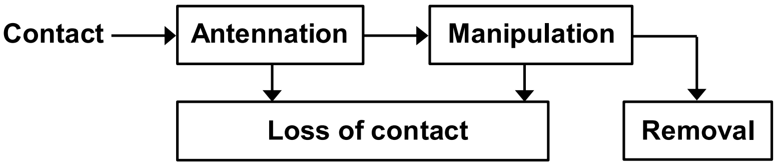
145. ***Crocus biflorus*** **Mill.** Iridaceae G bulb NE–Stenomedit.
146. ***Deschampsia cespitosa*** **(L.) P. Beauv.** Poaceae H caesp Subcosmop.
147. ***Gagea bohemica*** **(Zauschn.) Schult. & Schult. f.** Liliaceae G bulb Eurimedit.
148. ***Gagea chrysantha*** **(Jan) Schult. & Schult. f.** Liliaceae G bulb Endem.
149. ***Gagea dubia*** **A. Terrac.** Liliaceae G bulb Medit.
150. ***Gagea fragifera*** **(Vill.) E. Bayer & G. López González** Liliaceae G bulb Eurosib.
151. ***Gagea granatellii*** **(Parl.) Parl.** Liliaceae G bulb W–Medit.
152. ***Gagea lacaitae*** **A. Terracc.** Liliaceae G bulb W–Medit.
153. ***Gagea lojaconoi*** **Peruzzi** Liliaceae G bulb C–Medit.
154. ***Gagea lutea*** **(L.) Ker–Gawl.** Liliaceae G bulb Eurosib.
155. ***Gagea mauritanica*** **Coss.** Liliaceae G bulb SW–Stenomedit.
156. ***Gagea pratensis*** **(Pers.) Dumort.** Liliaceae G bulb C–Europ.
157. ***Gagea sicula*** **Lojac.** Liliaceae G bulb Endem.
158. ***Gagea trinervia*** **(Viv.) Greuter** Liliaceae G bulb C–Medit.
159. ***Gagea villosa*** **(M. Bieb.) Sweet** Liliaceae G bulb Eurasiat. Temper.
160. ***Galanthus nivalis*** **L.** Amaryllidaceae G bulb Europ.–Caucas.
161. ***Galanthus reginae–olgae*** **Orph.** subsp. ***reginae–olgae*** Amaryllidaceae G bulb E–Medit.
162. ***Galanthus reginae–olgae*** **subsp.** ***vernalis*** **G. Kamari** Amaryllidaceae G bulb E–Medit.
163. ***Holcus lanatus*** **L.** Poaceae H caesp Circumbor.
164. ***Leucojum autumnale*** **L.** Amaryllidaceae G bulb Stenomedit.
165. ***Luzula campestris*** **(L.) DC.** Juncaceae H caesp Europ.–Caucas.
166. ***Luzula forsteri*** **(Sm.) DC.** Juncaceae H caesp Eurimedit.
167. ***Luzula multiflora*** **(Retz.) Lej. subsp.** ***multiflora*** Juncaceae H caesp Circumbor.
168. ***Luzula multiflora*** **subsp.** ***congesta*** **(Thuill.) Arcang.** Juncaceae H caesp Circumbor.
169. ***Luzula sylvatica*** **subsp.** ***sicula*** **(Parl.) K. Richt.** Juncaceae H caesp SE–Europ.
170. ***Luzula sylvatica*** **subsp.** ***sieberi*** **(Tausch) K. Richt.** Juncaceae H caesp Orof. S–Europ.
171. ***Melica ciliata*** **L. subsp.** ***ciliata*** Poaceae H caesp Eurimedit.
172. ***Melica ciliata*** **subsp.** ***magnolii*** **(Godr.) K. Richt.** Poaceae H caesp Stenomedit.–Turan.
173. ***Melica cupanii*** **Guss.** Poaceae H caesp Eurimedit.–Turan.
174. ***Melica minuta*** **L.** Poaceae H caesp Stenomedit.
175. ***Melica nebrodensis*** **Parl.** Poaceae H caesp Endem. Sic.
176. ***Melica uniflora*** **Retz.** Poaceae H caesp Paleotemp.
177. ***Nectaroscordum siculum*** **(Ucria) Lindl.** Alliaceae G bulb Endem.
178. ***Scilla bifolia*** **L.** Hyacinthaceae G bulb Europ.–Caucas.

The myrmecochorous species recognized in this study account for approximately 6% of the total number of Angiospermae (*n* = 3,173) present in Sicily. Over 70% of myrmecochores are reported in this study for the first time.

Fifteen taxa are endemisms of Sicily (*Acinos minae*, *Carduus nutans* subsp. *nutans*, *Cynara cardunculus* subsp. *zingaroensis*, *Euphorbia exigua* var. *pycnophylla*, *Euphorbia gasparrinii*, *Euphorbia melapetala*, *Euphorbia papillaris*, *Knautia calycina*, *Melica nebrodensis*, *Rhamnus lojaconoi*, *Symphytum gussonei*, *Viola aetnensis*, *Viola nebrodensis*, *Viola tineorum*, and *Viola ucriana*, *Melica nebrodensis*).

These plant species are mainly herbaceous; the families most represented are the family of Euphorbiaceae (37 species) followed by the family of Asteraceae (15 species), the family of Liliaceae (13 species), the family of Portulacaceae and Ranunculaceae (each of 11 species) (***FIGURE 7***, ***8***, and ***9***).

For each diaspore contacted by ants, we quantified the following three types of behavioural parameters: a) ANTENNATION: the ant merely touched the diaspore with antennas not close to elaiosome, and then continued to explore the foraging area – b) MANIPULATION: the ant contacted the diaspore with antennas from elaiosome, held it between mandibles, bit it and then released it, without any removal – c) REMOVAL: after having antennated and manipulated the diaspore from elaiosome, the ant took it away. These behavioural parameters are summarized in the following flow chart (SERVIGNE & DETRAIN, 2008) (***FIGURE 10*** and ***11***).



Seed dispersal by ants is often studied empirically (CANNER, 2010) because it is a phenomenon difficult to observe in strictly natural conditions. The most effective method is to collect the seeds of a myrmecochorous plants, place them near the entrance of an ants nest, and wait that the workers will find them. This method involves the intervention of the observer and not a true natural observation (BLATRIX *ET AL.*, 2013).

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***FIGURE 1* – STUDY AREA 1. A**, **B**, and **C** Satellite images at different altitudes: **A** European–Mediterranean region (3,414.78 km); **B** Sicily, island of Southern Italy, in the Mediterranean Sea (300.60 km); **C** Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago (14.63 km). Date of acquisition of images: **A** and **B** = 04/10/2013; **C** = 07/29/2013. **D** Map of the ONR. (Images © **A**, **B**, and **C** Landsat US Dept of State Geographer – Google Earth, 2014; **D** Internet source www.altobelicecorleonese.com, 2008)

***FIGURE 2* – STUDY AREA 2.** Satellite images at different altitudes: **A** European–Mediterranean region (3,414.78 km); **B** Sicily, island of Southern Italy, in the Mediterranean Sea (300.60 km); **C** Madonie Regional Natural Park (54.96 km). Date of acquisition of images: **A** and **B** = 04/10/2013; **C** = 07/15/2011. **D** Map of Madonie RNP. (Images © **A**, **B**, and **C** Landsat US Dept of State Geographer – Google Earth, 2014; **D** Internet source www.linksicilia.it, 2013)

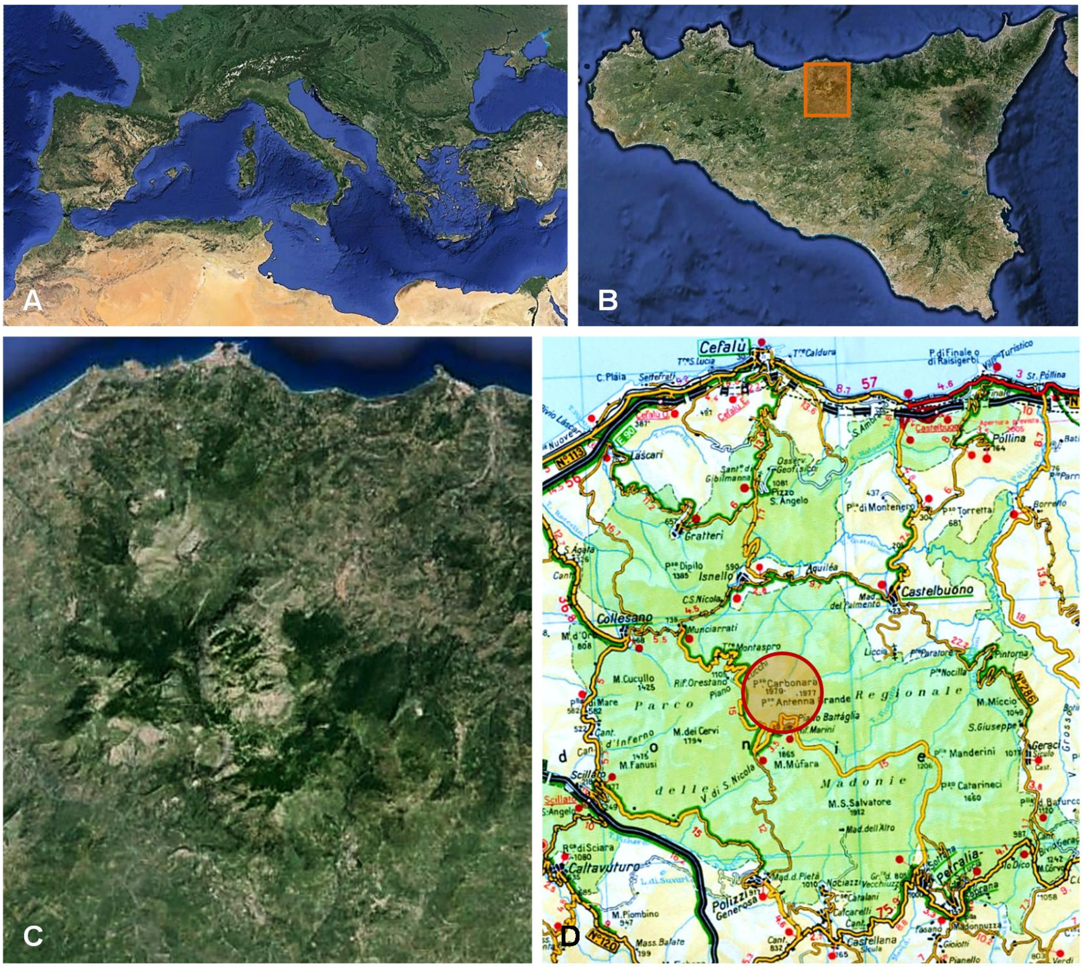

FIGURE 3 – MEDITERRANEAN CLIMATE REGIONS OF THE WORLD. In Mediterranean basin the biome is known as maquis; in Chile matorral; in South Africa fynbos; in California matorral and in Australia mallee. From: *Plant Life in the World's Mediterranean Climates* by DALLMAN, 1998 (adapted from: DI CASTRI *ET AL.*, 1981). © San Luis Obispo Botanical Garden, California (2012). Internet source image: www.slobg.org/mediterranean–climate (2014).

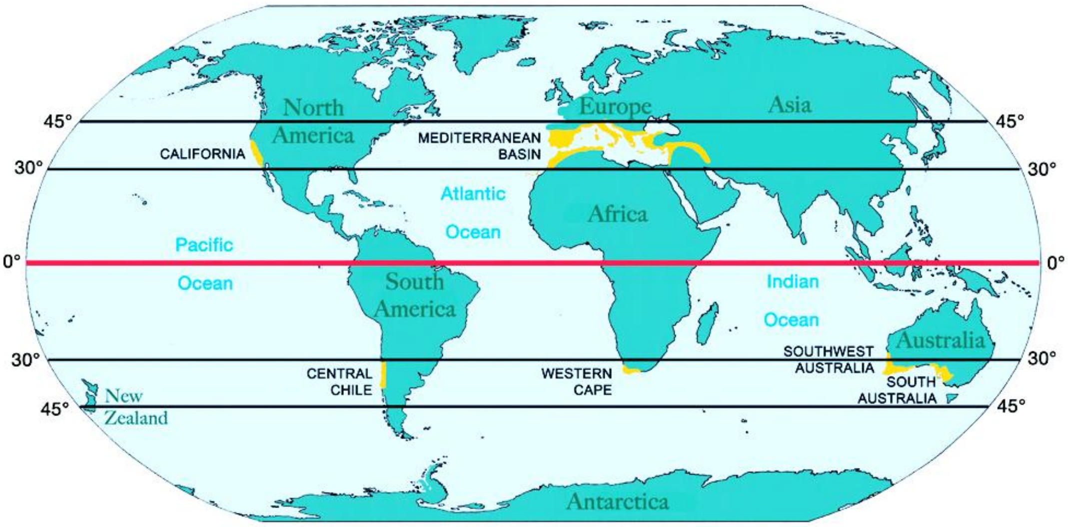

FIGURE 4 – MEDITERRANEAN CLIMATE LIMITS AND CLIMATE ZONES IN THE MEDITERRANEAN BASIN. Isolines of the mean minimum temperature for the coldest month (m = 7, 3, 0, –3 and –7 °C), which define the following climatic environments (including typical/potential vegetation). Areas with m < –7 ºC (oro–mediterranean, m = < –7; dwarf–shrubs) are small areas at the tops of mountains (not shown). From: Quézel & Médail (2003).

🟥 Infra–mediterranean.
🟧 Thermo–mediterranean: shrublands.
🟩 Meso mediterranean: evergreen oak forests.
🟨 Supra–mediterranean: winter semi–deciduous forests.
⬛ Mountain–mediterranean: conifer forest.

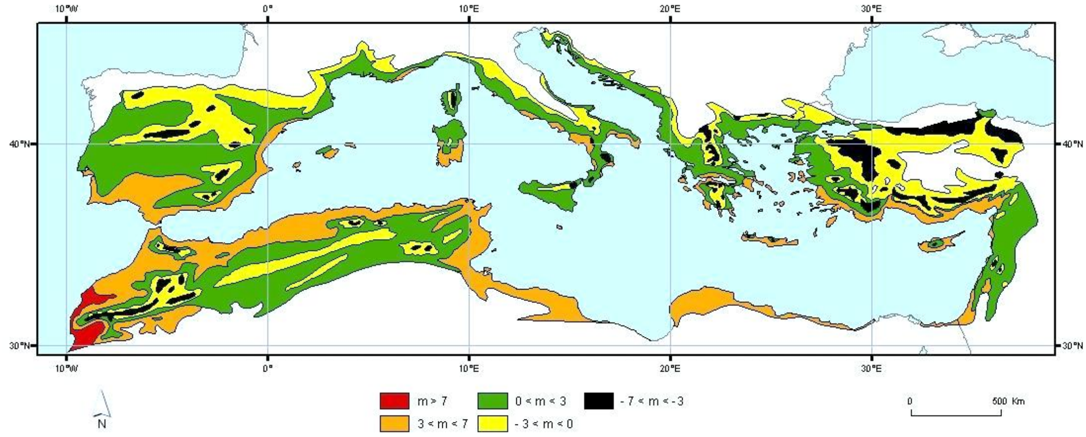

***FIGURE 5* – ELAIOSOMES.** 1 – Seed with strophiole of *Chelidonium majus* L. 2 – Seed with small caruncle of *Portulaca oleracea* L. 3 – Seed with small caruncle of *Portulaca grandiflora* Hook. 3a – Small caruncle in proximity of micropyle of the the seed of *P. grandiflora* Hook. 4 – Seed with caruncle of *Viola odorata* L. 5 – Fruit with elaiosome of *Borago officinalis* L. 6 – Seed elaiosomic coating of *Cyclamen repandum* Sm. subsp. *repandum*. 6a – Small elaiosomes of the seed of *Cyclamen repandum* Sm. subsp. *repandum*. (Photo © Li Vigni, 2014)

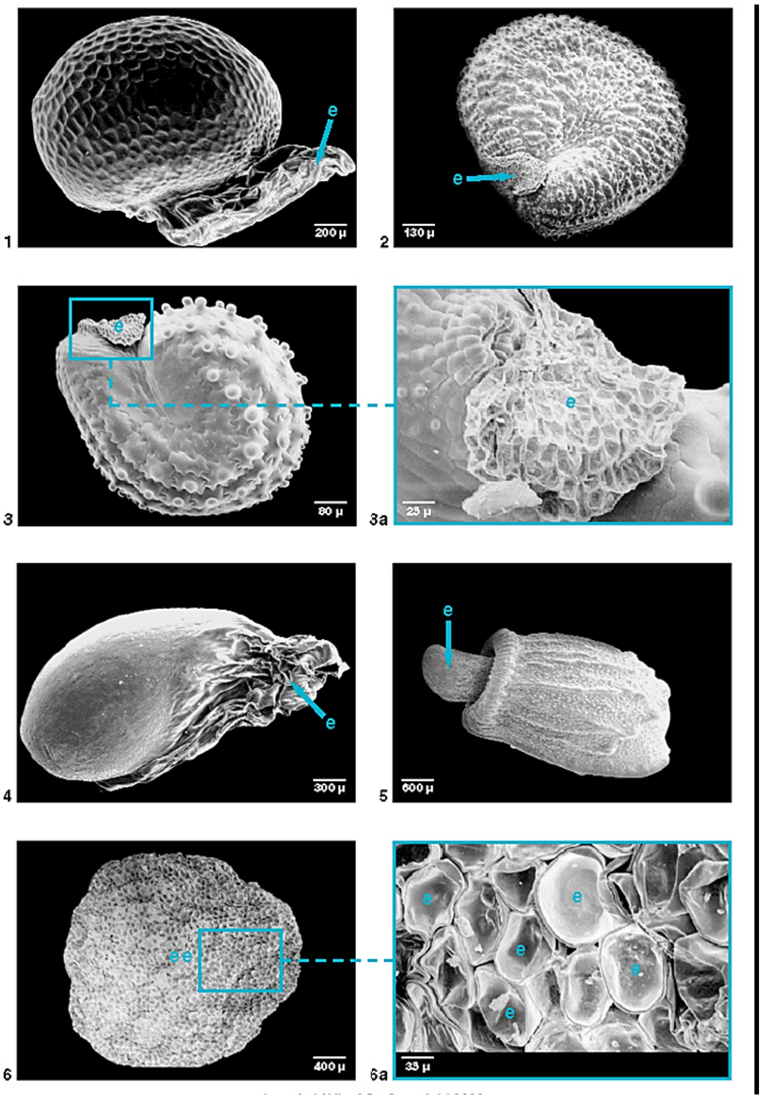

TABLE 1 – STATUS QUAESTIONIS OF ITALIAN RESEARCH ON MYRMECOCHORY.

Most of the research on myrmecochory made in the Italian territory is presented, in chronological order according to the dates of publication.

	AUTOR/S	PUBLICATION YEAR	TITLE
1.	**Calestani V.**	1933	Note sulla disseminazione delle piante. I: l'azione delle formiche del gen. *Messor.* *Bollettino della Società Veneziana di Storia Naturale, Venezia, 1 (2–3), 27–40.*
2.	**Li Vigni I.**	1986	Biologia della disseminazione mirmecocòra. *Premio di Merito al: 18° Concorso Philips per i Giovani Ricercatori Europei 1985/86, Milano, 1–50.*
3.	**Pacini E., & Grieco L.**	1989	Is the caruncle the only part oif the seed perceived by ants? *Giornale Botanico Italiano, 123, 162–162.*
4.	**Baroni–Urbani C., & Nielsen M. G.**	1990	Energetics and foraging behaviour of the European seed harvesting ant *Messor capitatus*: II. Do ants optimize their harvesting? *Physiological Entomology, 15 (4), 449–461.*
5.	**Pacini E.**	1990	*Mercurialis annua* L. (Euphorbiaceae) seed interactions with the ant *Messor structor* (Latr.), Hymenoptera: Formicidae. *Acta Botanica Neerlandica, 39 (3), 253–262.*
6.	**Urbani C. B.**	1991	Evolutionary aspects of foraging efficiency and niche shift in two sympatric seed–harvesting ants (*Messor*) (Hymenoptera Formicidae). *Ethology Ecology & Evolution, 3 (1), 75–79.*
7.	**Urbani C. B.**	1992	Factors affecting seed preference among Old World harvester ants of the genus *Messor*. *Ethology Ecology & Evolution, 4 (1), 73–80.*
8.	**Bianchini M., & Pacini E.**	1993	Form and function of elaiosome castor oil plant (*Ricinus communis* L.). *Giornale Botanico Italiano, 127, 618–618.*
9.	**Aronne G., & Wilcock C. C.**	1994	First evidence of myrmecochory in fleshy–fruited shrubs of the Mediterranean region. *New Phytologist, 127 (4), 781–788.*
10.	**Bianchini M., Lisci M., & Pacini E.**	1994	What are the functions of the elaiosome? *Plant Biosystem, 128 (1), 221–221.*
11.	**Aronne G., & Wilcock C. C.**	1995	Reproductive lability in pre–dispersal biology of *Rhamnus alaternus* L. (Rhamnaceae). *Protoplasma, 187 (1–4), 49–59.*
12.	**Bianchini M., & Pacini E.**	1995	The stage of dehydration of seeds of *Ricinus communis* L.

			Giornale Botanico Italiano, 129 (2), 16–16.
13.	**Bianchini M., & Pacini E.**	1996	The caruncle of *Ricinus communis* L. (castor bean): its development and role in seed dehydration, rehydration, and germination. *International Journal of Plant Sciences, 157, 40–48.*
14.	**Li Vigni I., & Melati M. R.**	1996	Policoria ed interazioni ecologiche in *Ricinus communis* L. *Atti della Accademia di Scienze Lettere e Arti di Palermo, 5/16 (1), 113–184.*
15.	**Li Vigni I., Melati M. R., & Vitale M. C.**	1996	Analisi comparativa cito–morfologica dei semi con elaiosoma di *Portulaca* sp. osservati al SEM. *Plant Biosystems, 130 (1), 402–402.*
16.	**Lisci M., Bianchini M., & Pacini E.**	1996	Structure and function of the elaiosome in some angiosperm species. *Flora – Morphologie, Geobotanik, Oekophysiologie, 191, 131–141.*
17.	**Li Vigni I., Melati M. R., Vitale M. C., & Trapani S.**	1997	Elaiosomi ed inquinanti. *92° Congresso della Società Botanica Italiana, Riassunti, Cagliari, 112–112.*
18.	**Lisci M. & Pacini E.**	1997	Fruit and seed structural characteristics and seed dispersal in *Mercurialis annua* L. (Euphorbiaceae). *Acta Societatis Botanicorum Poloniae, 66 (3–4), 379–386.*
19.	**Li Vigni I., & Melati M. R.**	1999	Examples of seed dispersal by entomochory. *Acta Botanica Gallica, 146 (2), 145–156.*
20.	**Li Vigni I., Paternostro B., & Giusquiano V.**	1999	Le strategie evolutive della dispersione mirmecocòra in *Primula acaulis* (L.) Hill (= *P. vulgaris* Huds.). *94° Congresso della Società Botanica Italiana, Riassunti, Ferrara, 122–122.*
21.	**Li Vigni I., Paternostro B., & Giusquiano V.**	2001	Myrmecochorous plants in Mediterranean region and their dispersal by ants. *X OPTIMA Meeting, Abstracts, Palermo, 199–199.*
22.	**Li Vigni I.**	2002	La dispersione dei semi di *Euphorbia characias* L. (Euphorbiaceae). *97° Congresso della Società Botanica Italiana, Riassunti, Lecce, 163–163.*
23.	**Viegi L., Vangelisti R., & Pacini E.**	2003	The achene pappi and elaisomes of *Centaurea* L.: dispersal and germination in some Italian species. *Israel Journal of Plant Sciences, 51 (1), 45–54.*

24.	**Li Vigni I.**	2004	Seed dispersal by ants in Mediterranean region. *XIV Congresso della Società Italiana di Ecologia, Siena, 35–35.*
25.	**Ciccarelli D., Andreucci A. C., Pagni A. M., & Garbari F.**	2004	The role of the elaiosome in the germination of seeds of *Myrtus communis* L. (Myrtaceae). *Atti della Società Toscana di Scienze Naturali, Memorie. B, 111, 143–146.*
26.	**Ciccarelli D., Andreucci A. C., Pagni A. M., & Garbari F.**	2005	Structure and development of the elaiosome in *Myrtus communis* L. (Myrtaceae) seeds. *Flora–Morphology, Distribution, Functional Ecology of Plants, 200 (4), 326–331.*
27.	**Guarino R., Ferrario B., & Mossa L.**	2005	A stochastic model of seed dispersal pattern to assess seed predation by ants in annual dry grasslands. *Plant Ecology, 178 (2), 225–235.*
28.	**Li Vigni I., & Paternostro B.**	2005	La dispersione dei semi da parte delle formiche negli ecosistemi mediterranei erbacei ed arbustivi della Sicilia (Italia). *Informatore Botanico Italiano, 37 (1A), 222–223.*
29.	**Casazza G., Borghesi B., Roccotiello E., & Minuto L.**	2008	Dispersal mechanisms in some representatives of the genus *Moehringia* L. (Caryophyllaceae). *Acta Oecologica, 33 (2), 246–252.*
30.	**Pacini E., Viegi L., & Franchi G. G.**	2008	Types, evolution and significance of plant–animal interactions. *Rendiconti Lincei, 19 (1), 75–101.*
31.	**Minuto L., Roccotiello E., & Casazza G.**	2011	New seed morphological features in *Moehringia* L. (Caryophyllaceae) and their taxonomic and ecological significance. *Plant Biosystems, 145 (1), 60–67.*

***FIGURE 6* – MYRMECOCHOROUS PLANTS OF SICILY.** (Photos © Li Vigni, 2014)

***Acinos alpinus* subsp. *meridionalis* (Nyman) P. W. Ball**

Ajuga chamaepitys* (L.) Schreb. subsp. *chamaepitys

***Ajuga tenorii* C. Presl**

***Allium pendulinum* Ten.**

***Allium triquetrum* L.**

***Anchusa azurea* Mill.**

Anemone coronaria* L. var. *coronaria

***Anemone hortensis* L.**

***Anemone palmata* L.**

***Aremonia agrimonoides* (L.) DC.**

***Borago officinalis* L.**

Carduus nutans* L. subsp. *nutans

Carduus pycnocephalus **L. subsp.** ***pycnocephalus***

Centaurea calcitrapa **L.**

Centaurea jacea **L.**

Chelidonium majus **L.**

Cirsium vulgare **(SAVI) TEN. subsp.** ***vulgare***

Colchicum bivonae **GUSS.**

Colchicum cupanii **GUSS.**

Corydalis densiflora **C. PRESL**

Crocus biflorus **MILL.**

Cyclamen hederifolium **AITON subsp.** ***Hederifolium***

Cyclamen repandum **SM.**

Cynara cardunculus **L. subsp.** ***cardunculus***

Cytisus scoparius **(L.) LINK**

Euonymus europaeus **L.**

Euphorbia bivonae **STEUD.**

Euphorbia ceratocarpa **TEN.**

Euphorbia characias **L.**

Euphorbia corallioides **L.**

Euphorbia dendroides L.

Euphorbia exigua L. var. *exigua*

Euphorbia helioscopia L.

Euphorbia myrsinites L.

Euphorbia rigida M. BIEB.

Euphorbia terracina L.

Fumaria officinalis var. *pycnantha* LORET & BARRANDON

Gagea fragifera (VILL.) E. BAYER & G. LÓPEZ GONZÁLEZ

Galanthus reginae–olgae ORPH. subsp. *reginae–olgae*

Helleborus bocconei subsp. *intermedius* (GUSS.) GR. & BURD.

Lathraea squamaria L.

Leucojum autumnale L.

Luzula campestris **(L.) DC.**

Moehringia trinervia **(L.) CLAIRV.**

Myrtus communis **L.**

Nectaroscordum siculum **(UCRIA) LINDL.**

Polygala preslii **SPRENG.**

Portulaca grandiflora **HOOK.**

Portulaca oleracea* L. subsp. *oleracea

***Primula acaulis* L.**

Ranunculus ficaria* L. subsp. *ficaria

***Rhamnus alaternus* L.**

***Ricinus communis* L.**

***Scilla bifolia* L.**

Silybum marianum **(L.) GAERTN.**

Symphytum gussonei **F. W. SCHULTZ**

Thesium humile **VAHL**

Viola aetnensis **(DC.) STROBL subsp. *aetnensis***

Viola tineorum **ERBEN & RAIMONDO**

Viola ucriana **ERBEN & RAIMONDO**

***FIGURE 7* – PREDOMINANT BIOLOGICAL FORMS.**

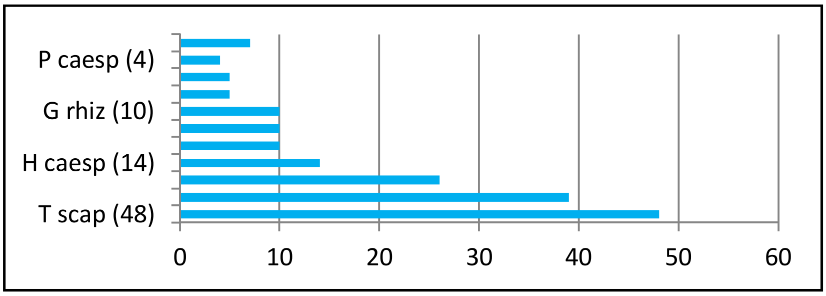

***FIGURE 8* – RAUNKIÆR SYSTEM OF LIFE FORMS.** **Phanerophyte** (1): any woody plant that carries its dormant buds openly on branches above the ground. **Chamaephytes** (2–3): any low perennial plant whose buds overwinter just above soil level. **Hemicryptophyte** (4): any plant whose buds are situated on herbaceous shoots on the soil surface, protected by foliage or dead leaves. **Geophytes** (cryptophytes) (5–6): resting buds are subterranean, often on storing organs protected within the soil. **Therophyte** (not represented): summer annuals, which can only reproduce by means of generative diaspores. **Hydrophyte** (not represented): resting buds are situated under water on the bed or in the mud.

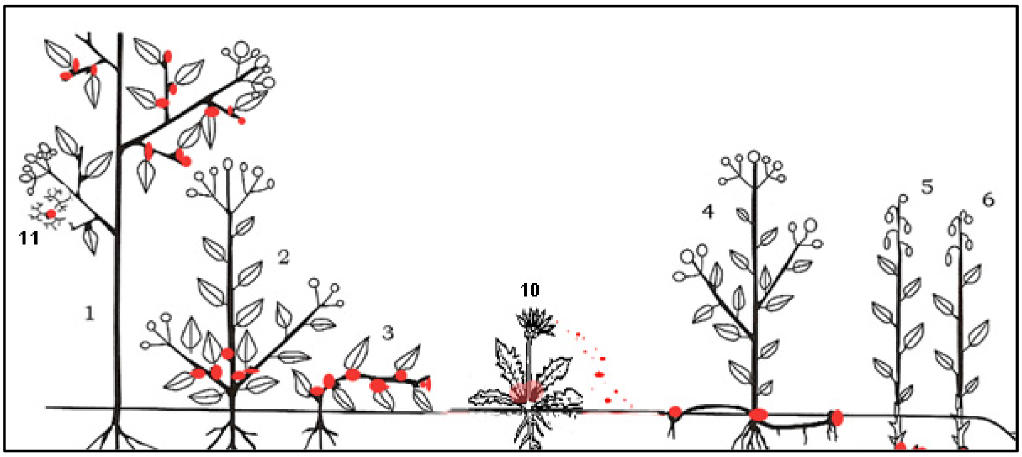

***FIGURE 9* – PREDOMINANT FAMILIES.**

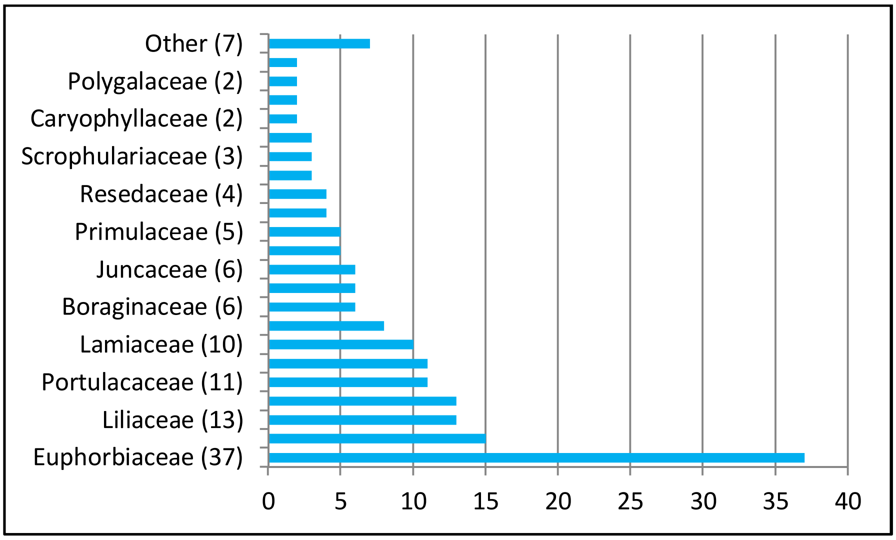

FIGURE 10 – SEQUENCE OF BEHAVIOURS. Arrows indicate elaiosomes. Worker ants = *Aphaenogaster semipolita* (NYLANDER, 1856) (Myrmicinae: Pheidolini) TL (n = 68) 5.52–7.48 (6.53) mm. Seeds with white elaiosomes = *Ricinus communis* L. (Euphorbiaceae) TL (n = 100) 11.03–14.08 (13.14) mm. Study site: margins of the Ficuzza Wood (Western Sicily) (Photos © LI VIGNI, 2014)

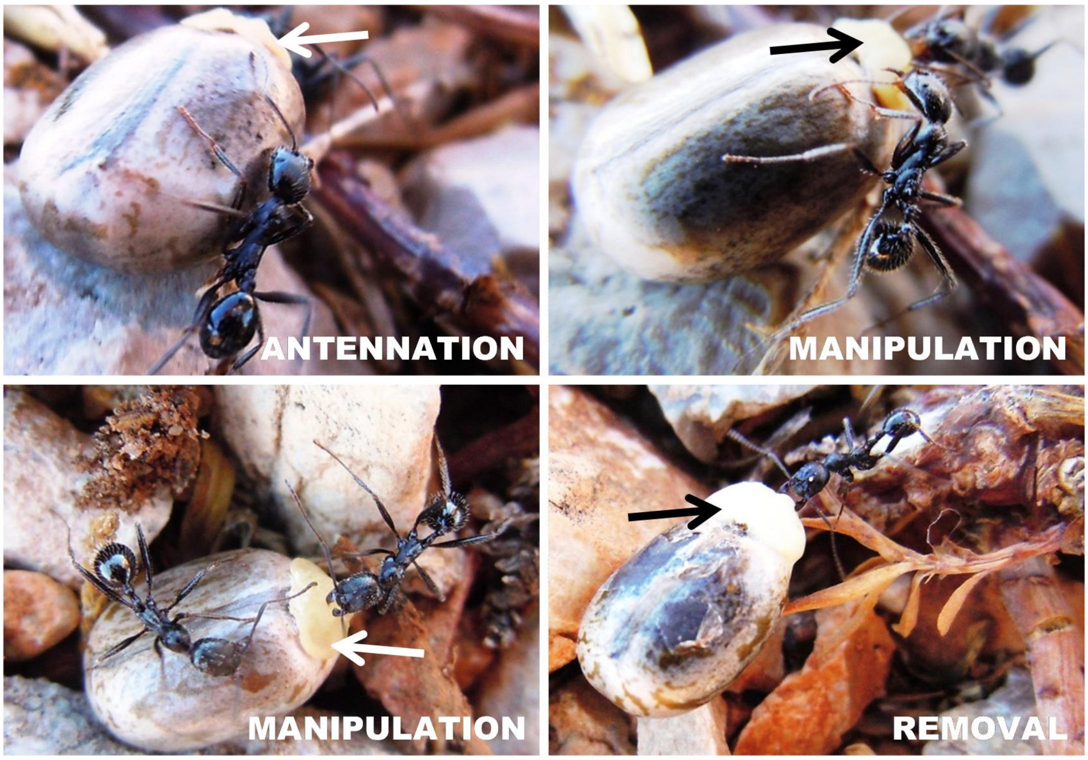

FIGURE 11 **– EXAMPLE OF NATIVES ANT–PLANT INTERACTIONS OF THE MEDITERRANEAN BASIN.** 1. *Messor capitatus* (Latreille, 1798) (Myrmicinae: Pheidolini) worker transport an elaiosome–fruit of *Borago officinalis* to the nest at Bosco del Cappelliere (ONR Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago). 2. *Messor capitatus* worker transport an elaiosome–seed of *Corydalis densiflora* to the nest at Pizzo Carbonara (Madonie RNP). 3. *Messor capitatus* worker transport an diaspore of *Aethionema saxatile* to the nest at Pizzo Carbonara (Madonie RNP). 4. *Pheidole pallidula* (Nylander, 1849) (Myrmicinae: Attini) worker transport an elaiosome–fruit of *Borago officinalis* to the nest at Bosco del Cappelliere (ONR Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago). 5. Worker ant of *Aphaenogaster semipolita* (Nylander, 1856) (Myrmicinae: Pheidolini) removes an elaiosome–seed of *Euphorbia characias*. 6. Worker ant *of Aphaenogaster sicula* Emery, 1908 (Myrmicinae: Pheidolini) removes an elaiosome–seed of *Euphorbia characias*. (Photos © Li Vigni, 2014)

Chapter 3

> “The introduction of a pitfall trap in the early 1930s
> formed the basis for the evolution of pitfallogy.”
>
> WAAGE B. E. (1985)

(•)Epigaeic myrmecofauna in a Mediterranean forest using a new integrated design of pitfall trap

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ABSTRACT

For over half a century, pitfall traps – introduced in 1931 by US entomologist BARBER – have been the standard assemblage method for epigaeic macroarthropods used to measure presence, species richness, diversity, abundance, and activity–density. The conventional model (cup trap) has proved timeless but, in parallel, it has undergone a number of changes and is still evolving. According to the studied taxon researchers have used a different design pitfall trap, justifying that: the material of construction may affect the capacity of the trap to collect insects, the nature of the preservative liquid can cause chemotactic phenomena, and the diameter of the trap is can counteract the digging–in effects. Other important variables are: the color, the presence of a roof, the use of a fence, and the arrangement of a set of traps. At current state–of–the–art a pitfall trap specific for epigaeic ants does not exist. This study describes in details a new design of pitfall trap aimed to maximize ant captures. The new trap combines the improvements made by different authors in an integrated model with additional changes. The Formicidae Design Pitfall Trap (FDPT) consists of two glasses (one with drainage holes and one with spillways), a short–stem funnel sprinkled with talc, a propylene glycol solution, a transparent roof (plastic plate) held up by a skewer, and a hexagonal mesh net. FDPT was tested in a European–Mediterranean forest (Western Sicily, Italy), dominated by *Quercus* spp., in comparison with conventional traps (CT) and proved highly selective towards Formicidae (total mean abundance of Formicidae collected was 15.00 with FDPT versus 11.21 with CT); this results in a significant increase in total sampling levels compared to other taxa. The obtained results suggest adopting FDPT as a specific method of sampling epigaeic macro–myrmecofauna.

KEYWORDS BARBER trap. Cup trap. Pitfallogy. Formicidae. Sicily.

CONTENTS

1. INTRODUCTION

In the studies on biodiversity which provide an inventory of arthropods, an important aspect is to establish standard sampling methods in order to obtain unbiased results, comparable to other studies. Ants are the subject of numerous ecological studies; the most common methods to sample ground–foraging ants are: pitfall and bait traps, aspirators, litter sifting, BERLESE–TULLGREN or WINKLER funnels for litter or soil samples, hand collections with forceps or nets, fogging, and beating and sweeping (FOLGARAIT, 1998; WANG *ET AL.*, 2001).

The pitfall traps buried flush with the ground surface, are the most commonly employed census method to sample ants, especially in savanna–dominated landscapes (ROMERO & JAFFE, 1989; PARR & CHOWN, 2001; LOPES & VASCONCELOS, 2008; PACHECO & VASCONCELOS, 2012). Trapping success depends on nest density, ground vegetation cover, and species–specific traits (inhabited stratum, colony size, foraging distance) (SCHLICK–STEINER *ET AL.*, 2006). Pitfall traps are commonly used worldwide to self–sample epigaeic invertebrates; however, this technique can be ineffective for capturing some species, while capturing high numbers of non–target invertebrates (SELDON & BEGGS, 2010).

Many and diverse pitfall trap concepts have been introduced. Analysis of the literature reveals that there are few common denominators. A conventional pitfall trap (Cup Trap: CT) consists, essentially, in a small container that is buried in the soil with its rim at surface level so that it can intercept the arthropods moving on the ground surface. The container is filled with a liquid that both preserves and kills the animals falling into the trap (e. g. BESTELMEYER *ET AL.*, 2000; BĂNCILĂ & PLĂIAŞU, LEATHER, 2005; 2009; BUCHHOLZ *ET AL.*, 2010). Pitfall traps are frequently used as an inexpensive semi–quantity method (LEATHER, 2005) and they are very effective for sampling particularly of beetles (Coleoptera) and ants (Hymenoptera), for systematic and ecological studies (BOUCHARD *ET AL.*, 2000; BUCHHOLZ *ET AL.*, 2010; GILL *ET AL.*, 2012); they are easy to use and can be operated for relatively long periods of time without maintenance (BOUCHARD *ET AL.*, 2000). These traps can collect arthropods in numbers that are suitable for rigorous statistical analysis, although their efficiency is influenced by many biotic and abiotic variables (GREENSLADE, 1964; SPENCE & NIEMELÄ, 1994; BOUCHARD *ET AL.*, 2000).

The various epigaeic ant species from Mediterranean area are for the most part of small size (linear body length 2.30–4.30 mm: LI VIGNI *ET AL.*, unpublished data) and therefore only visible at close range. Sometimes ants are camouflaged with the environment, immersed in the undergrowth (where they forage) or they are not distinguishable in sunny meadows, due to reflection of their shiny bodies. Formicidae are often elusive, living in underground nests, under stones, bark or other substrates and come into full operation at night; they have intensive period of work in the summer when they store food for the winter. They are also fast runners – move with an average speed of 4 cm per second (HÖLLDOBLER & WILSON, 1994). The ants are, therefore, difficult to spot and capture without specific devices. Ants, by virtue of their unique social organization, are abundant and ecologically important components of virtually all ecosystems (HÖLLDOBLER & WILSON, 1990) constituting, by an ecological point of view, a privileged object of study that deserves a specific sampling method. Therefore, effective methods to collect, quantify and inventory are needed. In 2006 a literature review carried out by SCHLICK–STEINER *ET AL.* (411 studies from 53 countries of all continents, published between 1961 and 2004) revealed 23 methods of quantifying ant species or assemblages, with pitfall traps (32.4%) ranking first in frequency.

At the current state–of–the–art (literature review published between 1964 and 2014, comprising of more than 50 articles) specific design of pitfall traps to catch epigaeic Formicidae, despite the conceptual homogeneity of the taxon. In view of the growing literature on various pitfall types and techniques, KIRKLAND & SHEPPARD (1994) recommended to standardize pitfall traps techniques so that results of diverse studies could be compared. The objective of this study is to propose a new model of Formicidae Design Pitfall Trap (FDPT), more specific to catch ground–dwelling ants, combined with an appropriate sampling protocol. In order to ascertain the validity (greater selectivity towards the Formicidae and significant increase in sampling values compared to other taxa), the new method was tested in a typical European Mediterranean ecosystem, in comparison with the conventional BARBER method.

1.1. HISTORY OF INVESTIGATION

Until the middle of the twentieth century, entomologists studying epigaeic macroarthropods went down on their knees with their pincers and mouth aspirators ready to capture every fleeing insect that just had been deprived of its hiding place. This method, apart from being very time–consuming, yields variable results according to the collector's searching image and endurance, thus making the method unsuitable for quantitative investigations and very subjective (WAAGE, 1985).

The pitfall traps are conceptually steep–sided cavities in the ground used to capture animals. The larvae of particular insects such as the antlions (Neuroptera: Myrmeleontidae) dig conical pits in the sand of 6–8 cm in diameter and about 6 cm deep, to capture ants and other small arthropods. Early human–beings dug pitfalls to trap mammals for meat and hide. In this century zoologists have rediscovered the pitfall traps as a device for capturing a variety of animals, including gastropods, arthropods, amphibians, reptiles, and micromammals (HANDLEY & KALKO, 1993). The pitfall traps are especially popular among entomologists as standard technique for epigaeic macroarthropods capturing, because they are effective, easy to build, affordable, easy to install, and they sample continuously throughout the day, overcoming interspecific differences with regards to circadian rhythms (AGOSTI *ET AL.*, 2000; SOUTHWOOD & HENDERSON, 2000). The method is passive and totally depends on the epigeous activity of different species (WAAGE, 1985). This activity is among other things modified by the habitat structure (GREENSLADE, 1964), and the macro– and micro–climate (BRIGGS, 1961). The catches obtained by pitfall trapping do not necessarily give good estimates of the activity–density (= abundance of the different species) (WAAGE, 1985).

Pitfall traps for arthropods were described for the first time in 1896(1) and 1907(2) by DAHL, and published in the scientific German literature (STEYSKAL, 1977; BUCHHOLZ & HANNIG, 2009). Later, LAURENT in 1917(3) (LEMIEUX & LINDGREN, 1999) and HERTZ in 1927(4) (NIEMELÄ *ET AL.*, 1988), described the first real pitfall traps used to catch macroarthropods. However, their introduction in the studies of terrestrial ecology refers to BARBER that best described them in *Traps for cave–inhabiting insects*, published in 1931(5) on *Journal of the Elisha Mitchell Scientific Society* (WAAGE, 1985; NIEMELÄ *ET AL.*, 1988; SOUTHWOOD & HENDERSON, 2000; BUCHHOLZ & HANNIG, 2009) (***FIGURE 1***). Since the mid–fifties of the last century several modifications were tested (SOUTHWOOD & HENDERSON, 2000). While originally conceived as a qualitative technique, the potential of the method for quantitatively sampling epigaeic invertebrate a population was soon realized (FICHTER, 1941). A detailed description of the technique was given by BALOGH in 1958(6); new developments were presented by MELBER in 1987(7) and GRELL in 1997(8) (BUCHHOLZ & HANNIG, 2009). The evolution of the design is well documented by a burgeoning international literature published in the last half century that has increased even more in the last decade (***TABLE 1***).

Several types of pitfall trap for macroarthropods, characterized by different designs are known. The conventional trap (CT) is commonly called pitfall trap (of Anglo–Saxon authors) or BARBER trap (of German authors). This trap has proved quite efficient as capture method and, therefore, timeless, but in parallel it has also undergone over time throughout a number of changes and it is still evolving. For several years, the cup–funnel trap was commercially available: it consists of a glass to be buried in the soil with its rim at surface level, with a funnel and a glass inside. Other type of traps are, for example: the bottle–funnel trap, consisting of a bottle with a funnel entrance, buried flush with the ground surface (OBRIST & DUELLI, 1996); the NORDLANDER trap, consisting of a container with a lid, provided with small holes distributed around to the upper rim, to be buried at the level of the holes (NORDLANDER, 1987); the ramp trap, consisting of a raised access container, reachable by a ramp (BOUCHARD *ET AL.*, 2000). The choice of the type of trap depends on the objectives of the study. The ramp traps, for example, seem to be the most effective method for sampling Araneae (Arachnida); the NORDLANDER trap and the BARBER trap instead appear to be, respectively, the best systems for sampling Carabidae (Coleoptera) and Formicidae (Hymenoptera) (PEARCE *ET AL.*, 2005; BĂNCILĂ & PLĂIAŞU, 2009) (***FIGURE 2***).

Diverse methodologies have been used to collect Formicidae and each of them has its own limitations given that no single method is able to collect all species inhabiting a given area (at least not in tropical and subtropical habitats where ant diversity is typically high), since these species commonly have a wide diversity of foraging and nesting habits. As a result, many ant inventories employ more than one sampling technique, as their use in combination often increases sampling efficiency (ROMERO & JAFFE, 1989; LONGINO & COLWELL, 1997; BESTELMEYER *ET AL.*, 2000; DELABIE *ET AL.*, 2000; PACHECO & VASCONCELOS, 2012). Pitfall traps, for instance, tend to be more efficient for the collection of relatively large ants that are active on the soil surface, whereas the WINKLER method favors the collection of smaller and often cryptic species that forage or nest in the litter layer (OLSON, 1993). The combined use of pitfall traps and the WINKLER samples has been proposed in the "Ants of Leaf Litter" (ALL) sampling protocol; this protocol has been employed successfully in ecological studies and inventories of tropical forest ants (BESTELMEYER *ET AL.*, 2000; LONGINO *ET AL.*, 2002; LOPES & VASCONCELOS, 2008; PACHECO & VASCONCELOS, 2012). GREENSLADE &

GREENSLADE (1971) and GREENSLADE (1973) describe their modification of pitfall traps used to sample ants and other epigaeic invertebrates in Southern Australia and elsewhere. They suggest that a tube with an inner diameter of 18 mm and 75 mm long, containing alcohol with a trace of glycerol is a suitable non–attractant design for sampling ants. MAJER (1978) refines the GREENSLADE model (***FIGURE 3***). In 2000 KASPARI has proposed a model of pitfall trap for capturing arboreal ants (***FIGURE 4***). In 1996 YAMAGUCHI & HASEGAWA and in 2010 ANDERSEN & BRAULT, and SCHMIDT & SOLAR, proposed pitfall trap for capturing hypogaeic ants. Recently, the subterranean trap was refined by PACHECO & VASCONCELOS (2012); in their study, the two researchers have tested these traps, inground at a depth of 20 or 50 cm, in comparison with the conventional traps placed on the soil surface, proving that 16% of the species collected in subterranean traps were unique and most of these had cryptobiotic morphology (***FIGURE 5***).

2. MATERIALS AND METHODS

2.1. STUDY AREA AND SITES

This study has been realized in Western Sicily (Southern Italy, European–Mediterranean region), inside in the Oriented Nature Reserve (ONR) Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago. The reserve territory includes five municipalities of the Province of Palermo: Corleone, Godrano, Marineo, Mezzojuso, and Monreale. This reserve, extended 7,397.49 ha, is the largest ONR of Southern Italy (FEDERICO, 2009). The altitude of the area ranges between 350 m ASL at Contrada Drago and 1,613 m at Rocca Busambra peak (GIANGUZZI *ET AL.*, 2004): a calcareous–dolomitic mountain that combined with Rocca Ramosa massif, makes up a massive ridge extending over 16 km from West to East and marks Southern border of the reserve (***FIGURE 6***). The Euro–Mediterranean region is part of the Mediterranean macrobioclimate, in the Mediterranean Pluviseasonal Oceanic bioclimate (MPO), characterized by hot and dry summer and mild and rainy winters that denotes different thermotypes and ombrotypes according to the particular thermal characteristics and rainfall (RIVAS–MARTÍNEZ, 1996, 2007). The bioclimate of the reserve has been referred to as thermotypes: a) thermo–mediterranean (mean annual temperature [T] = 17 °C; mean maximum temperature of the coldest month [M] = 16 °C; mean minimum temperature of the coldest month [m] = 7 °C) lower subhumid ombrotype (mean annual precipitation [P] = 700 mm) – b) meso–mediterranean (T = 14.5 °C, M = 11.5 °C, m = 2 °C) from lower to upper (P = 900 mm) subhumid ombrotypes – c) supra–mediterranean (T = 10.5 °C, M = 6 °C, m = –2.5 °C) upper subhumid and lower humid (P ≥1000 mm) ombrotypes (GIANGUZZI *ET AL.*, 2004). The samplings of the Formicidae were performed in three different sites of the reserve: Val dei Conti, Gorgo del Drago, and Alpe Ramosa (***FIGURE 7*** and ***TABLE 2***).

There are essentially four reasons why we selected this study area and sites: a) it is the richest area in terms of Sicilian fauna – b) it is an area with a known flora (GIANGUZZI *ET AL.*, 2004 and FEDERICO, 2009 see references), which facilitated the possibility of studying the ecological interactions with the Formicidae (e. g. foraging, pollination, seed dispersal, etc.) – c) sites are located in different altitudes (in general, temperature, pressure and humidity decrease with altitude, while sun radiation and, up to a certain altitude, rainfall are increased), which has allowed us to take samples from different climatic zones – d) we worked in sites within Zone A of the reserve, which essentially includes native forests that were less affected by anthropic disturbance and is rather distant from the most frequently trodden trails (so as to minimize disturbing elements).

2.2. FDPT'S CONSTRUCTION BACKGROUND

CONSTRUCTION MATERIAL. The traps should be kept very clean and have smooth internal surface, since the dirt wall of the enclosure could provide handholds and facilitate the escape of smaller individuals (MORRISON & PORTER, 2003, 2005). Glass has higher capture efficiency than plastic due to its ability to prevent the escape of arthropods because of its higher slipperiness (LUFF, 1975; WAAGE, 1985). Despite this greater retention capacity of the glass, plastic containers have maximum convenience of use. The biggest advantage of plastic is that it can be easily molded and allows a whole series of modifications which improve the efficiency (LUFF, 1996; LEMIEUX & LINDGREN, 1999). The plastic containers are also easier to transport, both for their lightness for the possibility to be stacked one inside the other occupying the minimum space possible. Finally, they are easier to install, where the soil is particularly difficult to dig, less expensive and safer for the operator in case of breakage (SPENCE & NIEMELÄ, 1994). Currently, the most used are the glasses in PP (temperature of use –5/+90 °C x 2 h) which are flexible and resilient and will not break even after crushing.

SHAPE AND SIZE. ABENSPERG–TRAUN & STEVEN (1995) have shown that the diameter of the mouth of the traps affect the capture efficacy for ants: a very small diameter may bias against larger ant species and collect fewer of the species present in an area compared to larger traps. Traps 40–70 mm wide are easier to use and best for studies concerning exclusive ants. Traps are normally circular. However, no advantage, in terms of overall trap efficiency, seems to be gained by deviating from this standard circular design (SPENCE & NIEMELÄ, 1994). Independently of the shape there is considerable variation in the size of traps. For circular traps, diameter may vary from as little as 1.8 cm (GREENSLADE & GREENSLADE, 1971; GREENSLADE, 1973; ABENSPERG–TRAUN & STEVEN, 1995) to over 25 cm (MORRILL *ET AL*., 1990). A modal diameter determined from the literature is found to be around 6–8 cm. Trap depth is variable but tends to be at least 8–10 cm (LEATHER, 2005); anything below this is likely to be particularly prone to escape. The size of the container plays a decisive role in determining the effectiveness of capture. The larger traps capture a greater number of individuals, since the possibility that the trajectories of these intersect the mouth of the container, increases as the circumference of the latter (LUFF, 1975; ADIS, 1979; KOIVULA *ET AL*., 2003). However, large traps, if not protected, should be used sparingly and with care as they are able to capture even small vertebrates (VAN DEN BERGHE, 1992; KOIVULA *ET AL*., 2003). Deep containers are more efficient in retaining individuals caught; they are also able to receive a greater amount of water (the sliding surface and/or rain), and debris (e. g. leaves and earth), before losing their capture efficiency (VAN DEN BERGHE, 1992).

CONSERVATION LIQUID. The use of a liquid that kills (by drowning or toxic effect) and preserves the specimens collected improves the efficiency of the traps (LEMIEUX & LINDGREN, 1999). The liquid prevents the escape from the trap and the predation occurring inside the trap on smaller individuals by the larger ones. A further increase in efficiency could be due to the masking of pheromones that could cause the aggregation of some species in the traps that become a source of attraction (THOMAS & SLEEPER, 1977). The liquid must provide clean samples, must not be very volatile, must maintain its effectiveness even when diluted by rain or concentrated by evaporation, and must be readily available. CALIXTO *ET AL*. (2007) have demonstrated that traps with a propylene glycol/water mixture collected significantly higher numbers of ants compared to traps with water only. For a collection interval of up to two weeks, a saturated solution of kitchen salt (LEMIEUX & LINDGREN, 1999), or wine vinegar saturated with salt (KOIVULA *ET AL.*, 2003), can be used; both are cheap and non–toxic. The disadvantage of these products lies in the fact that they are poor preservatives (LEMIEUX & LINDGREN, 1999). Besides salt deposits can be formed on the samples and must then be removed for the determination of the specimens. Wine vinegar, although it is a weak acid (6%), can corrode the samples. For long sampling intervals (1 month) the use of substances with a higher preservative capacity is required, such as ethylene glycol (GE) and MPG (e. g. VAN DEN BERGHE, 1992; LEMIEUX E LINDGREN, 1999). For long sampling intervals, CLARK & BLOM (1992) recommend to use GE 100% since it may be diluted as a result of precipitation, for shorter periods a concentration of 50% is suggested. For some taxa, the GE appears to be more attractive than repellant (ADIS, 1979). The GE should be used with care because of its sweet taste, which makes it also attractive to vertebrates, which may ingest lethal quantities (MARSHALL & DOTY, 1990). In recent years the use of MPG as the liquid in the traps to catch falling specimens is more and more encouraged. This substance, despite showing the same attractiveness to vertebrates (MARSHALL & DOTY, 1990), is relatively less toxic (LEMIEUX & LINDGREN, 1999). WEEKS & MCINTYRE (1997) comparing the two substances found no difference in the number of arthropod species caught. MPG is ideal for genetic studies because it preserves the DNA of the specimens in good condition (RUBINK *ET AL.*, 2003).

FUNNEL AND TALC. The use of a funnel is recommended by several authors, e. g. HOUSEWEART *ET AL.* (1979) and OBRIST & DUELLI (1996). Between the two claws of the Formicidae pretarsus there is the arolium: this is a membranous folder and flexible expansion, whose viscous product secreted by the arolium gland (present in the pretarsus) allows it to function as a kind of sucker allowing the ant to walk upright, upside down or on smooth surfaces (ORIVEL *ET AL.*, 2001). The arolium is present and more or less developed in almost all the worker ants with the exception of the species belonging to the subfamily Leptanillinae and some species of the subfamily Ponerinae (FREELAND *ET AL.*, 1982). Talc disrupts the good functioning of the arolium and, therefore, the ant is no longer able to climb up the rim of the funnel.

DOUBLE GLASS. Numerous authors recommend the use of two glasses inserted one inside the other, to minimize the disturbance caused by the repeated sampling over time (e. g. THOMAS & SLEEPER, 1977; SPENCE & NIEMELÄ, 1994). The bottom glass, in fact, remains permanently in the ground, while the upper one is periodically extracted for the collection of the sample and repositioned at each sampling. This foresight, besides saving time to relocate the trap prevents the digging–in effects (GREENSLADE, 1973). To avoid that the upper cup, after heavy rains, can exit from its seat due to the effect of hydrostatic pressure, it is recommended to make large drainage holes in the bottom of the bottom glass (LUFF, 1975; SPENCE & NIEMELÄ, 1994; PORTER, 2005).

SPILLWAYS. To avoid that rain can dilute the capture liquid (thus making the trap ineffective for subsequent catches) and drain individuals first captured, two opposite holes (Ø ≤1 mm) can be drilled as spillways on the vertical walls of the container just above the level of the conservation liquid. These allow the escape of excess liquid and then the good functioning of the trap (VAN DEN BERGHE, 1992).

COVER AND FENCE. The roofs are hold 3–4 cm from the soil surface to allow free access to the traps (LEATHER, 2005). BUCHHOLZ & HANNIG (2009; 2010) have shown that there are significant differences in the number of ants captured with traps with black, brown or green covers. Only traps with yellow or white covers show a slight increase in the number of ants captured.

THE TRAP RIM. The protrusion of the trap rim can repel invertebrates, although this is dependent on invertebrate size. It has been suggested that the trap rim should be placed 1–7 mm below the level of the substrate surface (LEATHER, 2005). However, this is normally awkward, and for large numbers of traps may be impractical. As a general rule it is necessary to at least get the rim of the trap at the level of the substrate surface (LEATHER, 2005).

TRAP SET PLACEMENT. In the literature several configurations of pitfall traps in the ground are reported: a grid, hexagon, circle, triad, transect, random (HANDLEY & KALKA, 1993) (**FIGURE 8**). In practice it is necessary, in probabilistic terms, to have a geometric pattern that can intercept the largest number of insects that move on the soil surface and in all possible directions. Of course, the choices are also dictated by edaphic factors and soil characteristics of the station concerned, by time available, by the number of traps to implant and by other additional factors, such as, for example, the cost of the sampling and the availability of labor. Many examples from the literature show that the estimates are more precise than those obtained with a systematic sampling, using a regular grid in the shape of an equilateral triangle, as in this case, the negative effects are minimized due to crowding and spatial redundancy of the experimental points (CASTRIGNANÒ & LOPEZ, 2000).

2.3. FDPT'S METHODOLOGY

This trap can be used repeatedly for a long time, with minimal disturbance of the habitat, once the system has been implanted. This operation may take 15 minutes while the collecting container replacement may take around 3 minutes. The FDPT is made of two conical glasses in polypropylene (PP) color transparent, capacity 300 mL, maximum diameter 76 mm, and length 102 mm. A glass is permanently installed in the ground for the duration of the investigation and has drainage holes; the replaceable glass has spillways. The internal glass is replaced at each collection date. As killing/preservative fluid we used monopropylene glycol (MPG at 99%), in a 1:1 water solution; ≈40 mL were used per trap, with addition of a few drops of surfactant to reduce the surface tension of the fluid. The insects die by drowning. The MPG is an organic compound in the class of diols; it is a clear, colorless, viscous liquid with a characteristic odor and a sweet taste, highly hygroscopic and miscible with water. Due to its very low toxicity, it is used in a wide range of sectors, including food and pharmaceutical industries; due to its low freezing point (–60 °C) it is also used as anti–freeze in cooling systems. A short–stem transparent PP funnel for powders, capacity 50 mL, diameter of the mouth 65 mm, length 68 mm, diameter of the stem 16 mm, length of the stem 25 mm, sprinkled with talc is placed inside the collection glass. Finally, the trap is covered by a transparent PP plate, diameter 165 mm, held in place by a double stainless steel skewer 33 cm long and covered by a hexagonal mesh net 13 x 13 mm, in thin stainless galvanized iron (***FIGURE 9***). The CT consists of a single glass without any additional device and with ≈40 mL of the preservative solution described above.

2.4. FIELD SAMPLING PROTOCOL

The field work has been carried out in late spring and early summer, a period when the Mediterranean basin ants are most active (GÓMEZ *ET AL*., 2003). The sampling took place between May 23 and July 18, 2011, for a total of 56 days (8 weeks). The traps were placed at the respective sites (VC, GD, and AR) between 4.00 and 6.00 PM and removed weekly at the same time of the day. Each trap was replaced with a new one weekly. Two trap models were compared: FDPT and CT; each one was replicated 3 times at each surveyed site (3). Therefore, a total of 144 (analyzed 141) were placed during the 8 week sampling period. In three cases traps have been disturbed, probably by large wild mammals or grazing: the samples were discarded and their contents were not taken into account during data analysis.

In each sampling station selected, consisting of a rectangular area of about 75 m^2 flat and with few trees, two different sets of traps were positioned. Each set consisted of 3 traps per type, placed at the vertices of an equilateral triangle, spaced 3 m apart. The active area of each set of sampling was 3.897 m^2. Two sets of traps were spaced apart approximately 10 m, measured from the upper vertices of each of the two ideal triangles (***FIGURE 10*** and ***11***). In order to reduce the digging–in effects (these effects consist of high catches immediately after traps are established which subsequently decline, as it the ants are attracted to freshly dug soil: GREENSLADE, 1973) that may affect results in short–term pitfall trap studies, to perform an excavation in the ground of the size of the diameter of the glass a gardening bulb planter with automatic release was used. Furthermore, to avoid digging–in effects the traps were kept closed for one week prior to the trapping period (GREENSLADE, 1973; ANDERSEN, 1991).

2.5. SAMPLES PROCESSING AND ANTS IDENTIFICATION

In the laboratory, each container was emptied into a large strainer with fine mesh (about 0.75 mm) and rinsed thoroughly with running water to remove the soil debris. The contents of the strainer were then emptied into a wide and low container filled with water, so as to bring the collected specimens to the surface. With the help of entomological soft forceps, the collected material sorted, eliminating the larger plant components (leaves, bark, small branches), and separating specimens of Formicidae from specimens belonging to other groups. The specimens were observed under a stereomicroscope for identification and to count the total number of individuals collected. The samples were stored in glass tubes containing 70% alcohol and each sample was accompanied by a card bearing the number of the trap in which they were captured, the location, and the date of collection. If sorting was not possible within 12 hours after collection, the material was frozen and then moved to ethanol.

The ants were determined to specific level and other invertebrates were identified to order and then sorted as morphospecies. The identification was performed based on the dichotomous keys by KUTTER (1977), COLLINGWOOD (1979), SEIFERT (2007), and CZECHOWSKI *ET AL.* (2012) concerning the ants of Switzerland, Scandinavia, Central Europe, and Poland, respectively. All are largely useful for Northern Italy only. Existing keys to Balkan ants provided by AGOSTI & COLLINGWOOD (1987) are also relevant. BOLTON's dichotomous keys (2003) were also consulted. Voucher ant specimens have been deposited at the Museo Civico di Storia Naturale of Milan (Italy) and at the Banca di Germoplasma del Mediterraneo of Palermo (Italy), and other specimens are part of the personal collection of one of the authors (LI VIGNI). Vouchers of other adult arthropods were deposited at Dipartimento di Scienze Agrarie e Forestali, Laboratorio di Zoologia applicata, University of Palermo (Italy).

2.6. DATA ANALYSIS

The data of the samplings were sorted in a spreadsheet with all the information needed to process the data (i. e. sampling localities collection dates, trap position, and trap type). The abundance (i. e. the total number of specimens) of the Formicidae was quantified at the conclusion of the 56 days of sampling, proceeding in three ways: 1) by calculating the mean number of ants captured per site (= mean abundance), 2) by calculating the mean number of ants captured in all sites (= total mean abundance), and 3) by calculating the percentage of ants captured by the traps compared to the total number of taxa (=percentage of ants abundance). To determine whether total frequencies of the arthropod groups differed significantly between the two tested trap types we used the PEARSON's Chi–square test of goodness–of–fit and independence (χ^2) (that compares experimentally obtained results with those to expected theoretically on a given hypothesis). The test was performed using the GRAPHPAD SOFTWARE QUICKCALCS (http://graphpad.com/quickcalcs/contingency1/). *P*–values were calculated from Chi–square to test the null hypothesis that in FDPT there was, compared to CT, neither an increase of ant catches other taxa decrease.

χ^2 is a non–parametric test that can be used for nominal data (i. e. discrete categories) and ordinal sets of data, for draw conclusions about differences between populations. χ^2 statistic can be applied to tables of counts (i. e. contingency table) that have a certain size. χ^2 uses a measure of goodness–of–fit which is the sum of differences between observed and expected outcome frequencies (that is, counts of observations), each square and divided by the expectation according to the following formula (JAMES, 2006; CHOW *ET AL.*, 2008).

$$\chi^2 = \sum_{i=1}^{k} \frac{(fO_i - fE_i)^2}{fE_i}$$

Where: $\sum$ = sum of all cells, the number of cells in the table, fO_i = an observed frequency (i. e. count) for bin i, and fE_i = an expected (theoretical) frequency for bin i, asserted by the null hypothesis. As in our calculations only one degree of freedom was present, we have made YATES correction for continuity, subtracting 0.5 from the numerator (in absolute value) of the χ^2 formula.

The expected frequency counts are computed separately for each population at each level of the categorical variable, according to the following formula.

$$fE_{r,c} = \frac{(n_r \cdot n_c)}{n}$$

Where: $fE_{r,c}$ = the expected frequency count for population r at level c of the categorical variable, n_r = the total number of observations from population r, n_c = the total number of observations at treatment level c, and n = the total sample size.

The degrees of freedom (df) are equal to:

$$df = (r-1) \cdot (c-1)$$

Where: r = number of rows in the contingency table and c = number of columns in the contingency table.

There were no *a priori* hypotheses requiring comparisons of mean number of other taxa in addition to ants and thus a multiple comparison procedure was used. In order to highlight any possible significant differences between the mean numbers of ant captures with the two types of traps tested, we used the Analysis of Variance (ANOVA), performed with the STATISTICA 7.0 software (STATSOFT INC., 2004). ANOVA allows the simultaneous comparison between more than two means of replicated groups, consisting of data collected under different conditions and is based on the ratio of variances (*F*–test). The capture data with the two types of traps (FDPT and CT) in three different sites (VC, GD, AR) in number of three for each site, were analyzed by two–way ANOVA with repeated measures on catches, with "type of trap" and "site" as "between" variables and "weeks of capture" as "within" variable.

3. RESULTS

3.1. TRAPS EFFICIENCY

Both CT and the FDPT have captured a wide variety of arthropods, among which: Araneae, Isopoda, Orthoptera, Homoptera, and Coleoptera. In total, sixteen different orders of arthropods were captured in both types of trap. In addition, the CT has captured also small reptiles and small mammals. The 70 FDPT tested captured a total of 6,840 arthropods 1,050 (15.35%) of which were ants. The 71 tested CT captured a total of 10,108 arthropods 796 (7.87%) of which were ants. The observed frequency of ants in FDPT was significantly higher than the frequency observed in CT (χ_1^2 = 234.142, *P* <0.0001). The ant captures ranged between 8 and 26 in FDPT and between 5 and 23 for CT. The average number of ant (±*sem*) trapped in the FDPT was 15.00 ±0.53 compared to 11.21 ±0.45 in the CT. In total, 20 species were recorded, belonging to 12 genera representing 3 different subfamilies. In 70 FDPT and 71 CT a total of 20 and 14 different species of ants were found respectively. The factors analyzed by ANOVA as a source of variation were the type of trap and the site and only the effect of the type of trap was statistically significant ($F_{1,9}$ = 15.86, *P* = 0.003). The effect of the site as well as the interaction between the two effects (respectively: $F_{2,9}$ = 0.28, *P* = 0.76 and $F_{2,9}$ = 0.68, *P* = 0.53) were not statistically significant. No significant effect was produced by the variable "within" (weeks of capture) ($F_{7,63}$ = 0.66, *P* = 0.70) nor by the interaction between this and the two variables "between" (type of trap and site) ($F_{14,63}$ = 1.29, *P* = 0.24) (***TABLE 3***, ***4***, and ***5***).

3.2. ANT SPECIES COLLECTED

The most represented subfamily of ants in both types of traps was Myrmicinae (10 species), followed by Formicinae (6 species), and Dolichoderinae (4 species). Sixteen species have a Palearctic distribution, two species an Afrotropical distribution (*Camponotus nylanderi* and *Linepithema humile*), one species is distributed in the Nearctic Region (*Lasius niger*), and one species in the Neotropical Region (*Tetramorium semilavae*). The most abundant species was *Aphaenogaster semipolita*; the less abundant species was *Tapinoma nigerrimum*. A total of 20 ant species were captured (**FIGURE 12**):

1. ***Aphaenogaster sardoa* MAYR, 1853** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Algeria, Italy (type locality).
2. ***Aphaenogaster semipolita* (NYLANDER, 1856)** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Georgia, Italy (type locality).
3. ***Aphaenogaster sicula* EMERY, 1908** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Italy (type locality), Malta, Montenegro.
4. ***Camponotus aethiops* (LATREILLE, 1798)** – Subfamily: Formicinae; Tribe: Camponotini. Distribution – Palaearctic Region: Albania, Andorra, Armenia, Austria, Balearic Islands, Bulgaria, China, Croatia, Czech Republic, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Iran, Israel, Italy, Malta, Montenegro, Portugal, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Switzerland, The former Yugoslav Republic of Macedonia, Turkmenistan, Turkey, Ukraine.
5. ***Camponotus barbaricus eubarbaricus* CAGNIANT, 1970** – Subfamily: Formicinae; Tribe: Camponotini. Distribution – Palaearctic Region: Algeria (type locality).
6. ***Camponotus lateralis* (OLIVIER, 1792)** – Subfamily: Formicinae; Tribe: Camponotini. Distribution – Palaearctic Region: Albania, Algeria, Andorra, Armenia, Balearic Islands, Bulgaria, Croatia, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Iran, Israel, Italy, Malta, Monaco, Montenegro, Portugal, Romania, Russian Federation, Slovakia, Slovenia, Spain, Switzerland, The former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Ukraine.
7. ***Camponotus nylanderi* EMERY, 1921** – Subfamily: Formicinae; Tribe: Camponotini. Distribution – Afrotropical Region: United Arab Emirates.
8. ***Crematogaster scutellaris* (OLIVIER, 1792)** – Subfamily: Myrmicinae; Tribe: Crematogastrini. Distribution – Palaearctic Region: Andorra, Austria, Balearic Islands, Bulgaria, Croatia, Denmark, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Israel, Italy, Malta, Montenegro, Portugal, Romania, Slovenia, Spain, Switzerland, Tunisia, United Kingdom of Great Britain and Northern Ireland, The former Yugoslav Republic of Macedonia, Turkey.

9. ***Formica cunicularia*** **LATREILLE, 1798** – Subfamily: Formicinae; Tribe: Formicini. Distribution – Palaearctic Region: Albania, Andorra, Armenia, Austria, Belarus, Belgium, Bulgaria, Channel Islands, China, Croatia, Czech Republic, Denmark, Estonia, Finland, France (type locality), Georgia, Germany, Greece, Hungary, Iberian Peninsula, Iran, Italy, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Mongolia, Montenegro, Morocco, Netherlands, Norway, Poland, Portugal, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, The former Yugoslav Republic of Macedonia, Turkey, Ukraine, United Kingdom of Great Britain and Northern Ireland.

10. ***Lasius niger*** **(LINNAEUS, 1758)** – Subfamily: Formicinae; Tribe: Lasiini. Distribution – Nearctic Region: Canada, United States. Neotropical Region: Falkland Islands (Malvinas), Mexico. Oriental Region: India, Maldives, Nepal, Pakistan. Palaearctic Region: Afghanistan, Albania, Andorra, Armenia, Austria, Belarus, Belgium, Bulgaria, Channel Islands, China, Croatia, Czech Republic, Democratic Peoples Republic of Korea, Denmark, Estonia, Faeroe Islands, Finland, France, Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, Monaco, Mongolia, Montenegro, Netherlands, Norway, Poland, Portugal, Republic of Korea, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, The former Yugoslav Republic of Macedonia, United Kingdom of Great Britain and Northern Ireland.

11. ***Linepithema humile*** **(MAYR, 1868)** – Subfamily: Dolichoderinae. Distribution – Afrotropical Region: Lesotho, Namibia, Saint Helena, United Arab Emirates. Australasian Region: Australia, New Zealand, Norfolk Island. Indo–Australian Region: Hawaii, Vanuatu. Nearctic Region: United States. Neotropical Region: Argentina (type locality), Bermuda, Brazil, Colombia, Ecuador, French Guiana, Mexico, Paraguay, Uruguay. Palaearctic Region: Balearic Islands, Belgium, Bulgaria, Canary Islands, Channel Islands, Democratic Peoples Republic of Korea, France, Germany, Gibraltar, Greece, Iberian Peninsula, Iran, Japan, Malta, Monaco, Montenegro, Poland, Portugal, Spain, United Kingdom of Great Britain and Northern Ireland.

12. ***Liometopum microcephalum*** **(PANZER, 1798)** – Subfamily: Dolichoderinae. Distribution – Palaearctic Region: Albania, Austria (type locality), Balearic Islands, Bulgaria, Croatia, Czech Republic, Georgia, Greece, Hungary, Iran, Israel, Montenegro, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Switzerland, The former Yugoslav Republic of Macedonia.

13. ***Messor bouvieri*** **BONDROIT, 1918** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Balearic Islands, France (type locality), Gibraltar, Iberian Peninsula, Malta, Portugal, Spain.

14. ***Messor capitatus*** **(LATREILLE, 1798)** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Balearic Islands, Bulgaria, Croatia, France (type locality), Greece, Iberian Peninsula, Italy, Malta, Monaco, Montenegro, Portugal, Romania, Slovenia, Spain, The former Yugoslav Republic of Macedonia.

15. ***Messor structor*** **(LATREILLE, 1798)** – Subfamily: Myrmicinae; Tribe: Stenammini. Distribution – Palaearctic Region: Albania, Andorra, Armenia, Austria, Balearic Islands, Belgium, Bulgaria, Canary Islands, China, Croatia, Czech Republic, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Iran, Israel, Italy, Kazakhstan, Kyrgyzstan, Malta, Monaco, Montenegro, Poland, Portugal, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Switzerland, The former Yugoslav Republic of Macedonia, Turkmenistan, Ukraine, Uzbekistan.

16. ***Pheidole pallidula*** **(NYLANDER, 1849)** – Subfamily: Myrmicinae; Tribe: Attini. Distribution – Palaearctic Region: Afghanistan, Albania, Algeria, Andorra, Armenia, Austria, Balearic Islands, Bulgaria, Canary Islands, Croatia, France, Georgia, Gibraltar, Greece, Iberian Peninsula, Iran, Israel, Italy (type locality), Kazakhstan, Kyrgyzstan, Malta, Monaco, Montenegro, Portugal, Romania, Russian Federation, Slovenia, Spain, The former Yugoslav Republic of Macedonia, Turkey, Turkmenistan.

17. ***Tapinoma erraticum*** **(LATREILLE, 1798)** – Subfamily: Dolichoderinae. Distribution – Palaearctic Region: Albania, Andorra, Armenia, Austria, Balearic Islands, Belarus, Belgium, Bulgaria, Canary Islands, Channel Islands, Croatia, Czech Republic, France (type locality), Georgia, Germany, Gibraltar, Greece, Hungary, Iberian Peninsula, Iran, Israel, Italy, Kazakhstan, Kyrgyzstan, Liechtenstein, Lithuania, Luxembourg, Malta, Monaco, Montenegro, Netherlands, Poland, Portugal, Republic of Moldova, Romania, Russian Federation, Slovakia, Slovenia, Spain, Sweden, Switzerland, The former Yugoslav Republic of Macedonia, Turkmenistan, Ukraine, United Kingdom of Great Britain and Northern Ireland, Åland Islands.

18. ***Tapinoma nigerrimum*** **NYLANDER, 1856** – Subfamily: Dolichoderinae. Distribution – Palaearctic Region: Algeria, Balearic Islands, France (type locality), Gibraltar, Greece, Italy, Montenegro, Morocco, Spain.

19. ***Temnothorax rottenbergii*** **(EMERY, 1870)** – Subfamily: Myrmicinae; Tribe: Crematogastrini. Distribution – Palaearctic Region: Canary Islands, Croatia, Greece, Italy (type locality).

20. ***Tetramorium semilavae*** **ANDRÉ, 1883** – Subfamily: Myrmicinae; Tribe: Crematogastrini. Distribution – Neotropical Region: Mexico. Palaearctic Region: Armenia, Austria, Balearic Islands, Canary Islands, Croatia, France, Georgia, Gibraltar, Greece, Hungary, Iberian Peninsula, Italy, Malta, Montenegro, Portugal, Romania, Russian Federation, Slovenia, Spain, Switzerland, The former Yugoslav Republic of Macedonia.

4. CONCLUSION

The pitfall traps are used to measure ant presence, species richness, diversity, abundance, and activity–density (e. g. WAAGE, 1985; PORTER & SAVIGNANO, 1990; WANG *ET AL.*, 2000); however, they are a non–selective sampling method and normally they catch many different organisms (sometimes in high numbers) (BUCHHOLZ *ET AL.*, 2010). Pitfall traps are a very frequently used for sampling in terrestrial ecology (NEW, 1998). Despite criticisms (e. g. BOMBOSCH, 1962; HALSALL & WRATTEN, 1988; BORGELT & NEW, 2005), pitfall traps are suitable for studying the occurrence and relative abundance of ground–dwelling arthropods (BUCHHOLZ *ET AL.*, 2010). The performance of ant sampling methods deserves further study. Applied biodiversity research and nature conservation requires assessing ant species and assemblages reliably with the minimum effort, as does the analysis of ecosystem function. Conflicting statements in the literature complicate the design of ant–ecological investigations. Key questions include: what picture do pitfall traps draw of one and the same habitat? Do the results change with size and shape of the sampling plot? And ultimately: are data from the literature collected with different sampling methods comparable? (SCHLICK–STEINER *ET AL.*, 2006). Despite criticism of its possible influence on the distortion of the sampling data (ADIS, 1979) and despite the lack of standardization of the protocol, the value of this technique, most likely, will have to endure over time (NEW, 1999). The value of pitfall traps is due to the numerous advantages they offer over other methods. They are inexpensive, easy to build and to use, efficient, take up a lot of species and individuals in sufficient number for rigorous statistical analysis (GREENSLADE & GREENSLADE, 1971). The traps are easily replicable and many different sites can be sampled simultaneously and repeatedly over time. The materials they are made of are readily available. Ethically, however, it is advisable to consider getting a design alternative to the standard pitfall traps to reduce the mortality of small mammals and amphibians that is otherwise captured. The accidental fall on small vertebrates, also affect the capture of arthropods and makes the trap inefficient (PEARCE *ET AL.*, 2005). The trap presented in this study is the result of the integration and improvement of previous models of pitfall traps experienced by various authors for the capture of epigaeic Formicidae (e. g. GREENSLADE, 1973; ABENSPERG–TRAUN & STEVEN, 1995; AGOSTI *ET AL.*, 2000; CALIXTO *ET AL.*, 2007). Designed in ideal phase in order to be able to have a more selective method of capture of Formicidae with respect to the classical model (CT), the FDPT was tested in comparison to CT, with results that meet the expectations, suggesting, therefore, its use for the collection of ants living on the ground in the framework of research programs studying aboveground myrmecofauna. The FDPT complements and in some cases improves, some of the changes made over time by various authors to BARBER basic–model (CT) used in the last sixty years for the study of epigaeic arthropodofauna and adds unpublished changes. FDPT can be used repeatedly for a long time, with minimal disturbance of the habitat once the system is stabilized.

The largest number of species captured by the FDPT is due, probably, to the use of the internal funnel and the presence of protective net. These two expedients act as a selective filter for larger species. In addition, the funnel sprinkled with talc prevent Formicidae any escape. Both types of traps have captured only ant species from epigaeic habits. Results suggest adopting FDPT as a specific method of sampling epigaeic macro–myrmecofauna.

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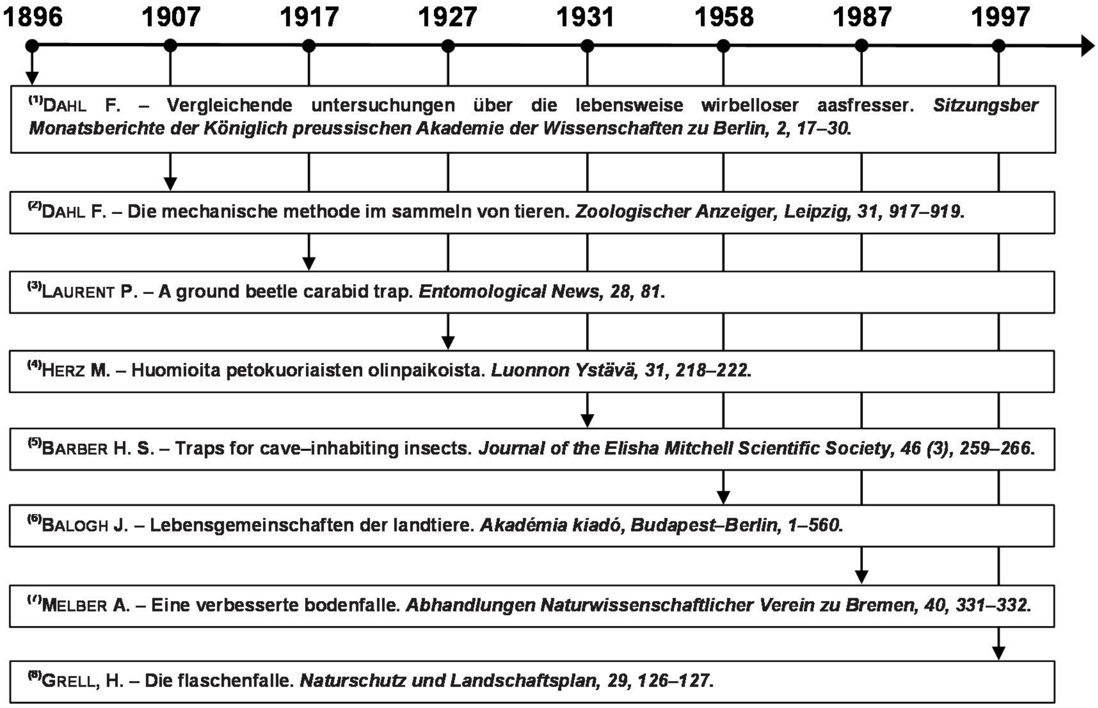
***FIGURE 1* – THE INVENTOR OF PITFALL TRAP.** In 1931 the US entomologist HERBERT SPENCER BARBER (1882–1950) – pictured in a photo of the 20's – has introduced the use of pitfall trap in studies of terrestrial ecology concerning the arthropodofauna. In his honor, this trap has been called, especially by German authors, "BARBER trap". (Photo © Internet source www.howderfamily.com, 2004)

***TABLE 1* – EVOLUTION OF PITFALLOGY.** Fundamental temporal events of the first publications on pitfall traps for arthropods.



(1)DAHL F. – Vergleichende untersuchungen über die lebensweise wirbelloser aasfresser. *Sitzungsber Monatsberichte der Königlich preussischen Akademie der Wissenschaften zu Berlin, 2, 17–30.*

(2)DAHL F. – Die mechanische methode im sammeln von tieren. *Zoologischer Anzeiger, Leipzig, 31, 917–919.*

(3)LAURENT P. – A ground beetle carabid trap. *Entomological News, 28, 81.*

(4)HERZ M. – Huomioita petokuoriaisten olinpaikoista. *Luonnon Ystävä, 31, 218–222.*

(5)BARBER H. S. – Traps for cave–inhabiting insects. *Journal of the Elisha Mitchell Scientific Society, 46 (3), 259–266.*

(6)BALOGH J. – Lebensgemeinschaften der landtiere. *Akadémia kiadó, Budapest–Berlin, 1–560.*

(7)MELBER A. – Eine verbesserte bodenfalle. *Abhandlungen Naturwissenschaftlicher Verein zu Bremen, 40, 331–332.*

(8)GRELL, H. – Die flaschenfalle. *Naturschutz und Landschaftsplan, 29, 126–127.*

***FIGURE 2* – ARTHROPOD TRAPS. A** Cup–funnel trap (Carolina Biological Supply Company, USA). **B** Bottle–funnel trap (Science Kit & Boreal Laboratories International, USA). **C** Nordlander trap (container in polypropylene, size 32 oz – Thermo Scientific Nunc, Germany; holes made by 6.35 mm paper punch: Fiskars, Finland). Scale bar = 10 cm. **D** Ramp trap: height = 140 mm, length = 155 mm, width = 155 mm (Insect Science, Southern Africa). (Photos © **A**, **B**, and **C** Li Vigni, 2014; **D** Internet source www.insectscience.co.za, 2005)

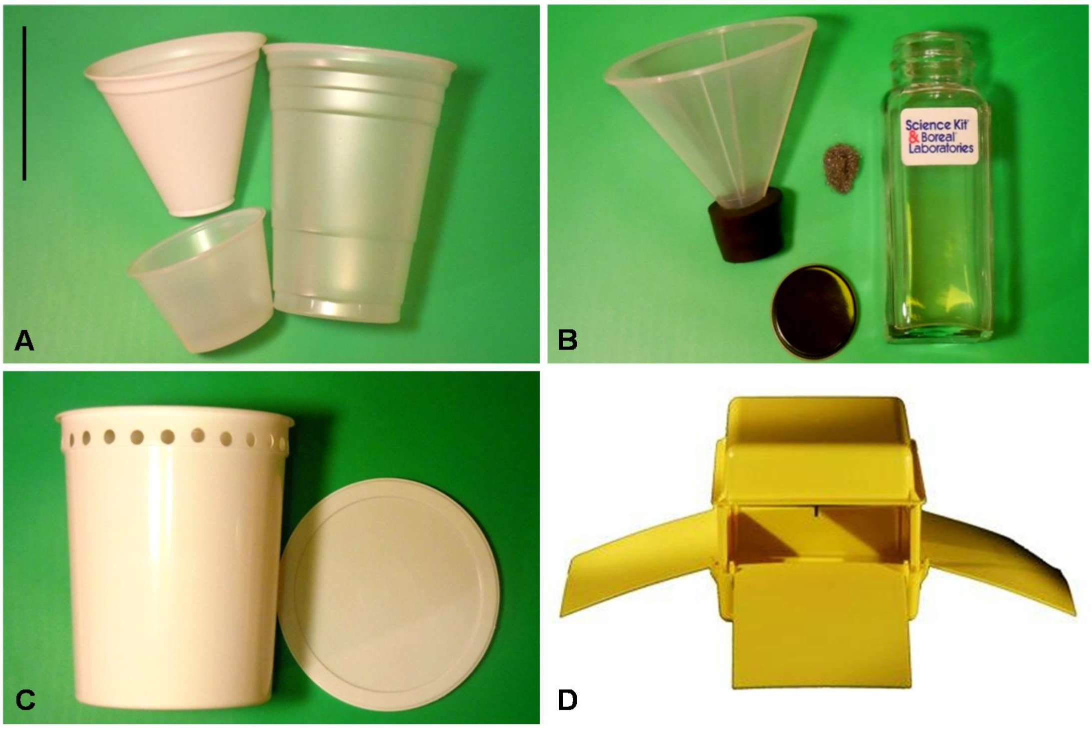
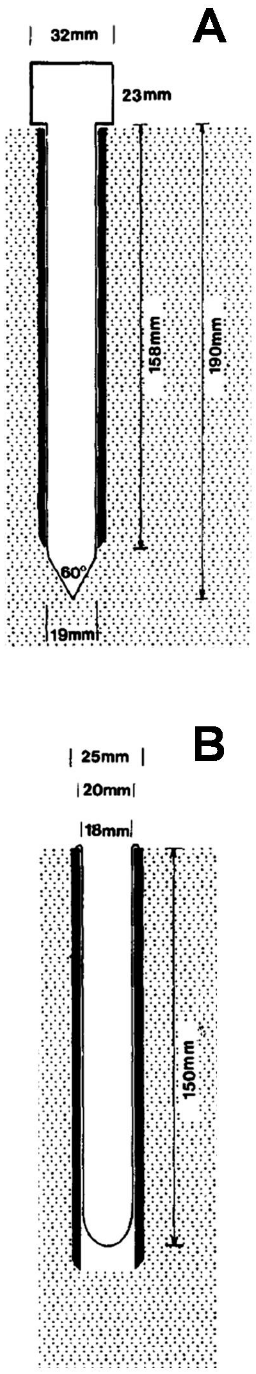


***FIGURE 3* – ANTS EPIGAEIC TRAP (MAJER). A** The inserter tool is used for placing the sleeve in the soil. It is turned from aluminium bar and has a pointed end. The trap sleeve, beveled at the lower end, is first placed over the inserter tool. The resulting unit can be forced into soil by driving the inserter tool with a mallet. **B** The trap consists of Pyrex test tube (150 mm deep, 19 mm external diameter and 18 mm internal diameter), set in a 158 mm polyvinyl chloride sleeve constructed from 25 mm diameter underground electrical conduit. The 8 mm space at the base of the sleeve acts as a reservoir to accommodate soil or detritus which may fall in during changing of test tube. Three mL of ethanol with a trace of glycerol as preservative are used in the trap. (Original drawings © MAJER, 1978)

***FIGURE 4* – ANTS ARBOREAL TRAP (KASPARI). A** The trap samples insects crawling along tree branches, by providing a platform a 10 x 30 cm strip of canvas positioned flush with the branch. A 25 x 200 mm test tube half–filled with ethylene glycol is inserted through a slit at one end of the canvas strip. The trap is draped over a tree branch, where the test tube dangles to one side. **B** The line is sent over a branch and controlled using a modified slingshot. **C** The trap is hung from branch and monofilament lines are staked down, holding it in place. (Original drawings © KASPARI, 2000)

***FIGURE 5* – ANTS HYPOGAEIC TRAP (PACHECO & VASCONCELOS).** The trap consists of a closed plastic container of 250 mL with four radial holes of 1 cm diameter made in the side of the container, which allow ants to access the trap. A 70 cm long rope is attached to the lid of each container to identify its location and facilitate removal. The traps are baited using sardine mixed with vegetable oil. About 5 mL of this mixture is poured onto a small lid of 2.5 cm diameter and the lid is fixed in the interior of each container suspended by a plastic frame. About 50 mL of alcohol and glycerin are poured on the bottom of the traps to act as killing and preservative agents. **A** Schematic external view. **B** Schematic internal view. (Original drawings © PACHECO & VASCONCELOS, 2012)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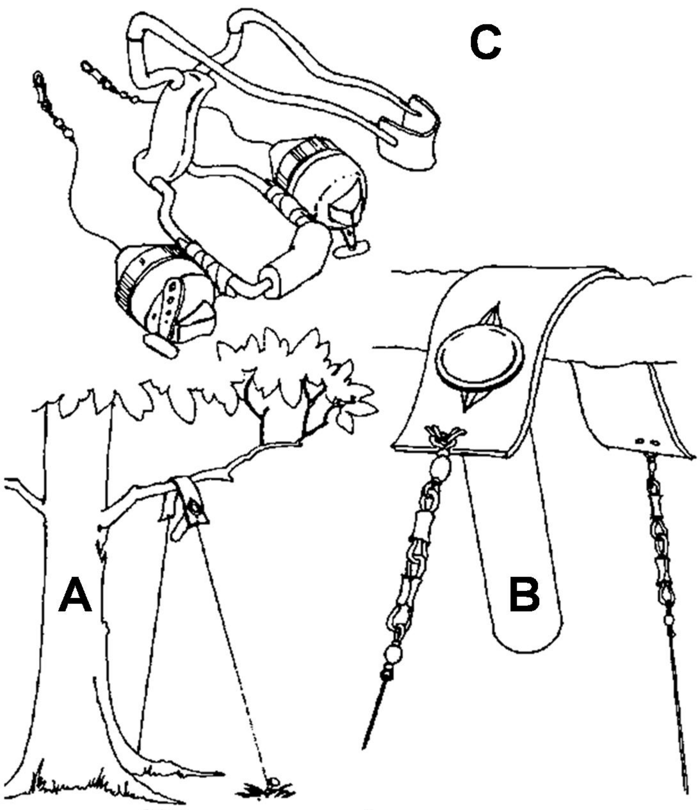
3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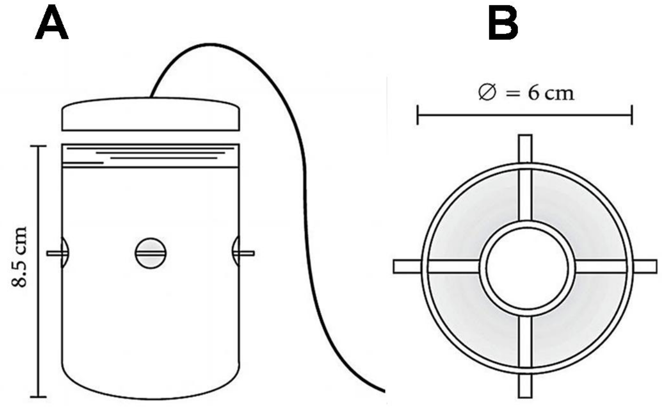
A
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8.5 cm
∅ = 6 cm

5

***FIGURE 6* – STUDY AREA AND SITES. A**, **B**, and **C** Satellite images at different altitudes: **A** European–Mediterranean region (3,414.78 km); **B** Sicily, island of Southern Italy, in the Mediterranean Sea (300.60 km); **C** Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere, and Gorgo del Drago (14.63 km). Date of acquisition of images: **A** and **B** = 04/10/2013; **C** = 07/29/2013. **D** Map of the ONR with the highlighted study sites: Val dei Conti (VC), Gorgo del Drago (GD) both in the Cappelliere forest, and Alpe Ramosa (AR) in the Ficuzza forest. (Images © **A**, **B**, and **C** Landsat US Dept of State Geographer – Google Earth, 2014; **D** Internet source www.altobelicecorleonese.com, 2008)

FIGURE 7 **– PANORAMIC VIEW OF THE STUDY SITES. A** The Cappelliere forest, dominated by the carbonate massif of Rocca Busambra (1,613 m ASL). **B** The Gorgo del Drago area in midsummer, reduced to a rainwater puddle. **C** A strip of Ficuzza forest located in Alpe Ramosa, dominated by the Rocca Ramosa massif (1,276 m ASL). (Photos © Li Vigni, 2011)

***TABLE 2* – SHORT DESCRIPTION OF THE SAMPLING SITES.** Geographic coordinates (longitude and latitude in Degrees, Minutes, and decimal Minutes) and altitudes (m Above Sea Level), recorded at the center of the sampling stations, were obtained using a cartographic GPS unit provided with altimeter, by Garmin (USA), model eTrex Vista HCx, with software Land Navigator Italy, with digital topographic maps, scaling 1:50,000 complete of geographical locations.

SITE	COORDINATES	ALTITUDE	HABITAT
Val dei Conti locality (Bosco del Cappelliere) Monreale (PA)	Long. 13°22.816′ E Lat. 37° 55.093′ N	660	Oak forest with a predominant presence of leaf leaves oaks (*Quercus amplifolia* GUSS.)
Alpe Ramosa locality (Bosco della Ficuzza) Marineo (PA)	Long. 13°22.444′ E Lat. 37°52.423′ N	925	Oak forest with a predominant presence of holm–oaks (*Quercus ilex* L.)
Gorgo del Drago area (Bosco del Cappelliere) Godrano (PA)	Long. 13°24.692′ E Lat. 37°54.069′ N	1,019	Oak forest with a predominant presence of cork oaks (*Quercus suber* L.)

***FIGURE 8* – DIAGRAMS OF VARIOUS CONFIGURATION OF PITFALLS.** Frequently used spatial arrangements of pitfall traps. (Drawing © LI VIGNI, 2014)

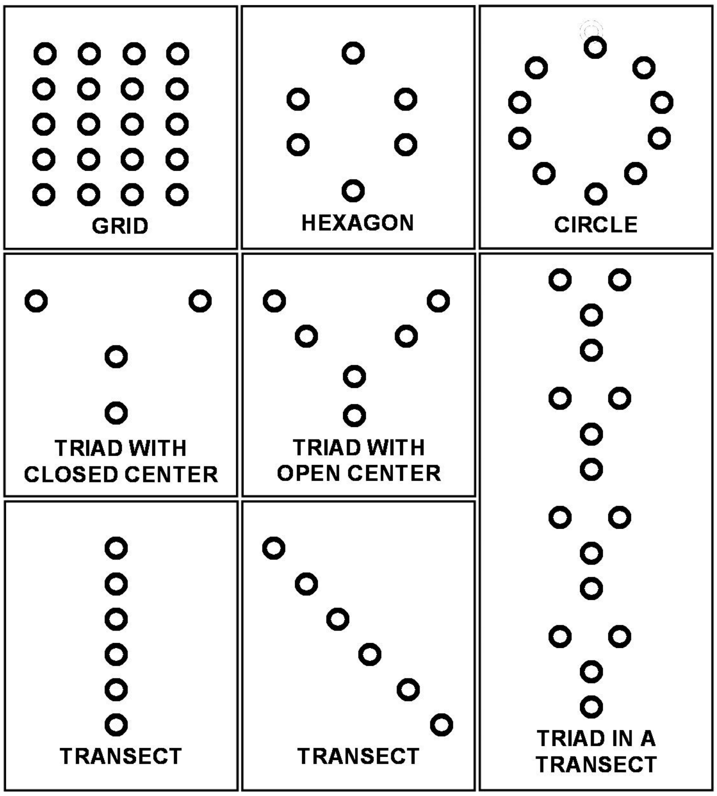


***FIGURE 9* –THE DESIGN OF THE FDPF.** This design is a guideline for sampling ants in the Mediterranean basin.

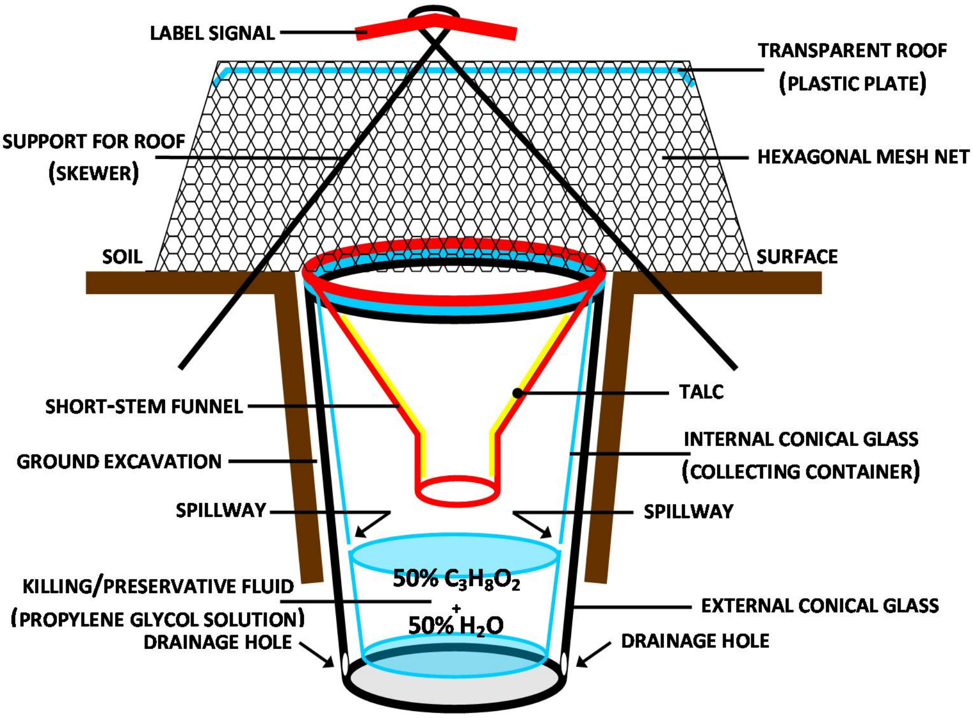


***FIGURE 10* – SCHEME OF TRAPS IN THE GROUND.** In each study site (VC, GD, AR), a long flat station with few trees and wide about 7 x 14 m was chosen, with the longest side facing North. Each type of sample set consists of three traps, placed at the vertices of a hypothetical equilateral triangle. Angles between the traps: 60°; height of the triangle: 2.598 m; active sampling area: 3.897 m^2; *l* = side: 3 m; *d* = distance between the two sets: 10 m; ○ = FDPT; ● = CT; 1, 2, 3 = position of the traps in each set. (Drawing and photo © Li Vigni, 2011)

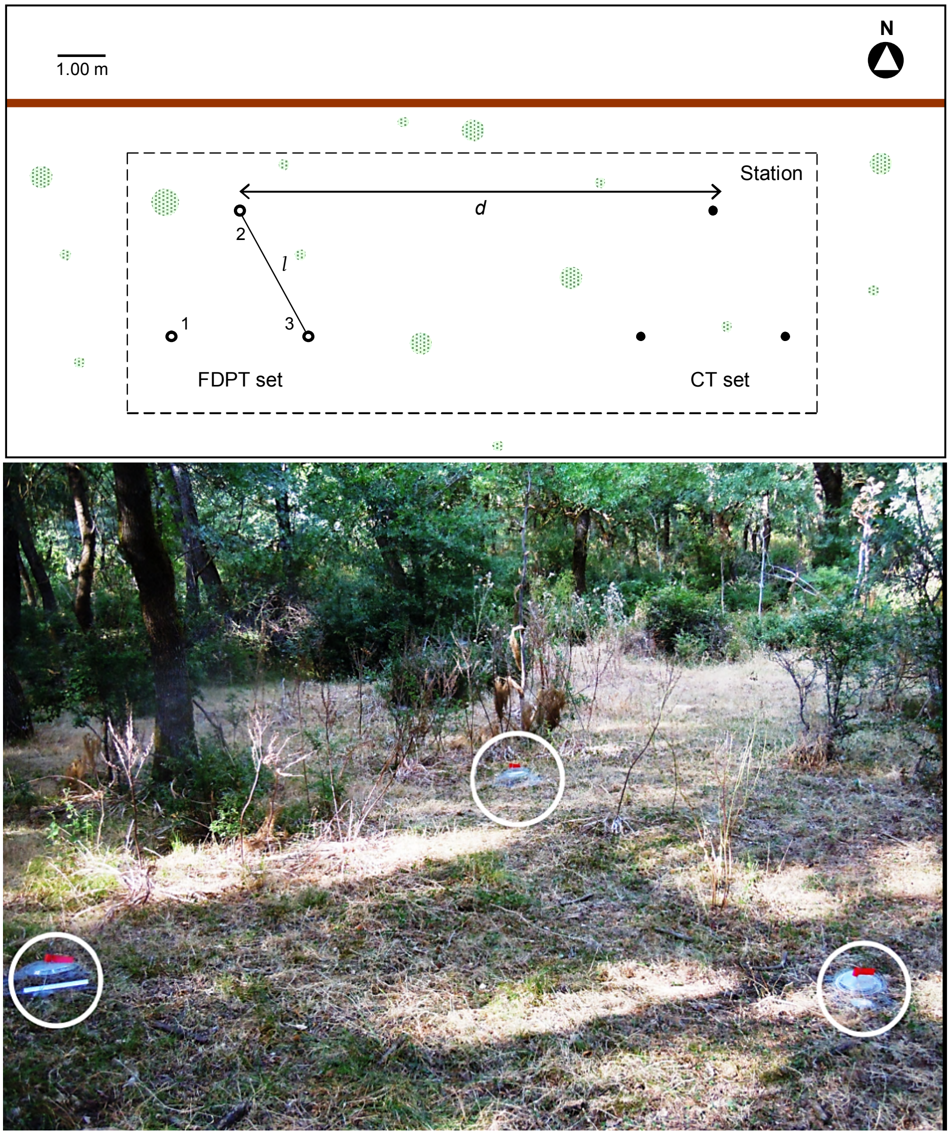


FIGURE 11 **– TRAPS IN THE GROUND.** It is often advantageous to use an obvious marker for each trap, such as a flag, to aid in relocation. (Photos © LI VIGNI, 2011)

***TABLE 3* – ANT FREQUENCIES AND PERCENTAGES IN THE TRAP TYPES.** χ^2 calculations two–tailed 2 x 2 contingency table. Observed frequencies in normal font–style. Expected frequencies *in italic font–style*. Individual χ^2 values in parentheses. *P*–value = probability, *n* = sample size.

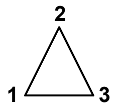
TRAP TYPES	**FREQUENCY**			**ANTS PERCENTAGE**
	ANTS	**NON–ANTS**	***n***	
FDPT	1,050 *745.02* (124.84)	5,790 *6,094.98* (15.26)	6,840	15.35%
CT	796 *1,100.98* (84.48)	9,312 *9,007.02* (10.33)	10,108	7.87%
Total	1,846	15,102	16,948	
$\chi^2_1 = 234.142, P < 0.0001$				

TABLE 4* – ABUNDANCE OF TAXON IN THE TWO TRAP TYPES AND STATISTICAL COMPARISON.** Total abundance of the major arthropod groups present in the two trap types compared using χ^2 with YATES correction. The taxa are at the level of order unless otherwise indicated; taxonomy follows BORROR ET AL. (1989). *P* is considered: extremely statistically significant (**in bold font–style**); very statistically significant (in normal font–style); statistically significant (in bold italic font–style***); not quite statistically significant (*in italic font–style*); not statistically significant (in parentheses).

	TYPE OF TRAP		MEAN		±*sd*		±*sem*		CHI–SQUARE	
TAXON	1 FDPT *n* 70	2 CT *n* 71	1	2	1	2	1	2	χ^2_1	*P*
I. Acari	83	225	1.19	3.17	0.87	1.73	0.10	0.21	22.875	**<0.0001**
II. Araneae	1,407	1,289	20.10	18.15	6.93	4.01	0.83	0.48	185.807	**<0.0001**
III. Opiliones	170	150	2.43	2.11	1.53	1.35	0.18	0.16	21.546	**<0.0001**
IV. Pseudoscorpiones	24	74	0.34	1.04	0.56	0.73	0.07	0.09	9.660	0.0019
V. Isopoda										
Suborder Oniscidea	402	845	5.74	11.90	2.92	5.20	0.35	0.62	36.520	**<0.0001**
VI. Class Diplopoda	34	69	0.49	0.97	0.63	0.68	0.08	0.08	2.028	(0.1544)
VII. Class Chilopoda	83	166	1.19	2.34	0.95	1.34	0.11	0.16	4.890	***0.0270***
VIII. Collembola	405	835	5.79	11.76	2.41	4.08	0.29	0.48	32.589	**<0.0001**
IX. Orthoptera										
Family Acrididae	37	125	0.53	1.76	0.72	1.10	0.09	0.13	20.128	**<0.0001**
Family Gryllidae	431	821	6.16	11.56	3.25	3.91	0.39	0.46	19.510	**<0.0001**
Family Gryllotalpidae	209	242	2.99	3.41	1.76	1.87	0.21	0.22	6.637	0.0100
Other Orthoptera	205	414	2.93	5.83	1.69	2.38	0.20	0.28	13.683	**0.0002**
X. Blattaria	96	210	1.37	2.96	1.21	2.09	0.14	0.25	10.078	0.0015
XI. Hemiptera	149	278	2.13	3.92	1.41	2.16	0.17	0.26	5.203	***0.0225***
XII. Homoptera	260	309	3.71	4.35	1.66	1.60	0.20	0.19	6.736	0.0095
XIII. Coleoptera										
Family Carabidae	631	1,079	9.01	15.20	2.08	5.75	0.25	0.68	9.290	0.0023
Family Chrysomelidae	63	176	0.90	2.48	0.92	1.83	0.11	0.22	19.151	**<0.0001**
Family Curculionidae	127	182	1.81	2.56	1.56	1.49	0.19	0.18	0.044	(0.8339)
Family Nitidulidae	115	222	1.64	3.13	1.17	1.54	0.14	0.18	5.290	***0.0214***
Family Scarabaeidae	94	150	1.34	2.11	1.02	1.37	0.12	0.16	0.273	(0.6013)
Family Staphylinidae	189	274	2.70	3.86	1.47	1.93	0.18	0.23	0.025	(0.8749)
Family Tenebrionidae	78	167	1.11	2.35	0.86	1.62	0.10	0.19	7.146	0.0075
Family Trogidae	72	155	1.03	2.18	0.72	1.53	0.09	0.18	6.777	0.0092
Other Coleoptera	80	191	1.14	2.70	0.86	1.78	0.10	0.21	12.987	**0.0003**
XIV. Diptera	83	157	1.19	2.21	0.92	1.51	0.11	0.18	3.135	*0.0766*
XV. Lepidoptera	120	162	1.71	2.28	1.38	1.61	0.17	0.19	0.485	(0.4863)
XVI. Hymenoptera										
Non–Formicidae	143	345	2.04	4.86	1.43	2.61	0.17	0.31	25.043	**<0.0001**
Family Formicidae	1,050	796	15.00	11.21	4.43	3.80	0.53	0.45	234.142	**<0.0001**
TOTAL	6,840	10,108								

***TABLE 5* – TRAP SAMPLING DATES AND ABUNDANCE OF ANTS FOR SITE.** Comparison between abundance of ants collected with the FDPT, and with the CT in *Quercus* spp. dominated mixed forests in three Euro–Mediterranean ecosystems (VC, GD, and AR), in the months of May–July 2011. *n* = sample size; *sdm* = standard deviation; *sem* = standard error of the mean; ▬ = trap discarded because compromised by external factors. Below: the box plots of the data.

TRAP SETS FOR STATION



		MODE OF DATA COLLECTION					
		(total individuals)					
		FDPT				**CT**	
		GROUPS					
REPLICAS	**SAMPLING DATES YEAR 2011**	**1 VC**	**2 GD**	**3 AR**	**4 VC**	**5 GD**	**6 AR**
1	23–30 May	$(15+19+16)_{11}$	$(14+25+18)_{21}$	$(18+10+19)_{31}$	$(13+15+11)_{41}$	$(07+12+\text{▬})_{51}$	$(\mathbf{05}+09+06)_{61}$
2	30 May–06 June	$(21+14+11)_{12}$	$(13+19+09)_{22}$	$(17+19+14)_{32}$	$(10+16+13)_{42}$	$(09+\mathbf{05}+15)_{52}$	$(10+12+05)_{62}$
3	06–13 June	$(24+11+16)_{13}$	$(13+11+09)_{23}$	$(22+\text{▬}+12)_{33}$	$(08+07+10)_{43}$	$(08+10+14)_{53}$	$(17+15+18)_{63}$
4	13–20 June	$(12+09+19)_{14}$	$(12+18+15)_{24}$	$(09+16+20)_{34}$	$(12+14+06)_{44}$	$(05+15+09)_{54}$	$(20+07+08)_{64}$
5	20–27 June	$(17+15+18)_{15}$	$(08+19+17)_{25}$	$(11+18+\mathbf{26})_{35}$	$(13+\mathbf{05}+06)_{45}$	$(16+09+12)_{55}$	$(14+11+10)_{65}$
6	27 June–04 July	$(12+13+16)_{16}$	$(15+14+20)_{26}$	$(\mathbf{08}+11+09)_{36}$	$(09+\mathbf{23}+12)_{46}$	$(14+07+12)_{56}$	$(13+17+14)_{66}$
7	04–11 July	$(15+10+18)_{17}$	$(11+19+09)_{27}$	$(13+12+\text{▬})_{37}$	$(11+13+10)_{47}$	$(13+16+14)_{57}$	$(10+06+09)_{67}$
8	11–18 July	$(22+12+10)_{18}$	$(16+11+18)_{28}$	$(09+16+23)_{38}$	$(12+13+11)_{48}$	$(11+06+10)_{58}$	$(11+12+15)_{68}$
8 weeks	**56 days**	**TOT. 365**	**TOT. 353**	**TOT. 332**	**TOT. 273**	**TOT. 249**	**TOT. 274**
n		**24**	**24**	**22**	**24**	**23**	**24**
MEAN ABUNDANCE		**15.21**	**14.71**	**15.09**	**11.38**	**10.83**	**11.42**
±*sdm*		**4.02**	**4.31**	**5.13**	**3.80**	**3.45**	**4.23**
±*sem*		**0.82**	**0.88**	**1.09**	**0.78**	**0.72**	**0.86**
VARIANCE		**16.1721**	**18.5634**	**26.2770**	**14.4184**	**11.8774**	**17.9057**
Two–way $\boldsymbol{ANOVA}_{1df}$: $SS = 325.03, MS = 325.03, F = 15.8641, P = 0.003191$							

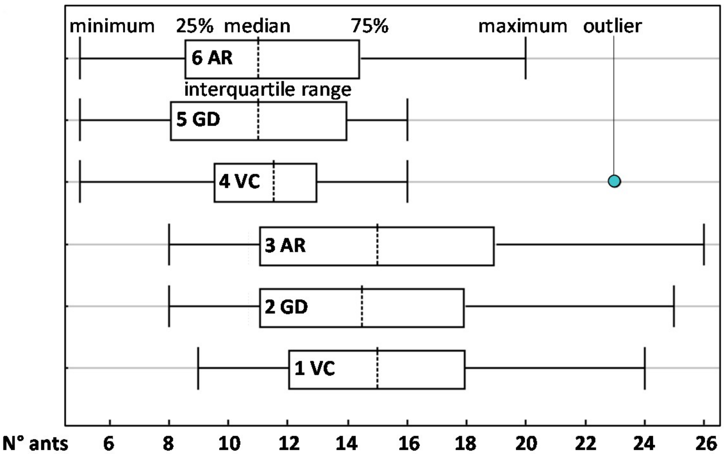


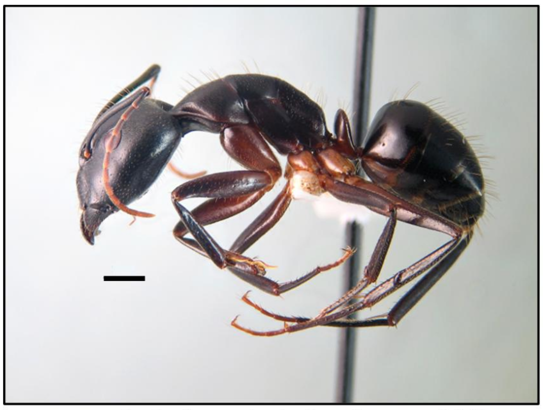
***FIGURE 12* – ANT SPECIES.** Scale bar = 1 mm. (Photos ©: *Aphaenogaster sardoa* PERRY/ANTWEB.ORG, 2012; *Pheidole pallidula* NOBILE/ANTWEB.ORG, 2007; all other PIRAINA/RIGATO/LI VIGNI, 2013)

***Aphaenogaster sardoa* MAYR, 1853**

***Aphaenogaster semipolita* (NYLANDER, 1856)**

***Aphaenogaster sicula* EMERY, 1908**

***Camponotus aethiops* (LATREILLE, 1798)**



***Camponotus barbaricus eubarbaricus* CAGNIANT, 1970**

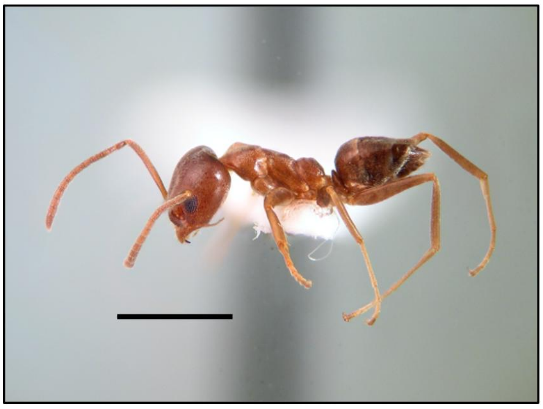
***Camponotus lateralis* (OLIVIER, 1792)**

Camponotus nylanderi **EMERY, 1921**

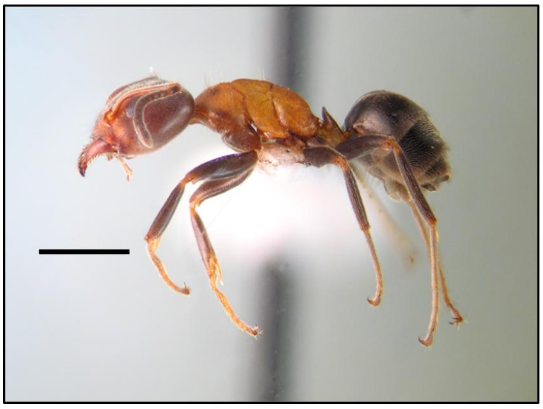
Crematogaster scutellaris **(OLIVIER, 1792)**

Formica cunicularia **LATREILLE, 1798**

Lasius niger **(LINNAEUS, 1758)**

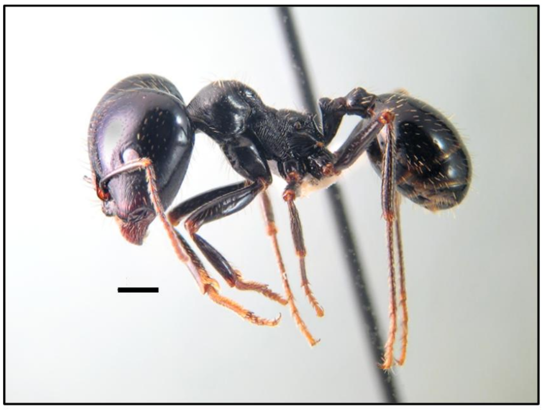


Linepithema humile **(MAYR, 1868)**



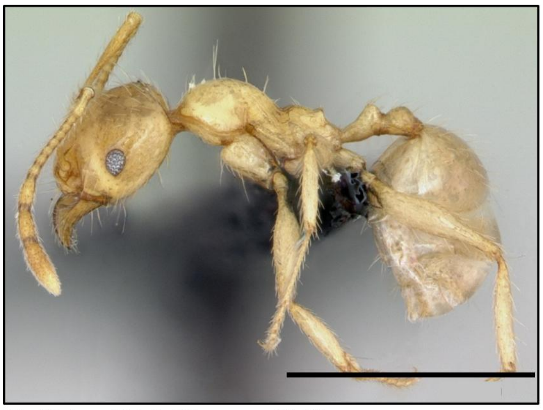
Liometopum microcephalum **(PANZER, 1798)**

Messor bouvieri **BONDROIT, 1918**



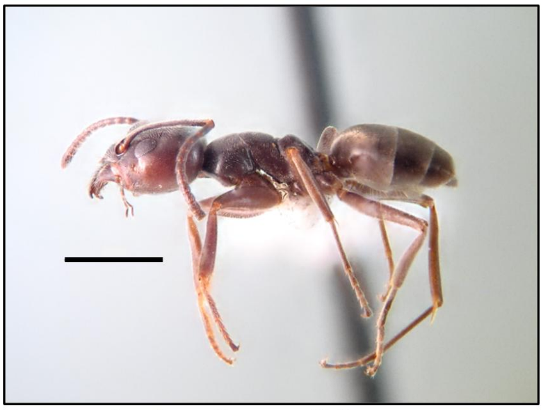
Messor capitatus **(LATREILLE, 1798)**

Messor structor **(LATREILLE, 1798)**



Pheidole pallidula **(NYLANDER, 1849)**

Tapinoma erraticum **(LATREILLE, 1798)**



Tapinoma nigerrimum **NYLANDER, 1856**

Temnothorax rottenbergii **(EMERY, 1870)**

Tetramorium semilavae **ANDRÉ, 1883**

Chapter 4

"The Prince bowed and watched an ant carrying a seed with great effort.
– Why do you do it? – He asked.
– I do not know – Said the ant.
The Prince crushed it with his boot and he mused:
– The effort does not serve unless it has a purpose –"

The seven truths, Slovenian folk tale (about 1590)

(•)Biological aspects and myrmecochory of *Corydalis densiflora* C. PRESL (Papaveraceae: Fumarioideae), endemic taxon of Southern Italy and Sicily

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ABSTRACT

Corydalis densiflora is a spring ephemeral tuberous herb with pallid pink flowers, which lives mainly in deciduous forest habitats. It is endemic in Central and Southern Italy, where it is not common. The majority of the species belonging to the genus *Corydalis*, have a conspicuous elaiosome connected to the basal part of the seed body (i. e. micropyle–hilum–arilbase region = true aril). Myrmecochory, i. e. seed dispersal by ants, is a common seed dispersal syndrome. In general, ants collect the entire diaspore (i. e. seed + elaiosome), carry it to their nest and feed the larvae with the elaiosome while the seed still viable is rejected either outside the nest or in an abandoned chamber. The bio–ecology of *C. densiflora* was understudied, while its elaiosome and myrmecochory have never been studied. The aim of this research was to investigate *C. densiflora* distribution in Sicily, some key biological aspects (morphology and germination), and the ecology of seed dispersal. We investigated *in situ* the relative effectiveness of myrmecochory by means of "cafeteria" experiments, offering to the ants seeds with elaiosome and seeds of the same species deprived manually of the elaiosome. In total 81.25% seeds with elaiosome tested were collected by the ants, while they ignored nearly totally seeds without elaiosome (2.98%). These results show that myrmecochory is the principal seed dispersal system of this species. The seed dispersers of *C. densiflora* observed in the study site (Pizzo Carbonara: Madonie Mountains) were the Mediterranean harvester ant *Messor capitatus*. This species is likely to be the main source of removal diaspores in all treatments because no other invertebrates were observed removing seeds. The entire seeds with elaiosomes were shown in tests significantly more effective for ants than the other two tested baits (seeds without elaiosome and elaiosomes alone): χ_2^2 = 1,708.167, *P*–value = 0.0000. We also observed that these worker ants take the seeds by elaiosomes during transport. The seed removal by ants was positively correlated with the presence of elaiosome and elaiosome removal increased germination success by at least 27% (χ_2^2 = 151.651, *P*–value = 0.0000).

KEYWORDS Seed dispersal by ants. Dense–flowered corydalis. *Messor capitatus* (LATREILLE, 1798). Elaiosome. True aril. Cafeteria experiment. Endemic plants of Sicily.

CONTENTS

1. INTRODUCTION

Corydalis densiflora C. PRESL (dense–flowered corydalis) is an ephemeral herb with pallid pink flowers, perennial tuberous, spring–green, that prefers to grow in the herb layer of nutrient–rich deciduous forests habitats, on slopes. Is present in Italy (CONTI *ET AL.*, 2005), Algeria (LIDÉN, 2011), and it has also been reported in Greece (GERASIMIDIS & KORAKIS, 2009), where it is not common and restricted to areas at an altitude ranging between 800 and 1,900 m above sea level. In Italy it is endemic in Abruzzo (doubtful report) and Umbria (Central Italy), Basilicata, Calabria, and Sicily (Southern Italy) (CONTI *ET AL.*, 2005). This taxon is closely related to *C. solida* (L.) CLAIRV. subsp. *solida*, a species that shows a large distribution in Europe; it is mainly found in Central and Eastern Europe, with a range extending to France and Belgium in the West, and to the Caucasus in the East (HULTÉN & FRIES, 1986), but it is not present in Sicily (CONTI *ET AL.*, 2005).

Corydalis DC. is a genus of about 470 species (SWAGAT–SHRESTHA *ET AL.*, 2013) that comprises annual, biennial, and perennial herbs. It is distributed mainly in the temperate regions of the Northern Hemisphere, mostly in Eurasia (DAR *ET AL.*, 2011). One species in is reported for subarctic Russia (*C. magadanica* A. P. KHOKHR.: BERKUTENKO & LUMSDEN, 2000), about ten species for Northern America (STERN, 1997), one species in the mountains of Eastern Africa (*C. cornuta* ROYLE: LIDÉN, 1993), and three species in subtropical Indo–China (ZHANG *ET AL.*, 2008). *Corydalis* is one of the largest genera in China, being represented by 357 species (ZHANG *ET AL.*, 2008), in Russia, being represented by about 50 species (POPOV, 1937), and in India, being represented by 53 species (ELLIS & BALAKRISHNAN, 1993). Most of *Corydalis* species in India occur in alpine and subalpine ranges of Himalaya, constituting an important component of the Himalayan angiosperm flora; in Kashmir Himalaya 31 species are reported (DAR *ET AL.*, 2011). The European flora includes 39 species (LIDÉN, 2011). Six taxa are recorded in Italy (CONTI *ET AL.*, 2005). There are also diverse cultivar and hybrids used as perennial garden plants for the abundant flowering and ease of cultivation.

Many seeds have a specialised external appendage (fat body): a rich pluricellular supplement of nutritional substances, above all lipids, but also proteins, amino acids, vitamins, and carbohydrates (BRESINSKY, 1963), called elaiosome. In all cases studied to date, these nutritious mature appendages attract worker ants, by playing a crucial role in the dispersal process (e. g. HANDEL & BEATTIE, 1990; LIDÉN & ZETTERLUND, 1997;

GORB & GORB, 2003). An alternative proposal is that elaiosomes may help the seed to absorb and retain moisture for germination (STERN, 2000). The ants discard seeds which are too hard, but which have an elaiosome. This alimentary bounty urges ants to gather the diaspores (i. e. seeds + elaiosome) and – soon after removing the elaiosome – to scatter them about along their way. This partial carpophagy of the diaspore with consequent indirect seed dispersal by ants (LI VIGNI & MELATI, 1999) is called myrmecochory *sensu stricto* (ULBRICH, 1928).

In the European–Mediterranean region worker ants of different genera disperse diaspores of many abundant herbaceous plants called myrmecochores, i. e. ant–dispersed plants that produce elaiosome–bearing seeds (the criterion for determining whether a plant is myrmecochorous is that its diaspores possess one elaiosome). The elaiosomes attract ants and elicit the transport of the diaspore usually to the nest by the ants as destiny. In the nest, ants consume the elaiosome or, more often, feed it to their larvae (because it provides various nutrients that are essential for insect reproduction and development). Seeds without elaiosomes are still viable (LI VIGNI *ET AL.*, 2001, LI VIGNI & PATERNOSTRO, 2005) and are then abandoned in garbage piles in chambers inside or outside the nest.

Nearly 98% of the dehiscent–fruited species of the Fumarioideae subfamily possess elaiosomes (FUKUHARA, 1999). Usually, *Corydalis* spp. have black seeds with conspicuous fleshy and whitish elaiosome; the clear colour of the elaiosome contrasts with the dark colour of diaspore body (GORB & GORB, 2003). In Chinese *Corydalis* spp. elaiosome is present, with the exception of two species (*C. heracleifolia* C. Y. WU & Z. Y. SU and *C. semenowii* REGEL & HERDER) (ZHANG *ET AL.*, 2008); Russian and North American *Corydalis* spp. are very rarely without elaiosome (POPOV, 1937; STERN, 1997); in Indian *Corydalis* spp. elaiosome is not quite constant (HOOKER & THOMSON, 1855) while in European species elaiosome is always present (TUTIN *ET AL.*, 1993). The taxonomic significance of these structures is demonstrated by the fact that *Corydalis* species with peculiar spirally twisted elaiosomes were grouped into the series *Helicosyne* LIDÉN by LIDÉN & ZETTERLUND (1997).

Myrmecochory was first studied in depth by SERNANDER, a botanist at the University of Uppsala in Sweden. In 1906 he published a distinguished review on Central European myrmecochorous shrubs (he recorded approximately 150 species). Seed dispersal by ants has been reported for many species in *Corydalis* genus (e. g. HANZAWA *ET AL.*, 1985;

NAKANISHI, 1994; GORB & GORB, 2003). This phenomenon in Italian species was studied by SERNANDER (1906), with the exception of *C. densiflora*. More recently, other authors were interested in some of these species, from the point of view of myrmecochory, so for example: FISCHER *ET AL.* (2005) showed that *Myrmica rubra* (LINNAEUS, 1758) larvae benefit from the nutritional gain of elaiosomes of *C. cava* subsp. *cava*; EHLERS (2012) demonstrated that in *C. intermedia* elaiosome mass must increase more than proportionally with increasing seed mass for the diaspore to remain attractive to ants and the direction of interspecific differences suggests that a plant–mating system may affect selection for dispersal.

Corydalis densiflora is very similar to *C. solida* subsp. *solida* and in fact, it is often regarded as *C. solida* subsp. *densiflora* (C. PRESL) HAYEK (RAIMONDO *ET AL.*, 2010). *Corydalis solida* subsp. *solida* presents great elaiosomes and the myrmecochory has been well studied (e. g. EHLERS, 2012; GORB & GORB, 1997; SERNANDER, 1906); it is therefore likely that both subspecies are myrmecochores. Recent evidence suggests that the traditional view of myrmecochory as a highly diffuse interaction between diaspores and a wide range of ant species attracted to their elaiosomes may not be correct. The effectiveness of dispersal varies markedly among ant species, and combined with differential attractiveness of diaspores due to elaiosome size and composition, this raises the potential for myrmecochorous plants to target ant species that offer the highest quality dispersal services (LEAL *ET AL.*, 2014). Given this perspective, it should be necessary to confirm experimentally that the elaiosome presence for a certain species is related or not at the myrmecochory (LI VIGNI *ET AL.*, unpublished observation). At the present state–of–the–art, it appears that the biology and ecology of *C. densiflora* were understudied, instead the elaiosome and myrmecochory have never been studied. This article reviews this taxon, with particular attention to its distribution in Sicily, to some key biological aspects (i. e. morphology and germination) and to the ecology of seed dispersal, in particular by ants.

2. MATERIALS AND METHODS

2.1. STUDY SITE

The study was carried out in Pizzo Carbonara, a carbonatic massif that is the highest point of the Madonie Regional Natural Park (North–Western Sicily), characterized by a supra–mediterranean bioclimate (mean annual temperature = 10.5 °C), from humid to subhumid (mean annual precipitation ≥1000 mm) (GIANGUZZI *ET AL.*, 2004). Beech forest, developing on calcareous ground, represents the climax forest vegetation. This cenosis is dominated by *Fagus sylvatica* L., while *Acer pseudoplatanus* L., and *A. campestre* L. are more sporadic. In the undergrowth *Ilex aquifolium* L. is frequent. The herbaceous layer includes several nemoral species such as *Allium ursinum* L. subsp. *ucrainicum* (KLEOPOW & OXNER), *Anemone apennina* L. subsp. *apennina*, *Cyclamen repandum* SM. subsp. *repandum*, *Cyclamen hederifolium* AITON subsp. *hederifolium*, *Gagea fragifera* (VILL.) EHR. BAYER & G. LÓPEZ, *Primula vulgaris* HUDS. subsp. *vulgaris*, *Scilla bifolia* L., and several endemic or rare plants, e. g. *Colchicum bivonae* GUSS., *Colchicum triphyllum* G. KUNZE, *Euphorbia myrsinites* L. subsp. *myrsinites*, *Gagea bohemica* (ZAUSCHN.) SCHULT. & SCHULT. F., *Knautia calycina* (C. PRESL) GUSS. and *Viola nebrodensis* C. PRESL, *Viola parvula* TINEO. The dispersal of these herbaceous plants is carried out by the ants (LI VIGNI *ET AL.*, unpublished data). However in the summit areas of Pizzo Carbonara this forest is present only in small fragments with a tree layer that rarely reaches the height of a real tree, either due to soil and climatic reasons but mainly because of old logging and grazing. Another peculiar aspect of this area is the vegetation that occupied the bottom of the dolines, typical expression of karst in Madonie Mountains (***FIGURE 1*** and ***2***).

2.2. STUDY SPECIES

Corydalis densiflora has aboveground growth for only a very short period between March and May, during which flowers are set and seeds ripen. The flowering and fruiting phenology was studied, in the spring 2012, by examining 35 marked plants. Seven sampling stations where small populations of dense–flowered corydalis grew, at an altitude between 1,633 and 1,887 m ASL were identified (***TABLE 1***). At each station five plants were randomly chosen. During the flowering and fruiting periods (April–May) we visited every weekly regularly the stations to record the number of flowers per inflorescence and the number of fruits, without disturbing the plants.

From a survey of the myrmecofauna conducted in the same period in the same site, through captures and direct observations (LI VIGNI *ET AL.*, unpublished data), *Messor capitatus* (LATREILLE, 1978) resulted abundant and it appears to be responsible of most seed collecting activity and our observations and experiments were concentrated on this species. Worker ants were captured using a portable electric aspirator for collecting insects and were stored in 70% ethanol. The determination was made in the Museo Civico di Storia Naturale of Milan (Italy). During the first and second week of May 2012, we collected the seed content of two nests of *M. capitatus*. The nests of *M. capitatus* are dug into the soil in depth, they are very large and have few exits concentrated. We carefully excavated the whole nest removing an area of soil approximately 50 cm^2 wide to a depth of 60 cm. *Ex situ*, we selected the fraction of the seeds of *C. densiflora*.

Seed sets were collected from the field when fruits were ripe and beginning to shed their seeds, during the first and second week of May 2012. In *Corydalis* spp. experiments in controlled conditions showed that temperature is the main factor controlling dormancy and germination (VANDELOOK & VAN ASSCHE, 2009). *In situ* seed germination was studied in the periods February–April 2011 and 2012, observing the development of the seedlings. In nature, seeds of *C. densiflora* are dispersed in spring and are exposed to a long period of relatively high temperatures in summer, followed by a period of intermediate temperatures in autumn, and a period of low temperatures in winter, and germinate in the following spring. In order to reproduce natural conditions with more fidelity, *ex situ* the seeds were stored, both with and without capsules, in cotton bags in incubator preset to standard temperatures summer (20 °C) for 3 months, autumn (15 °C) and high humidity for 3 months, required to initiate embryo growth, while a transfer to 5 °C (winter temperature) for 8 weeks is needed for completion of embryo growth and germination. At this stage the seeds begin to germinate. If the period of cold weather is not sufficient, the seeds will germinate the following year. The germination tests were conducted in PETRI dishes (diameter 100 mm, height 15 mm), and the seeds were placed on filter paper in the second week of January 2013. We humidified the seeds with distilled water whenever necessary. We used one germination camera with white light, with a constant temperature of 20 °C, under a photoperiod of 12 hour light and 12 hour darkness. In order to reproduce natural conditions with more fidelity, we did not perform any kind of pretreatment with seeds, such as application of fungicide. The seeds were placed to germinate in three different seeding conditions (seeds with elaiosome, seeds without elaiosome by hand removal in the laboratory, and seeds without elaiosome ant removal). The three types of seeds were placed to germinate in 30 PETRI dishes (10 PETRI dishes for each types), each containing 50 seeds. We considered a seed germinated when there was protrusion of the hipocotile–radicular axis.

In order to measure the length of flowering and fruiting pedicels, the length and the width of the fruits and the elaiosomes and – according to GORB & GORB (2003), seen that the shape of the seeds is almost circular – the maximum length (equivalent to the diameter) of the seeds, we have utilized an ocular micrometer (max error of measurement ±0.01 mm) under a Leica MS 5 stereomicroscope at 6–50× magnifications and a fiber ring lamp. The weight of the seeds was obtained with an analytic scale Orma model BC Memory (error ±0.001 g). All parameters were expressed as (minimum) **sample arithmetic mean** ±sample standard deviation of the mean (maximum) (*n* = sample size); all measures were rounded to two decimal placed for the width and length and to three decimal placed for the weight. The diaspores of *C. densiflora* were compared with the Italian diaspores of other five species: *C. capnoides* (L.) PERS.; *C. cava* (L.) SCHWEIGG. & KÖRTE subsp. *cava*; *C. intermedia* (L.) MÉRAT; *C. pumila* (HOST) RCHB., and *C. solida* (L.) CLAIRV. subsp. *solida*. The seeds of these species were provided by Banca di Germoplasma del Mediterraneo of Monreale PA, Italy (***FIGURE 3***).

2.3. FIELD EXPERIMENTS

We investigated *in situ* the relative effectiveness of the myrmecochory by means of "cafeteria" experiments (i. e. a type of "self–service" experiments, where food objects are presented to animals to record removal rates of the objects: GORB & GORB, 2003), by offering to ants three bait types of *Corydalis densiflora*: entire seeds with elaiosome (i. e. intact seeds), seeds previously deprived of the elaiosomes (i. e. seed without elaiosome), and elaiosomes alone (i. e. detached elaiosomes). Eight grey color metal plates (11 x 11 cm), each one inserted in a transparent tray with openings of 1 cm at the corners, were placed at a distance of 50 cm from one nest of *M. capitatus*, in the four cardinal directions. On four metal plates we have positioned, regularly spaced (≈1 cm), 42 fresh seeds with elaiosomes and on the other four plates 42 seeds without the elaiosomes, into six rows of seven columns of seeds. This procedure was reproduced twice a day, at the same hours (9.00–11.00 AM and 1.00–3.00 PM) for four days, in sunny and windless days during 2013 spring. To avoid influences of the microclimatic habitat we permuted the position of the metal plates (see ***FIGURE 4***). A total of 2,688 seeds

(1,344 seeds with elaiosome and 1,344 seeds without elaiosome) were tested. The final number of seeds on each plate was recorded after two hours. On the days preceding the setup of the experiment, all seeds were gathered as seed pods started to dehisce. *Ex situ* elaiosomes were carefully manually removed from half of the seeds under a stereomicroscope and using botany dissection kit. Care was taken to avoid damaging the seeds or scarifying them. In most of the cases the elaiosomes were separated cleanly from the seed coat. The diaspores were taken to the laboratory, in small airtight containers for foods, to about 5–10 °C using a small portable refrigerator. Before starting of the experiment several seeds of dense–flowered corydalis were scattered on the ground near the nest trays to verify whether they were still attractive. Workers manipulated the seeds with their mandibles and carried those seeds to their nests. 1,344 only fresh elaiosomes were also offered to the ants, with the same procedure (eight other metal plates, each one with 42 fresh elaiosomes, for a total of two replicates per day, for two consecutive days).

2.4. STATISTICAL ANALYSIS

To determine if the germination success of seeds having elaiosome removed by ants is significantly higher than the two other treatments, we used the PEARSON's Chi–square tests of goodness–of–fit and independence (χ^2), indicates if the frequency of germination obtained is random or not.

χ^2 compare experimentally obtained results with those to be expected theoretically on some hypothesis), using the CHI–SQUARE CALCULATOR TURNER (http://turner.faculty.swau.edu/mathematics/math241/materials/contablecalc/). χ^2 is a non–parametric test because use nominal data (i. e. discrete categories) and ordinal set of data. Non–parametric tests use rank or frequency information to draw conclusions about differences between populations. χ^2 statistic can be applied to tables of counts (i. e. contingency table) that have a certain size. χ^2 uses a measure of goodness–of–fit which is the sum of differences between observed and expected outcome frequencies (that is, counts of observations), each square and divided by the expectation according to the following formula (JAMES, 2006; CHOW *ET AL.*, 2008).

$$\chi^2 = \sum_{i=1}^{k} \frac{(fO_i - fE_i)^2}{fE_i}$$

Where: $\sum$ = sum of all cells, the number of cells in the table, fO_i = an observed frequency (i. e. count) for bin i, and fE_i = an expected (theoretical) frequency for bin i, asserted by the null hypothesis.

The expected frequency counts are computed separately for each population at each level of the categorical variable, according to the following formula.

$$fE_{r,c} = \frac{(n_r \cdot n_c)}{n}$$

Where: $fE_{r,c}$ = the expected frequency count for population r at level c of the categorical variable, n_r = the total number of observations from population r, n_c = the total number of observations at treatment level c, and n = the total sample size.

The degrees of freedom (df) is equal to:

$$df = (r - 1) \cdot (c - 1)$$

Where: r = number of rows in the contingency table and c = number of columns in the contingency table.

3. RESULTS AND DISCUSSION

3.1. Study species: *Corydalis densiflora*

DISTRIBUTION IN SICILY. In Sicily dense–flowered corydalis is not common, but relatively frequent in oak and beech forests of the Madonie Mountains, in North–Western Sicily (Cuprania, Mount Carbonara, Mount Ferro, Mount Mufara, Mount Spina Puci, Mandria del Conte, Piano Battaglia, Piano Lungo, Pizzo Scalonazzo, Zottafonda valley), generally at altitude above 1,000 m ASL. Instead it is more sporadic in the beech forests (Cutò, Mount Soro, Piano Cicogna, Pizzo Nero, Portella Miraglia) of Nebrodi Mountains, in North–Eastern Sicily and in some mountains of Western Sicily such as

Rocca Busambra and Mount Cammarata (Sicani Mountains) where it is found in the clearings of holm oak forest. A doubtful report of *C. solida* subsp. *solida* by RONSISVALLE & SIGNORELLO (1979) for the North–Eastern side of Mount Etna (Fornazzo Nido d'Aquila, Pietracannone) is probably to be referred to *C. densiflora* (GIARDINA *ET. AL*., 2007).

GUSSONE's localities, 1828: Madonie, Mount Cammarata. LOJACONO–POJERO's localities, 1883: Cuprania, Mount Cammarata, Mount Ferro, Mount Soro, Rocca Busambra (1,600 m ASL), Pizzo Nero. RONSISVALLE & SIGNORELLO's localities, 1979: Fornazzo, Nido d'Aquila, Pietracannone. RAIMONDO's localities, 1980: Mount Mufara, Mount Spina Puci, Slopes of Pizzo Scalonazzo, Zottafonda valley. FEDERICO's localities, 2001, 2009: Mount Carbonara, Piano Battaglia, Rocca Busambra. GIARDINA's localities, 2008: Cutò near the Enel central, Mount Soro, Piano Cicogna. AUTHORS' localities, 2014: Mandria del Conte, Mount Cammarata, Mount Carbonara, Mount Soro, Piano Battaglia, Piano Lungo, Portella Miraglia (***FIGURE 5***).

TAXONOMY AND PLANT MORPHOLOGY. Basionym: *C. densiflora* C. PRESL (nomenclature reference in: PRESL, 1882); homotypic synonyms: *C. solida* (L.) CLAIRV. subsp. *densiflora* (J. & C. PRESL) ARCANG. (CONTI *ET AL*., 2005); *C. solida* subsp. *densiflora* (C. PRESL) HAYEK; *C. solida* var. *densiflora* (C. PRESL) BOISS.; heterotypic synonyms = *C. solida* subsp. *bracteosa* (BATT. & TRAB.) GREUTER & BURDET; *C. solida* var. *bracteosa* BATT. & TRAB. (LIDÉN, 2011). Common names: Italian = colombina a fiori addensati; French = corydale à fleurs rammasées; English = dense–flowered corydalis (LI VIGNI *ET AL*., unpublished data).

The species is a perennial herbaceous plant (bulbous geophyte) with a yellow tuber, filled, 8–10 mm in diameter, covered by membranous tunics. Each plant has a single simple, unbranched stem. The stem is erect, 50–170 mm tall, with a long, ovate scale near base, which occupies one third of its length, glabrous. The lower cauline leaf is reduced to a scale, the two upper leaves have pedicels of 10–20 mm length and a triangular shape of 30–35 mm width, 2–ternate with leaflets very broad and apically incised or deeply divided into 3–5 lanceolate segments of about 3 x 10–12 mm, the last more narrow. The leaves are glaucous. The flowers are hermaphrodite, disposed in a single very dense raceme of 8–16 flowers, 50–60 mm long, with lower bract of about 13 x 9 mm, cuneiform at base, deeply lobed into 5 or more lacinies, which are also 3–lobed. The upper bracts are short and broad, with lobes apically divided into short lobules. The corolla is 15–20 mm long, with a pallid pink color (rarely white), violet in the tips, with a spur usually slightly curved. The lower petal is 8–13 mm long, broadly obtuse, cuneate. The style is often geniculate. The fruit is a pod–like capsule, in which both leaves open, dry dehiscent, pendulous, glabrous, ellipsoid–flattened, restricted to the extremities, terminated by the persistent style, very short and straight, with capitellate stigma, yellowidh green in the mature fruit, length (9.24) $\bar{x}$ **10.64** ±*sd* 1.07 (13.80) mm (*n* = 82), width (4.82) $\bar{x}$ **5.07** ±*sd* 0.75 (5.90) mm (*n* = 82), containing (3) $\bar{x}$ **6.67** ±*sd* 1.96 (10) (*n* = 96) seeds with a large elaiosome, connected to the basal part of the seed body (micropyle–hilum–arilbase region: FUKUHARA, 1999) (***FIGURE 6***). The fruiting pedicel is recurved, glabrous, inserted in leaf axil, in the flowering time it is long (3.98) $\bar{x}$ **6.64** ±*sd* 0.95 (7.97) mm (*n* = 80), later (6.30) $\bar{x}$ **7.50** ±*sd* 0.42 (9.47) mm (*n* = 82) during the fructification.

Corydalis densiflora (LIDÉN, 2011) previously considered only at subspecific rank of *C. solida* (ZANGHERI, 1976; PIGNATTI, 1984; TUTIN, 1993; CONTI *ET AL*., 2005), is clearly distinct from this for several morphological features, such as leaves shape, bracts and the number of flowers and colors. In particular, *C. densiflora* differs from *C. solida* for the leaves more closely divided and intensely glaucous and even closer to emerald green, for the bracts more deeply divided in 3–lobed segments and for the more compact raceme, with a greater number of flowers and lighter in color.

In modern terminology adopted by SPJUT (1994) the fruit of *Corydalis* genus is called *ceratium* that is: siliquiformis capsule, one–celled, many–seeded, superior, linear and dehiscent by two valves separating from the replum; seeds attached to two spongy placentae adhering to the replum and alternate with the lobes of the stigma. Differs from the siliqua in the lobes of the stigma being alternate with the placentae, not opposite (LYNDLEY, 1848) (***FIGURE 7***).

DIASPORE ANATOMY. Seeds of *Corydalis* Italian species are similar in shape (non–angular, from circular to circular–reniform, rarely reniform: *C. capnoides*; margin obtuse, slightly flattened at the sides, transverse section elliptic), surface (glabrous and smooth, rarely indistinct areolate: *C. pumila*) (BOJŇANSKÝ & FARGAŠOVÁ, 2007), hilum (conspicuous, protruding laterally, central or sub–central, arillate), color (shiny–black), and color of elaiosomes (fresh: whitish or translucent; dry: brownish–beige), but the sizes of seeds differ, as well as also the sizes and shapes of the elaiosomes.

Their mean values of the largest diameters are significantly different (seeds ordered from largest to smallest): *C. cava* = (2.12) $\bar{x}$ **2.72** ±*sd* 0.37 (3.15) mm (n = 100); *C. pumila* = (1.92) $\bar{x}$ **2.24** ±*sd* 0.23 (2.60) mm (n = 90); *C. intermedia* = (1.88) $\bar{x}$ **2.06** ±*sd* 0.17 (2.41) mm (n = 94); *C. densiflora* = (1.65) $\bar{x}$ **1.98** ±*sd* 0.21 (2.38) mm (n = 100); *C. capnoides* = (1.72) $\bar{x}$ **1.93** ±*sd* 0.15 (2.10) mm (n = 92); *C. solida* = (1.30) $\bar{x}$ **1.74** ±*sd* 0.27 (2.10) mm (n = 100). Weight of seeds of *C. densiflora* = (0.089) $\bar{x}$ **0.098** ±*sd* 0.007 (0.109) g (n = 100). Elaiosomes of *C. densiflora* are fleshy, flattened wing–shaped, tightly adhering to seeds, set below the hilum; length = (1.48) $\bar{x}$ **1.80** ±*sd* 0.84 (2.58) mm (n = 97); width: (0.92) $\bar{x}$ **1.24** ±*sd* 0.16 (1.45) mm (n = 97); weight: (0.025) $\bar{x}$ **0.031** ±*sd* 0.012 (0.037) g (n = 100). Weight ratio of mean elaiosome/seed of *C. densiflora* = 0.316.

Recent anatomical studies of Gorb & Gorb (2003) have revealed a funicular origin of the elaiosomes of *Corydalis* genus (a previous study of Fukuhara (1999) had assumed that in *Corydalis* genus the elaiosomes develop from a raphal protuberance). This fact led us to consider that the elaiosome of *C. densiflora* is a true aril in accordance to Gorb & Gorb (2003) and to Tahtadzhjan (1985). In *Corydalis* spp. longitudinal anatomical sections of diaspores, it is clear that the elaiosome is composed of various cell types: the basal part consist of small round–shaped cells, the apical part – of large elongated cells. A cell group with thickened perforated cell walls is situated in the central part of the elaiosome (Gorb & Gorb, 2003). The last cell group presumably serves a transporting function (Gorb & Gorb, 2003). Generative diaspores, adapted to ant–dispersal, usually bear a rather solid integument to avoid damage by worker ants. However, *Corydalis* seeds have relatively thin spermoderm which may be easily damaged by ant mandibles during manipulation of seeds. Anatomical studies have revealed a group of small thick–walled cells at the base of the elaiosome. Presumably, these cells provide easy breakage of the elaiosome and thus a decrease in the time of manipulation with the seed after its transport from the seed depot (Gorb & Gorb, 2003).

The morphology of the seeds plays a role in improving diaspores' removal rates by ants (Pfeiffer *et al.*, 2007). Gomez *et al.* (2005) have demonstrated that the structure of the elaiosome can serve as a handle for the ants and may improve the handling qualities of the seeds. Thus some of the seeds, e. g. *C. densiflora*, which have a completely smooth surface, are hard to be moved by the ants when the elaiosome is missing (Pfeiffer *et al.*, 2007).

SEED GERMINATION IN CONTROLLED CONDITIONS. The seeds of *C. densiflora* have germinated *ex situ* in about 60 days from seeding, as soon as they are ripe. Percentage of germination: intact seeds = 51%, seeds without elaiosome manually removed in laboratory = 40.6%, and seeds without elaiosome removed by ants = 78%. Elaiosome removal increased germination success by at least 27% (χ^2_{2df} = 151.651, *P*–value = 0.0000) (**FIGURE 8** and **TABLE 2**).

Timing and location of seedling emergence are crucial events in the plant life cycle and determine its future success (HARPER, 1977). The optimal timing for seedling emergence is species–specific and depends on several variables, such as the plant life cycle, habitat preference and geographical distribution (NIKOLAEVA, 1999). Seed dormancy is considered the most important mechanism for cueing germination and seedling emergence (FENNER & THOMPSON, 2005). It has long been known that some species have ripe seeds containing an underdeveloped embryo, which must reach a critical size inside the seed prior to germination (FINDEIS, 1917). The resulting lag time has been termed morphophysiological dormancy (MPD), in cases where an additional physiological mechanism delaying embryo growth is present (NIKOLAEVA, 1977). Studies on seed dormancy showed that seeds of species with MPD are frequent in perennial herbs of temperate deciduous forests (e. g. KONDO *ET AL.*, 2005; VANDELOOK & VAN ASSCHE, 2008). Following completion of embryo growth and prior to germination, seeds of *C. solida* go through a resting period (LIDEN & STAAF, 1995). A similar resting period during winter was observed in seeds of *C. ambigua* (KONDO *ET AL.*, 2005).The ripe seeds of *Corydalis* species contain an underdeveloped embryo at the moment of dispersal, consisting of no more than a clump of cells (RYBERG, 1959). In nature, seeds are dispersed in spring, while elongation of the embryo starts in the autumn, and continues in winter (LIDEN & STAAF, 1995). Germination starts in late winter, immediately after embryo growth is completed, resulting in seedling emergence in the following spring (VANDELOOK & VAN ASSCHE, 2009). Seeds of *C. solida* subsp. *solida* germinate irrespective of light conditions (JANKOWSKA–BLASZCZUK & DAWS, 2007). Germination and seedling emergence is similar in *C. solida* subsp. *solida*, *C. ambigua* CHAM. & SCHLTDL. (KONDO *ET AL.*, 2005), *C. ledebouriana* KAR. *ET* KIR. (LIDEN & STAAF, 1995), *C. cava* (FINDEIS, 1917), and *C. pumila* (STOLLE, 2004). The dormancy mechanism is very similar in *Corydalis* species studied thus far.

3.2. STUDY SPECIES: ANT DISPERSERS

The elaiosomes attract potential animal seed dispersers: probably small animals, e. g. insects and especially Formicidae (LI VIGNI & MELATI, 1999). It is known that the granivorous ants can act as seeds dispersers (GOMEZ & ESPADALER, 1998; RETANA *ET AL.*, 2004). Workers of *Messor capitatus* were commonly associated with the dense–flowered corydalis and their nests were frequently located at the base or near the plants. The seed dispersers observed in Pizzo Carbonara were substantially the Mediterranean harvester ant workers of this species. This species is likely to be the main source of diaspores removal in all treatments because no other invertebrates have been observed removing *C. densiflora* seeds.

Messor is a genus with more than 100 species, all of which are harvester ants; the generic name comes from the Roman god of crops and harvest, *Messor*. *M. capitatus* is the largest European species of the genus (total length (n = 93) 3.52–13.04 (10.32) mm). *Messor* spp. have a distinct caste of macrocephalic dinoergates whose role is of carrying and cutting the large seeds which comprise much of the colonies' subsistence. *M. capitatus* has a shiny black color and only in specimens not yet mature the head is brown. The propodeum angled is distinctiveness of the species.

Harvesting ants use various methods for seed transport. Method of seed holding during transport is determined by the relationship between the morphological feature and size of diaspores, and by the size of an ant worker (BERNARD, 1971; JOHNSONS, 1991; GORB & GORB, 2003). Since ant species of the deciduous forest differ in size, some interspecific differences in transporting methods exit. To remove the diaspore from the depot, an ant selects the region of the diaspore, the size of which best corresponds to an optimal span of the ant mandibles (GORB & GORB, 1995, 2003). In the case of *C. densiflora*, we observed that worker ants take the seeds by elaiosomes during transport (***FIGURE 9***).

Ants of the genus *Messor* inhabit dry areas and regularly include seeds as a primary element of their diet. Several species of this genus, like many other harvesting ants, employ a mixed foraging strategy consisting of both individual foraging and column retrieval: if seeds are dispersed or food items are small enough to be transported singly, the scout, after having located one of them, carries it back to the nest; when a patch of seeds is discovered or the food items are too large to be carried by a single ant, the scout returns home and recruits nestmates (HÖLLDOBLER & WILSON, 1990). *Messor capitatus* is a common seed–harvesting ant with a broad Mediterranean distribution (BERNARD, 1968). BARONI–URBANI *ET AL.* (1987, 1988) analyzed the behavior of successful scouts of *M. capitatus* which, once in the nest, performed a typical "motor display" (characterized by running, body vibrations, interindividual body contacts, self–grooming, food transmission) while recruiting nestmates. A peculiar element of the behavior of this species is sound production by stridulation, performed by the recruiting ants near the food source and probably also inside the nest. Sounds produced by *M. capitatus* workers increase the success of recruitment and the efficiency of food retrieval.

GRASSO *ET AL.* (1998) have verified that, when a conspicuous food source is discovered, the scouts of *M. capitatus* return home dragging their abdominal tip over the substrate and within a few minutes recruit nestmates. However, before following the trail to the food, ants inside the nest need to be elicited by the recruiter scout. In fact, these behavioral observations showed that successful scouts, once in the nest, ran among nestmates (often touching them) and shook their own body vigorously. As soon as a scout entered the nest and this motor display has been performed, the activity inside the nest increased rapidly; many ants rushed out and moved towards the bait. During this phase of the recruitment process, the ants are likely to produce acoustical signals by stridulation. However, sounds produced by ants generally do not convey specific signals but act to modulate signals transmitted through other sensory channels (HÖLLDOBLER & WILSON, 1990). Indeed, GRASSO *ET AL.* (1998) have verified that, in *M. capitatus*, the recruitment process inside the nest is also strongly affected by the emission of chemical signals from DUFOUR's gland that induced a significant increase of the number of exits. Once out of the nest, the ants moved towards the bait following the return path of the scout, which initially might also remain in the nest. Thus, in *M. capitatus*, the presence of a leader to guide the nestmates to the feeding site is not necessary, as is typical for mass–recruiting ants (BARONI–URBANI, 1993). The results of GRASSO *ET AL.* (1998) show that the DUFOUR's gland secretions of *M. capitatus* workers have both a recruiting and an orienting effect on nestmates. Finally, there are many evidences that *M. capitatus* foragers no longer rely on chemical substances but also on celestial (i. e. solar) orienting cues. Moreover, it is possible that these ants – as the foragers of *Pogonomyrmex* spp. – resort also to the memory of visual landmarks experienced during their journeys (GRASSO *ET AL.*, 1998).

3.3. FIELD EXPERIMENTS

At the end of the second hour after the start of the self–service experiments with diaspores, we observed that almost all seeds with elaiosome were collected by ants (in total 1,092 out of 1,344 – i. e. 81.25%), while the ants ignored nearly totally seeds without elaiosome (only 40 out of 1,344 – i. e. 2.98% – seeds without elaiosome were collected by the ants). Two hours after the beginning of the "cafeteria" experiments with detached fresh elaiosomes, about half (726 out of 1,344 – i. e. 54.02% of detached elaiosomes were collected by the worker ants). This last anomalous result could be explained by considering the lack of the visual stimulus of the elaiosome that contrast with the dark body of the diaspore. In this case, investigations are needed. The elaiosomes are also short lived and dry out quickly in a dry atmosphere; the ants we observed generally had not collected the seeds with dry elaiosome. The entire seeds with elaiosomes have been shown in tests significantly more effective for ants than the other two tested baits (seeds without elaiosome and elaiosomes alone): χ^2_2 = 1,708.167, *P*–value = 0.0000 (***FIGURE 10*** and ***TABLE 3***).

SEED DISPERSAL. Seed can be dispersed in various manners; this is normally dependant on the physical characteristics of the seed or seed coat or the fruit containing the seed. Seed dispersal modes are usually classified into five broad types (syndromes): unaided (e. g. passive, ballistic), by wind (anemochory), by water (hydrochory), by vertebrates externally (e. g. on fur: exozoochory) or internally (passing through the gut: endozoochory), and myrmecochory (by ants) (PIJL VAN DER, 1982).

Corydalis densiflora is a diplochorous plant, i. e. seed dispersal involves two steps. After fruit dehiscence, seeds are scattered around the parent plant on the ground (the first step = autobarochory: a type of autochory – which does not require dispersal agents – when diaspores fall off through gravity and fall off to the explosive launch of the ripe fruit: LEVINA, 1957, 1967). The seeds are then removed by ants from the soil surface (the second step = myrmecochory), as for almost all other species of *Corydalis*. Thus, each seed dispersal step gives advantages for seedlings: the autobarochory decreases the influence of the parent plant on seedlings, by dispersing the seeds 10–15 cm away from the parent plant, while the myrmecochory reduces competition between seedlings, by relocating the seeds at a distance of 200–300 cm from the seed depot (GORB & GORB, 1997, 2003; SERVIGNE & DETRAIN, 2008). The fruiting, which occurs in mid–spring (mid–May), produces capsules which explode elastically as soon the seeds mature (approximately 20 days after anthesis). After fruit ripening, pedicles decline to the soil surface. Seed producing capacity ranges from 30–90 seeds per plant. Seeds are usually accessible in group to ants (= obligate myrmecochores: GORB & GORB, 2003). The fruits are splits for dehiscing by two slender flexible valves. The ripe capsule explodes naturally or when disturbed, spreading seeds at a short distance (the presence of elaiosome alters the seed aerodynamic, reducing the distance of explosive dispersal: BEATTIE & LYONS, 1975).

In a similar way to ants (partial carpophagy of the diaspore with consequent indirect seed dispersal), various phytophagous insect, above all coleopteran, attracted by the nutritive properties (elaiosomes) of myrmecochorous seeds and fruits, can contribute to dispersal. Ants are likely to be the main source of diaspores removal in all treatments because no other invertebrates were observed removing diaspores. Ants are undoubtedly the major of invertebrate taxa seed dispersers (e. g. BEATTIE, 1985; BOULAY *ET AL.*, 2006; LI VIGNI & MELATI, 1999; PFEIFFER *ET AL.*, 2007; RICO–GRAY & OLIVEIRA, 2007). Cases of non–Formicidae entomochorous dispersal discovered in the course of bio–ecological observations in the Mediterranean regions are to be considered random and not specific, as opposed to the large insect's diversity of the tropical forests that contribute significantly to seed dispersal (LI VIGNI & MELATI, 1999).

It is probable that the wind (HANZAWA, 1985) and periodic precipitation may facilitate dispersal of *C. densiflora* seeds at maturity (LI VIGNI *ET AL.*, unpublished observation).

The function of the elaiosome in other species of *Corydalis* has been well–studied as a means to understand the evolution and importance of myrmecochory in temperate floras (BEATTIE & CULVER, 1981; FARNSWORTH, 2001; GORB & GORB, 2003; HANZAWA *ET AL.*, 1985; NAKANISHI, 1994). Ants transport the diaspores of *Corydalis* sp. pl. to nutrient–rich nest sites favorable for germination (BEATTIE *ET AL.*, 1979). BEATTIE & CULVER (1981) documented that 80% of the seeds of *C. flavula* (RAF.) DC. were dispersed by ants (*Aphaenogaster rudis* ENZMANN, J., 1947) in the mountains of Western Virginia. They saw no evidence that *C. flavula* is dispersed by agents other than ants. Many authors have suggested that ants facilitate seed germination and seedling establishment by moving seeds to nutrient–rich nests and burying intact seeds once they have consumed the elaiosome (BEATTIE *ET AL.*, 1979; HANZAWA *ET AL.*, 1985). A study of the evolution of myrmecochory in seven *Corydalis* species of Japan suggests the potential importance of ants to the dispersal and survival of propagules of annuals like *C. flavula*. *Corydalis* species that rely exclusively on ant dispersal are generally annuals or biennials (without a persistent seed bank) that inhabit warm–temperate Japan (NAKANISHI, 1994). The elaiosome of *C. aurea* serves a dual function, promoting dispersal by ants while repelling herbivory by its main seed predator, *Peromyscus manulatus* (WAGNER, 1845), the deer mouse (HANZAWA *ET AL.*, 1985).

Future studies on *C. densiflora* should address the effect of elaiosome predation by other ant species. Future studies should also assess the availability of suitable sites (e. g. through introduction experiments in apparently suitable but unoccupied sites: VAN DER VEKEN *ET AL.*, 2007) in an attempt to fully understand the factors responsible for its restricted distribution.

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***FIGURE 1* – STUDY SITE.** Satellite images at different altitudes: **A** European–Mediterranean region (3,414.78 km); **B** Sicily, island of Southern Italy, in the Mediterranean Sea (300.60 km); **C** Madonie Regional Natural Park (54.96 km). Date of acquisition of images: **A** and **B** = 04/10/2013; **C** = 07/15/2011. **D** Map of Madonie RNP with the highlighted study site (Pizzo Carbonara). (Images © **A**, **B**, and **C** Landsat US Dept of State Geographer – Google Earth, 2014; **D** Internet source www.linksicilia.it, 2013)

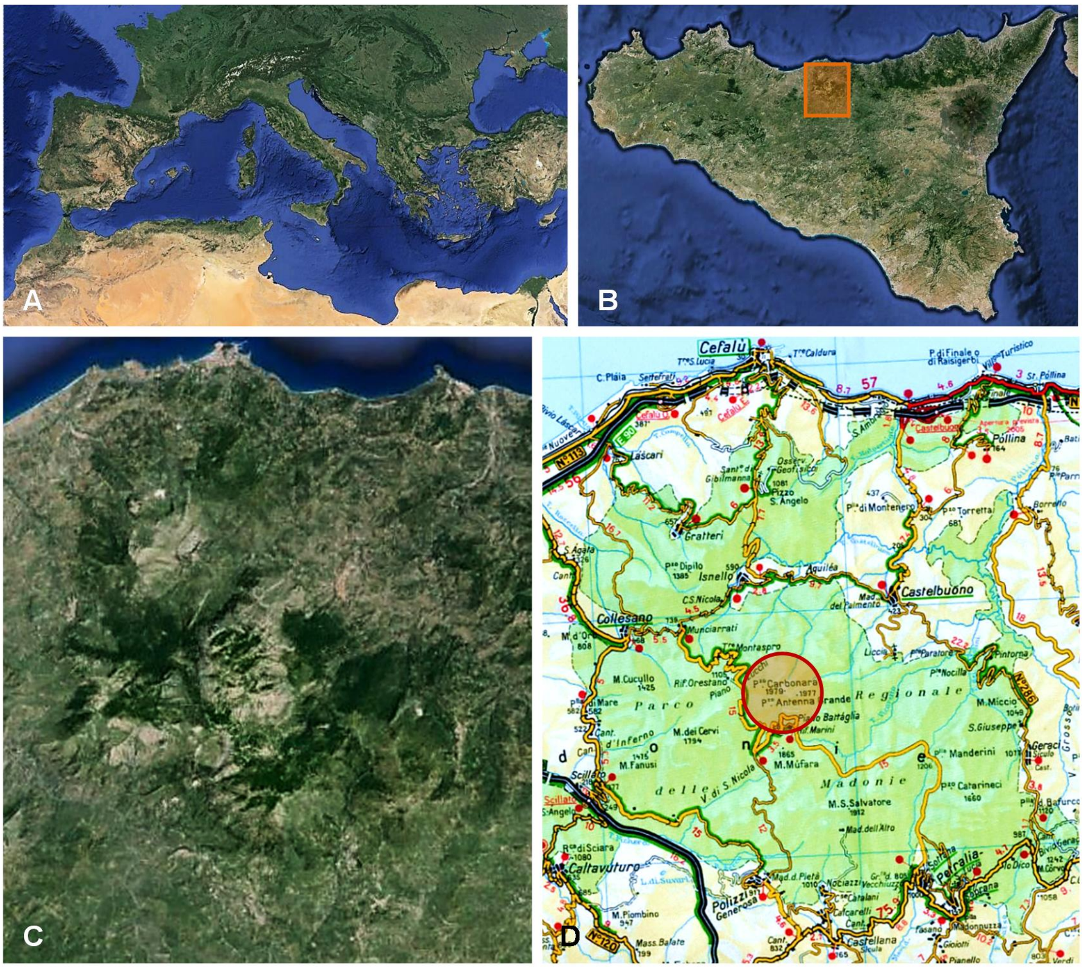

FIGURE 2 **– PANORAMIC VIEW OF THE STUDY SITE. A** Beech forest on of Pizzo Carbonara. **B** Still covered with snow dolines. **C** Community of *Cachrys ferulacea* (L.) CALEST. on the exposed slopes **D** Top of Pizzo Carbonara (1,979 m ASL). (Photos © CAMBRIA, 2013)

***TABLE 1* – SAMPLING STATIONS.** Geographic coordinates (longitude and latitude in Degrees, Minutes, and decimal Minutes) and altitudes (m Above Sea Level), recorded at the center of the sampling stations, were obtained using a cartographic GPS unit provided with altimeter, by Garmin (USA), model eTrex Vista HCx, with software Land Navigator Italy, with digital topographic maps, scaling 1:50,000 complete of geographical locations.

STATION	COORDINATES	ALTITUDE	HABITAT
Above Piano della Battaglietta	Long. 14°01.745' E Lat. 37°52.606' N	1,633	In the undergrowth of Beech forest, a mesophilous community where the dominant tree is *Fagus sylvatica* L., with the sporadic presence of *Acer pseudoplatanus* L. and *A. campestre* L.
Southern slopes of Pizzo Carbonara beetween Piano della Battaglietta and Fosse di San Gandolfo	Long. 14°02.206' EW Lat. 37°53.058' N	1,737	Calcareous soil in degraded communities of *Astragalus nebrodensis* (Guss.) Strobl
Southern slopes of Pizzo Carbonara	Long. 14°02.215' E Lat. 37°53.052' N	1,748	Degraded vegetation with *Geranium pyrenaicum* Burm. f. subsp. *pyrenaicum*, *Aethionema saxatile* (L.) R. Br. subsp. *saxatile*, *Valeriana tuberosa* L., *Helianthemum nummularium* (L.) Mill. subsp. *nummularium*
Southern slopes of Pizzo Carbonara	Long 14°02.270' E Lat 37°53.099' N	1,752	Beech forest with a rich undergrowth of *Muscari neglectum* Guss. ex Ten.
Southern slopes of Pizzo Carbonara	Long. 14°01.875' E Lat. 37°53.154' N	1,850	Beech forest with *Scilla bifolia* L.
Near a locality known as Fosse di San Gandolfo	Long. 14°01.693' E Lat. 37°53.301' N	1,875	At the edge of beech forest with *Gagea fragifera* (Vill.) Ehr. Bayer & G. López and *Scilla bifolia* L.
Near the top of Pizzo Carbonara	Long. 14°01.689' E Lat. 37°53.346' N	1,887	In an open site, on a poor substrate, with *Viola nebrodensis* C. Presl

FIGURE 3 **– SYNOPTIC COMPARISON OF THE DIASPORES OF ITALIAN *CORYDALIS* SPECIES. A** *C. cava* subsp. *cava* (hollow–root corydalis). **B** *C. pumila* (minor corydalis). **C** *C. intermedia* (intermediate corydalis). **D** *C. capnoides* (cream corydalis). **E** *C. solida* subsp. *solida* (solid–tubered corydalis). **F** *C. densiflora* (dense–flowered corydalis). Diaspores in lateral view; scale bar in mm. (Drawings © LI VIGNI, 2013)

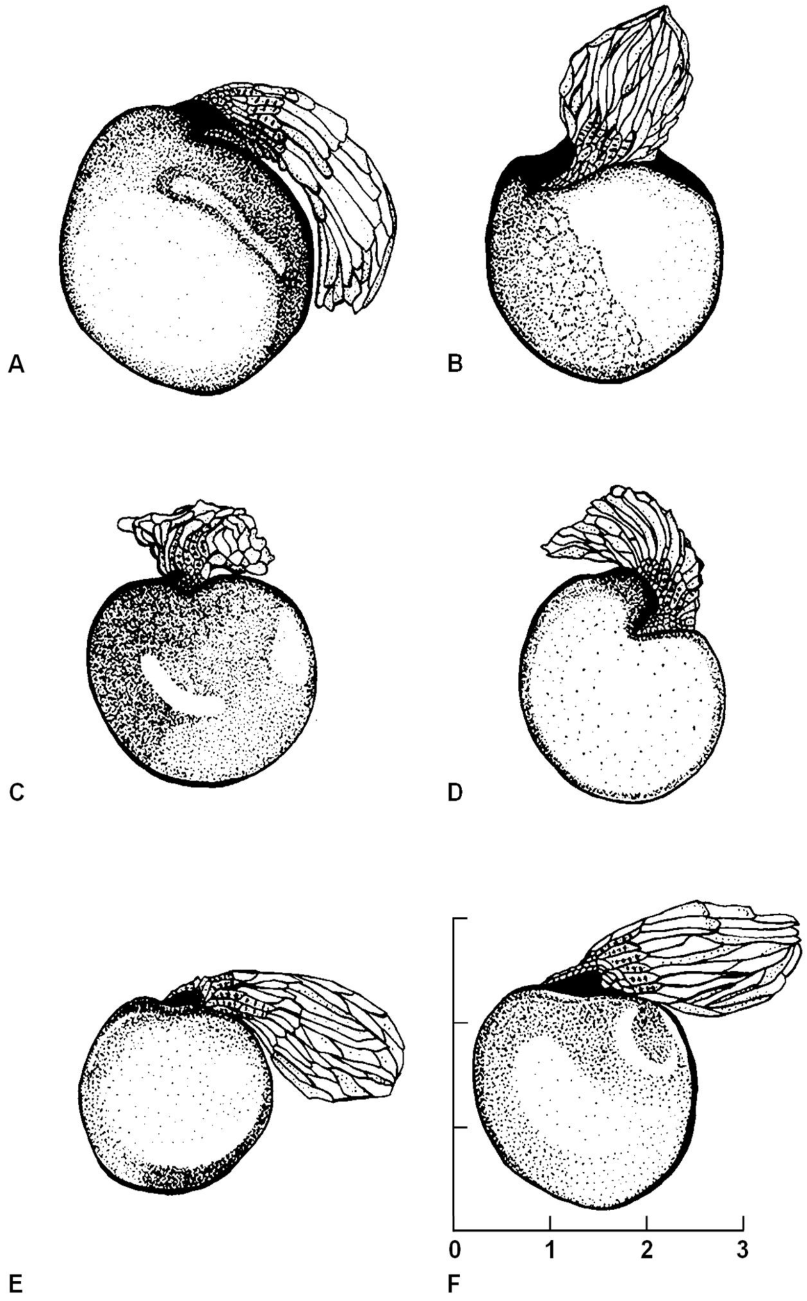

***FIGURE 4* – PHASES OF THE CAFETERIA EXPERIMENT. A** Elaiosomes are manually removed in the laboratory under a stereomicroscope, using forceps and needle mounted on the handle. **B** Diaspore of *Corydalis densiflora* in lateral view: dashed line = point of detachment of the elaiosome, near the protruding hilum; scale bar = 0.5 mm. **C** and **D** Explanation in the text. (Drawings © Li Vigni, 2013)

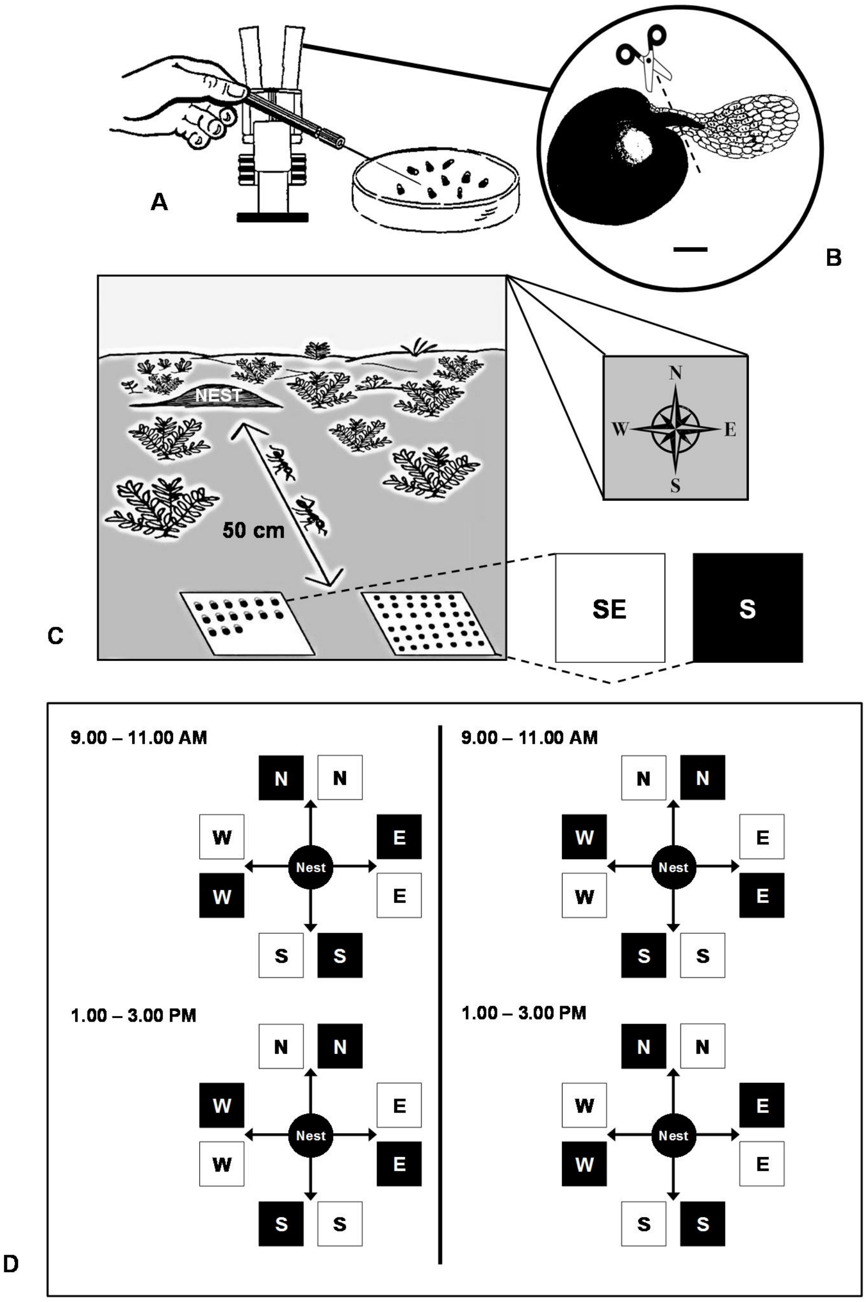

***FIGURE 5* – DISTRIBUTION OF *CORIDALYS DENSIFLORA* IN SICILY.** The representation on the map of localities reported in literature and field observed was done indicating the different degrees of geographical accuracy of the data.

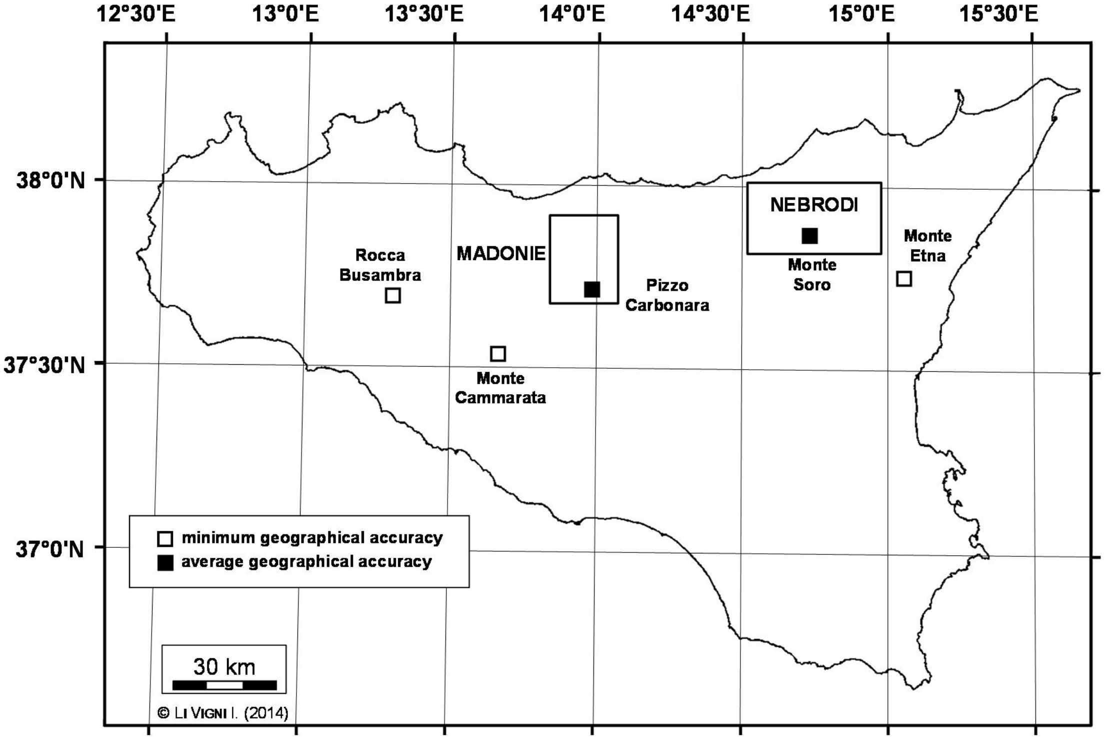

FIGURE 6* – DEHISCENT CAPSULE AND ARILLATE SEEDS OF *CORYDALIS DENSIFLORA. (Photos © LI VIGNI, 2013)

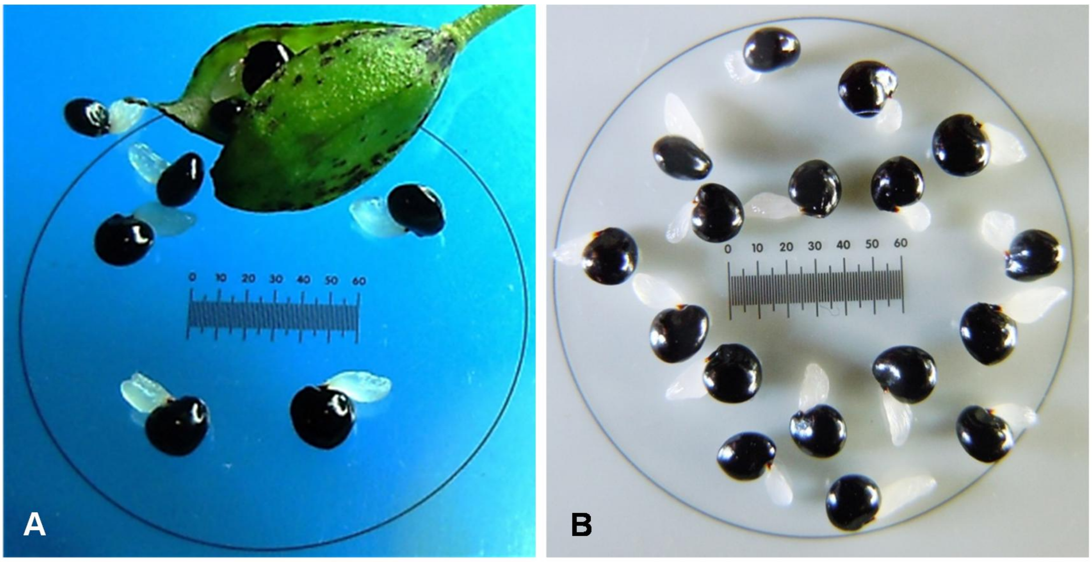

***FIGURE 7* – HABITUS OF *CORYDALIS DENSIFLORA*. A** Dense raceme of flowers. **B** Leaves closely divided. **C** Unripe fruits. **D** Ripe fruits with persistent style and capitellate stigma. (Photos © **A** and **C** Li Vigni, 2013; **B** and **D** Cambria, 2013)

***FIGURE 8* – GERMINATED SEEDS IN SEEDING DIFFERENT CONDITIONS (*IN VITRO*).**

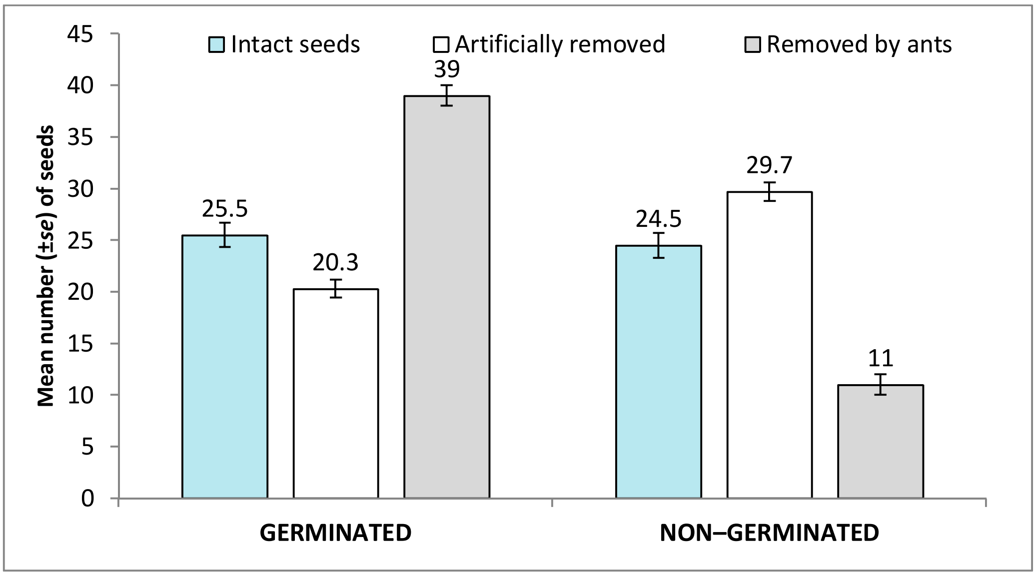

***TABLE 2* – GERMINATION FREQUENCIES AND PERCENTAGES OF *CORYDALIS DENSIFLORA* SEEDS WITH INTACT, ARTIFICIALLY, AND ANT–REMOVED ELAIOSOMES.** χ^2 calculations two–tailed 2 x 3 contingency table. Observed frequencies in normal font–style. Expected frequencies *in italic font–style*. Individual χ^2 values in parentheses. *P*–value = probability, *n* = sample size.

SEEDING CONDITIONS (ELAIOSOME MANIPULATION)	**GERMINATION FREQUENCY**		***n***	**GERMINATION PERCENTAGE**
	GERMINATED	**NON–GERMINATED**		
Intact seeds	255 *282.67* (2.71)	245 *217.33* (3.52)	500	51%
Seeds without elaiosome artificially removed	203 *282.67* (22.45)	297 *217.33* (29.20)	500	40.6%
Seeds without elaiosome removed by ants	390 *282.67* (40.76)	110 *217.33* (53.01)	500	78%
Total	848	652	1,500	
$\chi^2_2 = 1{,}708.167, P\text{–value} = 0.0000$				

FIGURE 9 **– METHOD OF DIASPORE TRANSPORTING IN *MESSOR CAPITATUS*.** *Messor capitatus* (Myrmicinae: Pheidolini) worker transport an elaiosome–seed of *Corydalis densiflora* to the nest at Pizzo Carbonara. **A** Worker in lateral view. **B** Worker in posterior–lateral view. **C** Worker in frontal view. Arrows indicate elaiosomes; S = seed; scale bar = 2 mm. (Photos © LI VIGNI, 2013)

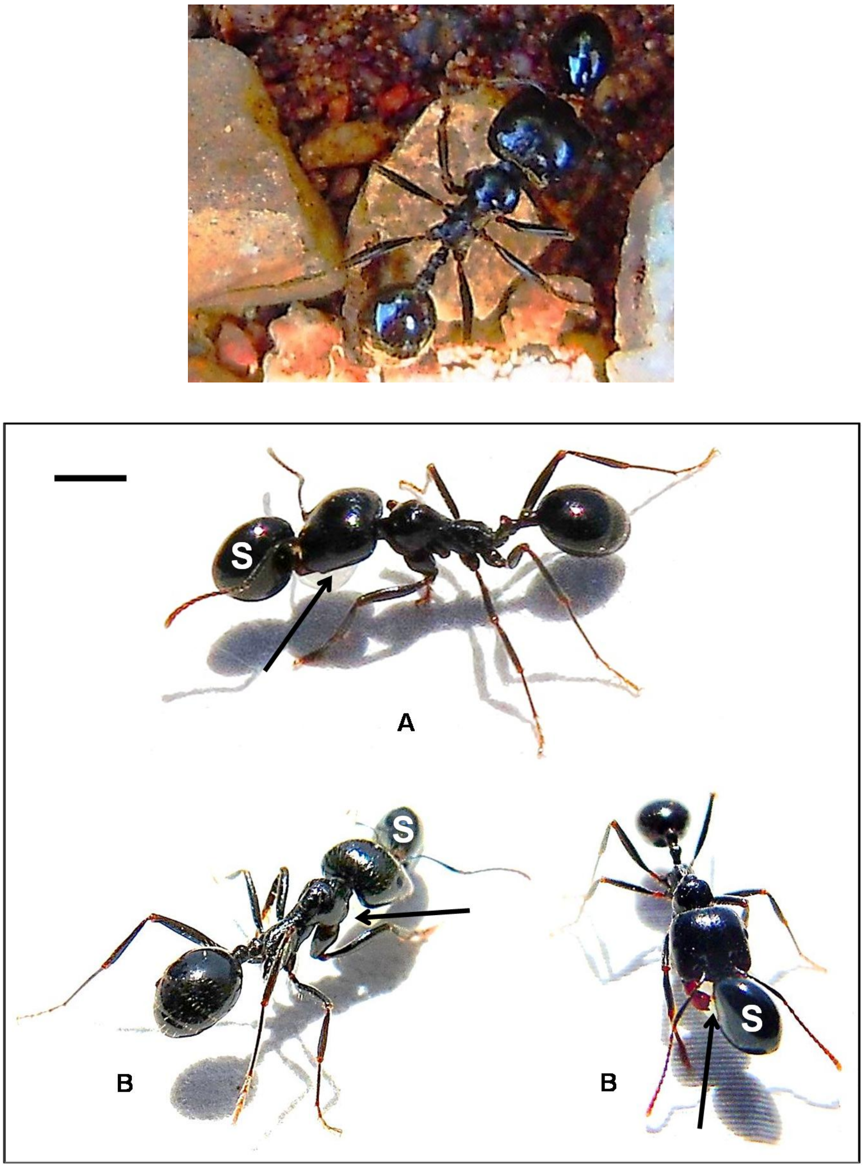

***FIGURE 10* – REMOVAL OF BAIT TYPES BY ANTS DURING THE SELF–SERVICE EXPERIMENTS.** Cafeteria experiment was reproduced twice a day, at the same hours for six days, in sunny and windless days, during 2013 spring. (Bar chart legend: SE = seeds with elaiosome, E = elaiosomes alone, S = seeds without elaiosome artificially removed. Error bars represent standard errors of the means)

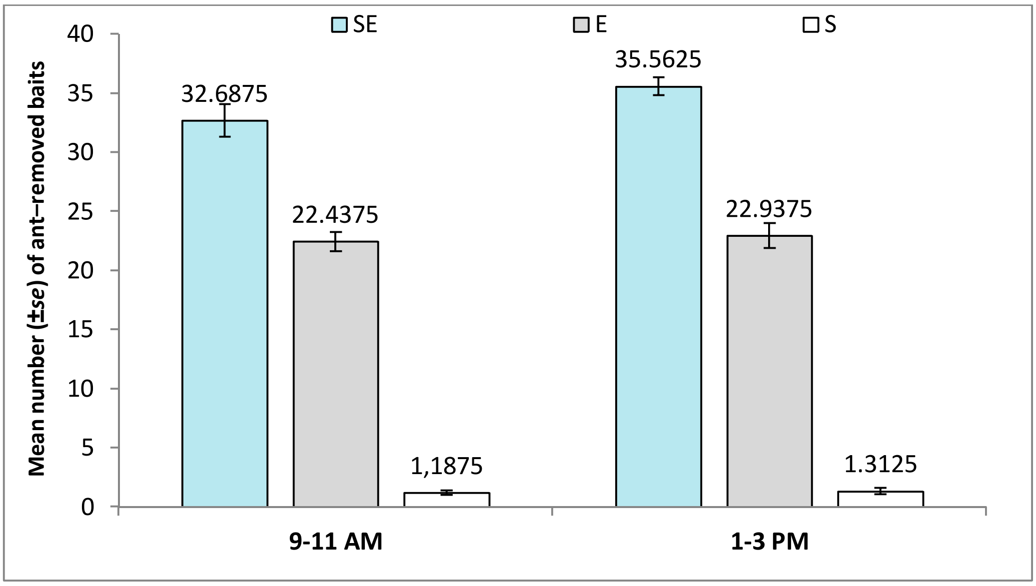

***TABLE 3* – REMOVAL FREQUENCIES AND PERCENTAGES OF *CORYDALIS DENSIFLORA* SEEDS WITH INTACT AND WITHOUT ELAIOSOME, AND ELAIOSOMES ALONE.**

CAFETERIA EXPERIMENT (BAIT TYPES)	REMOVAL FREQUENCY		*n*	REMOVAL PERCENTAGE
	REMOVED	IGNORED		
Intact seeds	1,092 *619.33* (360.73)	252 *724.67* (308.30)	1,344	81.25%
Elaiosomes alone	726 *619.33* (18.37)	618 *724.67* (15.70)	1,344	54.02%
Seeds without elaiosome artificially removed	40 *619.33* (541.92)	1,304 *724.67* (463.15)	1,344	2.98%
Total	1,858	2,174	4,032	
$\chi^2_2 = 1{,}708.167$, P–value = 0.0000				

Conclusions and future outlooks

> "The seeds or fruits of myrmecochorous plants are provided with an appendage, which serves as a bait to ants, called eleosome. This condition has greatly contributed to the diffusion of plant species, while those seed differentiations of plants were not essential to ants. We can say therefore, that the existence of myrmecochory has been helpful essentially to the plants, and that instead the ants have not modified at all."
>
> EMERY C. (1915)

From a seed's or seedling's point of view most sites of the landscape are unsuitable for establishment and the environment is hostile, especially in the vicinity of the parent plant. Therefore, plants have evolved sundry dispersal modes to transport seeds actively or passively to suitable microsites. Such dispersal vectors include wind, water, ballistic powers, gravity, man or animals such as birds, mammals, reptiles, and invertebrates with ants as the most important group, and many plants make use of several dispersal modes within the same seed or by the production of different seed types (polychory, mostly diplochory).

The structure of an ecosystem defines the predominant dispersal modes, e. g. the availability of animals, water or wind for dispersal. Animals can be effective dispersers, but their presence and abundance is often closely related to the conditions of an ecosystem. Seed dispersal can be limited by the availability of dispersers or by the high abundance of seed predators.

Anthropogenic–driven alterations of ecosystems can lead to the loss of these dispersers, which may have detrimental effects on the plant species lacking dispersal. Prior to or after initial dispersal, seeds may face a variety of possible destinies, including death due to various hazards (predation, loss to pathogens, fatal germination at depth, loss of viability with age), secondary dispersal, dormancy or germination. Again, the ecosystem has an impact on the fate of seeds.

Myrmecochory potentially provides various advantages for plants which suggest that traits enhancing diaspore collection may be under selection by ants. Plants may attain selective advantage from myrmecochory in several ways:

a) DIRECTED DISPERSAL – ants displace seeds to sites where the plant fitness is higher than it would be in a random location, most likely due to nutrient enrichment at or near the ant nest –

b) PREDATOR–AVOIDANCE – dispersal, and burial of seeds by ants reduce the ability of seed predators to locate, and obtain seeds –

c) DISTANCE DISPERSAL – seed dispersal reduces parent–offspring conflict and sibling competition –

d) FIRE–AVOIDANCE – seed burial by ant's increases survival through fire and/or post–fire performance of seeds, seedlings and adults –

e) NUTRIENT LIMITATION – the production of elaiosome, which has low potassium content, may be favored in environments such as the extremely nutrient–poor regions of Australia and Southern Africa, where production of potassium–rich dispersal units (e. g. fleshy fruits) is prohibitively costly.

The work carried out highlighted some interesting novelties and lays foundation for future researches:

An analysis carried out on a sample of myrmecochores of the Sicily has highlighted that they tend to flower earlier than other plant species of the flora. This would confirm that the ants may have exerted selective pressures on the phenology of myrmecochores towards an earlier flowering. Empirical data show that in the study areas the larger ant species (greater than 8 mm) disperse seeds further than do smaller ants. Besides, many Sicilian endemisms appear to be involved in this process of dispersion.

The ants carry the seeds to their nests, but discard them unharmed soon after removing the elaiosome. There are however other ant species that carry seeds to their nests with the purpose of preying them (granivorous species). These ants, named harvester ants, collect a large number of seeds to their nests and usually destroy almost all of them. A number of works has highlighted the impact of harvester ants as post–dispersal seed predators and their role in shaping plant communities composition and structure. Furthermore, several authors have found evidence of the negative impact of harvester ants on plant recruitment, particularly in several endangered. It would be interesting to clarify the real role as seed dispersers of harvester ants. It would seem that the harvester ants such as *Aphaenogaster* spp. and *Messor* spp. in the Mediterranean basin in relation to the diaspores with elaiosomes, contribute significantly to dispersal (in other areas of the World are, essentially, seed–predators).

Explanations are needed for the cases, such as in *Corydalis aurea* WILLD., when far more seeds are produced by the plants growing on ant nests.

The content of elaiosomes differs between species. Elaiosomes consist of sugar, lipids, and proteins and attract ants mainly through diglyceride and triglyceride components that mimic insect prey. In the elaiosomes of some species lack proteins or carbohydrates. Essential nutrients for ants, like the amino acids cystine and methionine, were found in *Hardenbergia violacea* (SCHNEEV.), and the fatty acids oleic and linoleic acid occur in *Ulex minor* ROTH., and *Ulex europaeus* L. Hence, future studies about myrmecochory in ants should consider the respective elaiosome nutrient content, since this might result in different adjustment responses for the colony.

There is limited knowledge about the presence of elaisomes and ant behaviour affecting seed dispersal and how ant–plant interactions evolved under various plant traits. In order to better understand the evolution of myrmecochory in many species, future studies should experimentally address how various plant traits affect the proportion and outcome of ant–plant interactions (including germination and establishment success after dispersal), and how they evolve across space and time. Further detailed studies focused on how the presence or absence of the elaiosome, and on how the quantitative variation of other seed traits affect seed removal, and ant behaviour are needed to better understand the seed removal patterns of many species. Additionally, future studies should also address the effect of elaiosome predation by other ant species. However it is highly unlikely that myrmecochory represents an outcome of diffuse or guild coevolution, as no reciprocity can be inferred. Elaiosomes are just food items to ants, which display no obvious adaptations to myrmecochory. Thus, this fascinating form of seed dispersal appears to be the result of plant evolution, as a result of selection from ants in general and not of coevolution of plants and specific ants.

Seed dispersal is a key process for the invasion of new areas by exotic species. Acacias present elaiosome–bearing seeds and – in their native range – seeds of Australian acacias are primarily dispersed by ants. Ants also play an important role on acacia invasions in Southern Africa. Their successful expansion in Southern Europe suggests that they could have found effective ant seed dispersers, but to date there is no data on the importance of ants on acacia seed dispersal out of Australia and Southern Africa. Only recently, have studied the role of ants in dispersing seeds of *Acacia longifolia* (ANDREWS) WILLD. and *A. dealbata* LINK in a coastal dune system on the central–Northern coast of Portugal (where the climate is Mediterranean with an Atlantic influence). The data suggests that invasive acacias have found effective ant seed dispersers in Europe and that the importance of such dispersers is higher at the invasion edges. Future research should fully describe ant community composition at different invasion densities and relate it to seed removal rates both for invasive acacias, for the remaining native plant community of the Mediterranean basin.

Ants are considered beneficial insects for their roles as predators, scavengers, and as dispersers of plant seeds. But when the seeds belong to a pest plant, the ants' role may change to that of accomplice in an unwanted biological invasion. As an example; *Formica exsectoides* FOREL, 1886 is a species of ant native to the Atlantic area of Northern America. Its range extends from Nova Scotia to parts of Georgia. *F. exsectoides* carries off seed of a non–native plant, *Euphorbia esula* L., so this ant species may be assisting the plant as it spreads across the great plains of the central United States. This aspect also needs to be considered and investigated.

The traditional pairwise perspective on mutualism has been recently shifted to admit a network of multiple interacting species, and whose evolution and maintenance are influenced by the biotic and abiotic contexts in which the interaction occurs. Recent evidence suggests that the traditional view of myrmecochory as a highly diffuse interaction between diaspores and a wide range of ant species attracted to their elaiosomes may not be correct. The effectiveness of dispersal varies markedly among ant species, and combined with differential attractiveness of diaspores due to elaiosome size and composition, this raises the potential for myrmecochorous plants to target ant species that offer the highest quality dispersal services. Faced with this prospect, it should be possible to investigate this aspect to confirm potential myrmecochores.

Glossary

> "The real voyage of discovery consists not in seeking new landscapes but in having new eyes."
>
> Proust M. (1871–1922) In search of lost time.

Angiospermae (from Greek *angeîon* = vessel + Greek *spérma* = seed). Etymologically, Angiospermae means a plant that produces seeds within an enclosure (the fruit), i. e. ovules enclosed in an ovary (instead, the Gymnospermae are at "naked seeds"); they are fruiting plants, although more commonly referred to as flowering plants.

Diaspore (from Greek *diasporā* = dispersion). The diaspore is a part of a plant that serves for reproduction and dispersal (TAHTADZHJAN, 1985). In flowering plants the sexual diaspore (or simply diaspores) is a dispersal unit, i. e. seed or fruit + any additional tissues – as more or less large parts of the plant itself (VITTOZ & ENGLER, 2007) – that assist dispersal.

Elaiosome (from Greek *elaion* = oil + Greek *soma* = body). Many seeds or seed and fruit together have a specialised external appendage (fat body): a rich pluricellular supplement of nutritional substances (vitamins – e. g. B_1, C –, amino acids – e. g. cystine and methionine –, proteins, monosaccharides, and starch), above all lipids (e. g. oleic acid and linoleic acid), called elaiosome (BRESINSKY, 1963; PIJL VAN DER, 1982; HOCKING & KORTT, 1987; LI VIGNI & MELATI, 1999; GAMMANS *ET AL.*, 2005). The elaiosomes attract potential animal seed dispersers: probably small animals, e. g. insects, and especially Formicidae (LI VIGNI & MELATI, 1999). In almost all cases studied to date, these nutritious mature appendages attract worker ants, by playing a crucial role in the dispersal process (e. g. SERNANDER, 1906; BERG, 1969; BEATTIE, 1985; HANZAWA *ET AL.*, 1988; HANDEL & BEATTIE, 1990; HÖLLDOBLER & WILSON, 1990; GORB & GORB, 2003). An alternative proposal is that elaiosomes may help the seed to absorb and retain moisture for germination (STERN, 2000).

Myrmechory myrmecochory sometimes (SIMBERLOFF & REJMÁNEK, 2011).

Myrmecochory (from Greek *myrmēko* = ant + Greek *chorein* = to disperse). The dispersal of fruits and seeds by ants. In the World worker ants of different genera disperse diaspores of many abundant herbaceous plants called myrmecochores, i. e. ant–dispersed plants that produce elaiosome–bearing seeds or seeds and fruits together (the criterion for determining whether a plant is myrmecochorous is that its diaspores possess one elaiosome).

Aril (from Latin *arillus* = pips of grapes) A seed appendage consisting of sappy and pulpy tissues or presenting a kind of a pellicle or a fringe (TAHTADZHJAN, 1985).
– **complex** An aril of a dual origin (WERKER, 1997).
– **false** An aril originating in the region of micropyle, chalaza or along raphe–antiraphe (TAHTADZHJAN, 1985).
– **micropylar** An aril originating in the region of micropyle (TAHTADZHJAN, 1985).
– **raphal** An aril originating along raphe–antiraphe (TAHTADZHJAN, 1985).
– **true** An aril originating on a funicle (TAHTADZHJAN, 1985).

Caruncle (from Latin *caruncula* = small piece of flesh) See Aril micropylar.

Chalaza (from Greek *khalaza* = hard lump, hailstone) A basal region of the ovule, where integuments arise and the funicle is attached (TAHTADZHJAN, 1985).

Chalazal process An aril originating in the region of chalaza (TAHTADZHJAN, 1985).

Funicle (from Latin *fūniculus* = thin rope) A stalk that attaches the ovule to the placenta (TAHTADZHJAN, 1985).

Hilum (from Latin *hīlum* = trifle) A scar on a seed that marks the point, at which it was attached to the funicle or placenta (TAHTADZHJAN, 1985).

Micropyle (from Greek *mīkrós* = small + Greek *pulē* = gate) A canal in the integuments on the apical part of the ovule, through which the pollen tube (a tube formed from a germinating pollen grain) passes during fertilization (TAHTADZHJAN, 1985).

Pseudoaril See Aril false.

Raphe (from Greek *rhaphē* = suture) A part of the funicle of an anatropous ovule adnate to the integuments and forming a ridge from the hilum to chalaza (TAHTADZHJAN, 1985).

Sarcotesta The outer and usually soft and sappy part of the seed cover (TAHTADZHJAN, 1985).

Strophiole (from Latin *strophiolum* = small wreath) See Aril raphal.

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Appendix

> "The real voyage of discovery consists not in seeking new landscapes but in having new eyes."
>
> PROUST M. (1871–1922)
> In search of lost time.

LIST OF USEFUL WEB–BASED ANT RESOURCES

Ant Species Catalogs, Literature, and Taxonomy

1. • Ant journal articles: *http://scholar.google.it/*
2. • Antbase (ant taxonomic authority and primary literature): *http://antbase.org/*
3. • AntCat (ant taxonomic authority and catalog): *http://www.antcat.org/*
4. • AntWeb (ant specimen images and regional species lists): *http://www.antweb.org/*
5. • AntWiki (ant taxonomy, morphology, etc.): *http://www.antwiki.org/wiki/Main_Page*
6. • Discover Life (ants album): *http://pick5.pick.uga.edu/20/q?search=Formicidae*
7. • Formis (bibliography of ants literature): *http://www.ars.usda.gov/Research/docs.htm?docid=10003*
8. • Global Ant Project (biography of 1000 myrmecologist and publications): *http://gap.entclub.org/*
9. • HOL (Hymenoptera online): *http://www.hol.osu.edu*
10. • Internet Archive (digital library offering free universal access to books): *http://archive.org/*
11. • William L. Brown Memorial Digital Library: *http://ripley.si.edu/ent/nmnhtypedb/wlb/*

Ant Distributions

12. • Ants from the Iberian Peninsula: *http://www.hormigas.org/*
13. • Ants of France: *http://antsmania.com/?p=254*
14. • Ants of France: *http://apef.france.pagesperso– orange.fr/Fourmis.htm*
15. • Formicarium (Italian site on the ants): *http://www.formicarium.it/*
16. • Fourmis: *http://fort.thomas.free.fr/*
17. • GBIF (Global Biodiversity Information Facility): *http://www.gbif.org/*
18. • Key to identifying common French: *http://cle.fourmis.free.fr/*
19. • The ants of Cachoeira Nature Reserve Brazil: *http://ants–cachoeira.net/*

Ants Morphology

20. • Ant eMuseum (ant morphology glossary): *http://projects.biodiversity.be/ants/glossary/termsList*
21. • Antkey glossary (ant morphology glossary): *http://antkey.org/lexicon/43*
22. • Glossary of Surface Sculpture: *http://antbase.org/ants/publications/harris1979.html*

Regional and Other Identification Guides

23. • Ants Down Under: *http://anic.ento.csiro.au/ants*
24. • Ants in Vietnam: *http://www.antist2007.com/Diversity/main.html*
25. • Ants of Costa Rica: *http://academic.evergreen.edu/projects/ants/AntsofCostaRica.html*
26. • Ants of Egypt: *http://www.discoverlife.org/proceedings/0000/7/orgs/*
27. • Ants of Illinois: *http://campus.lakeforest.edu/menke/Resources/AntGeneraKey/antkey_illinois.html*
28. • Ants of North America: *http://www.utep.edu/leb/antgenera.htm*
29. • Fiji Ants: *http://www.elisarnat.com/research/the–ants–of–fiji*
30. • Invasive Ants of the Pacific Islands: *http://www.piakey.com/*
31. • Japanese Ant Image Database: *http://ant.edb.miyakyo–u.ac.jp/E/index.html*
32. • New World Army Ants: *http://www.armyants.org/*
33. • Pheidole of Tiputini Ecuador: *http://www.amymertl.com/Pheidole.html*
34. • Pheidole Group: *http://academic.evergreen.edu/projects/ants/pheidoleworkinggroup/index.htm*

Miscellany

35. • Alex Wild Photography: *http://www.alexanderwild.com/ants*
36. • Ant Course: *http://research.calacademy.org/ent/courses/ant*
37. • Ant Genomics Database Fourmidable (ant molecular and genetic data): *http://antgenomes.org/*
38. • AntArk (an introduction to myrmecology): *http://www.antark.net/*
39. • Encyclopedia of Life (EOL): *http://eol.org/pages/699/overview*
40. • Insect photography by Alex Wild: *http://myrmecos.net/*