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ACKNOWLEDGMENTS

I warmly thank my tutor, Prof. Tullia Zetto, for her essential support and consideration during my period of study, for her invaluable suggestions on manuscript organization, for linguistic revision and human prop; Prof. Pietro Brandamayr for structuring my research program and for many ideas; Dr. Teresa Bonacci for field and laboratory help and numerous other forms of entomological supports, for encouraging me to continue my work on behavioural research, Dr. Antonio Mazzei for helping me during sampling in experimental sites and carabid beetles identification, Dr. Anita Giglio and Dr. Federica F. Talarico for their suggestions during histological preparations and photographical acquisitions; Dr. Alessandro Massolo, for his useful support in statistical analyses.

I am particularly grateful to Prof. Beatrice Bitonti, Dr. Leonardo Bruno for taking time to teach me DNA analysis laboratory methods.

I thank Dr. Nino Iannotta, C.R.A, and his co-worker, in particular Dr. Stefano Scalercio, for research facilities, and all the other numerous and wonderful persons that help, support and endure me during these demanding, absorbing and fascinating three years.

I also thank Prof. Alfio Raspi and Dr. Andrea Lucchi, for *Drosophila melanogaster* determination; Dr. Emilia Rota for earthworms determination.

This research was partially financed by RIOM Project, (Ricerca e Innovazione per l'Olivicoltura Meridionale) Ministero delle Politiche Agricole e Forestali and by project of research fund 'Giovani Ricercatori', Area Ricerca Scientifica e Rapporti comunitari, University of Calabria.

RIASSUNTO

La mosca delle olive (*Bactrocera oleae*) infesta gran parte degli uliveti del mondo. La larva si nutre della polpa dell'oliva. Molti meccanismi di controllo sono stati sviluppati contro questo fitofago, inclusi prodotti chimici e metodiche biologiche (lanci di imenotteri parassitoidi).

I coleotteri carabidi sono presenti in molti uliveti, sebbene il loro impatto sulle catene alimentari sia ancora poco noto. La maggioranza delle larve della mosca delle olive si impupa nel terreno sotto gli alberi, vulnerabile a molti predatori del suolo, compresi i carabidi.

Questa ricerca esamina l'esistenza della predazione sugli stadi pupali di *B. oleae* da parte dei carabidi in laboratorio. Obiettivo della ricerca è identificare quali specie di carabidi sono predatori naturali della mosca delle olive e quando predino le pupe della mosca delle olive durante la giornata, al fine di valutare il ruolo dei carabidi come predatori della mosca delle olive e la possibilità di impiegarli come naturali antagonisti della *B. oleae.*

Dati preesistenti sulle carabidocenosi degli uliveti, raccolti dal nostro gruppo di ricerca (Brandmayr-Zetto e collaboratori) in collaborazione con il C.R.A. Centro di Ricerca per l'Olivicoltua e l'industria olearia (Rende, CS), hanno fornito una lista delle specie più abbondanti negli uliveti calabresi, punto iniziale di questo studio. Le specie polifaghe e zoospermofaghe, potenziali predatori della pupa della mosca delle olive, sono state scelte tra queste specie.

I coleotteri carabidi includono nella loro dieta anche pupe di dittero, inoltre le specie di carabidi riproduttori autunnali e la mosca delle olive hanno una fenologia coincidente. In letteratura ci sono indicazioni di predazione della mosca delle olive probabilmente operata da parte di carabidi. Partendo da tali presupposti, ho utilizzato una combinazione di esperimenti di scelta alimentare, campionamenti in campo, analisi microscopiche e molecolari.

1- Le specie che predano le pupe della mosca delle olive sono state selezionate con uno screening preliminare di laboratorio consistente in test di scelta alimentare. Per testare le preferenze alimentari di carabidi adulti predatori generalisti sulla *B. oleae*, ho condotto degli esperimenti di laboratorio usando pupe della mosca delle olive, adulti di *Drosophila melanogaster* e pezzi di lombrico della specie *Nicodrilus caliginosus.* In ciascun test, le tre prede sono state offerte contemporaneamente ad un carabide tenuto a digiuno per due giorni.

2- Ulteriori analisi quantitative sono state condotte sui carabidi pesando le prede. Le preferenze alimentari sono state valutate contando la frequenza di esplorazione e l'ammontare del cibo ingerito (in grammi) pesando ciascuna preda prima e dopo il test. Le preferenze alimentari sono state individuate utilizzando l'Indice di Elettività (EI).

3- Inoltre il contenuto intestinale di due specie di carabidi (*P. melas* e *C. fuscipes*) catturati in uliveti, è stato analizzato usando il microscopio ottico per individuare frammenti appartenenti alla mosca delle olive.

4- Il contenuto intestinale dei carabidi è stato anche analizzato con metodi molecolari (PCR) per individuare la predazione sulle pupe. Riguardo all'analisi del contenuto intestinale, primer specifici per *B. oleae* sono stati disegnati da una regione non conservata, come verificato dal confronto delle sequenze di DNA di *Bactrocera oleae* pubblicate. La specificità dei primer è stata verificata usando differenti DNA di vari invertebrati prede (ditteri, chilopodi, diplopodi, ragni, lumache, chiocciole, lombrichi, opilioni, coleotteri, blatte, tisanuri). Successivamente i carabidi sono stati alimentati con alcune pupe per analizzarne il contenuto intestinale. I primers della *B. oleae* sono stati utilizzati per amplificare il DNA della mosca, contenuta nello stomaco dei carabidi predatori.

5- In aggiunta ai dati raccolti con le trappole a caduta, la densità dei carabidi è stata stimata al metro quadro in un uliveto, utilizzando dei recinti di 2 m^2 e trappolando all'interno in maniera esaustiva ("*Leerfang*"). Per valutare gli effetti della predazione dei carabidi sulla mosca delle olive è importante stimare l'abbondanza di predatori e prede. Infatti, un predatore può ridurre la popolazione della preda solo se esso stesso è abbondante.

6- Ho verificato quando i carabidi sono attivi e quando di solito predano analizzando il ritmo di attività giornaliero con un nuovo e moderno sistema di videoregistrazione.

7- L'abilità dei carabidi di trovare la pupa sotto la superficie del terreno è stata testata in laboratorio interrando le pupe. Inoltre ho verificato anche l'abilità di catturare le larve migranti che cadono delle olive.

Risultati – Esperimenti di laboratorio hanno mostrato che alcune specie di carabidi, *Pterostichus melas, Calathus fuscipes, Pseudoophonus rufipes, Laemostenus cimmerius, Distichus planus, Brachinus italicus, B. crepitans,* regolarmente mangiano le pupe di *B. oleae*, mentre altre specie (*Carabus coriaceus, Pseudoophonus griseus, Calathus cinctus, Nebria kratteri, Brachinus sclopeta*, *Anchomenus dorsalis*) quasi mai ingeriscono le pupe. Test quantitativi hanno confermato I test preliminari.

L'analisi dell'Indice di Elettività ha mostrato differenze tra i sessi in due specie, *P. melas* and *C. fuscipes*; le femmine di *P. melas* hanno mostrato preferenze significative per le pupe, mentre *C. fuscipes* preferisce i lombrichi. *L. cimmerius* ha mangiato le pupe, ma ha preferito i lombrichi. Diversamente *C. coriaceus* e *N. kratteri* non gradiscono le pupe. Nessuna delle specie testate ha preferito le drosofile.

Riguardo alla frequenza di esplorazione, per *P. melas* le esplorazioni seguite da predazione sono state maggiori per le pupe che per i lombrichi e le drosofile, anche i pupari sono stati più esplorati che le drosofile. In *C. fuscipes* esplorazioni seguite da predazione sono state minori per le drosofile che per le altre prede. Se la pupa è ingerita per prima *P. melas* consuma le pupe in quantità maggiore di lombrichi mentre *C. fuscipes* consuma i lombrichi in maggiore quantità delle pupe. C'è concordanza tra la prima preda esplorata e la prima preda ingerita per *P. melas* e *C. fuscipes.*

L'analisi del contenuto intestinale con il microscopio ottico ha rivelato un discreto gruppo di prede per le due specie esaminate, in due esemplari di *C. fuscipes* sono state individuati frammenti probabilmente appartenenti ad esemplari adulti di mosca delle olive,mentre nessuna traccia di pupe di *B. oleae* è stata trovata.

Quindi l'analisi molecolare risulta utile e necessaria per mettere in evidenza la predazione dei carabidi sulla pupa mosca delle olive. L'analisi del contenuto intestinale con PCR è stata applicata solo a carabidi nutriti in laboratorio con le pupe. I risultati sono incoraggianti. I primers sono stati disegnati nel gene *Bactrocera oleae Transformer* (*tra*), implicato nella determinazione del sesso di *Bactrocera oleae* con sei esoni e cinque introni. I primers sono specifici perchè non c'è stata amplificazione in altri invertebrati preda.

Riguardo alla densità di carabidi al metro quadro in uliveto 47 individui appartenenti a 12 specie sono stati campionati. La densità totale è stata stimata come 5.9 carabidi per m^2. Le densità media di *P. melas* è stata di 0.5 individui/m^2.

I dati raccolti sui ritmi di attività mostrano che le specie esaminate (*P. melas, C. fuscipes, C. coriaceus, P. rufipes, P. griseus, N. kratteri, L. cimmerius*) sono notturne e predano in prevalenza un'ora dopo il tramonto.

Discussione – Non tutte le specie di carabidi polifagi abbondanti negli uliveti hanno le stesse preferenze per le pupe di *B. oleae*. *Brachinus sclopeta, Anchomenus dorsalis* non hanno preferito le pupe e non le hanno mangiate durante i test. Queste specie hanno piccole dimensioni e anche mandibole ridotte, possono essere incapaci di rompere il pupario, probabilmente troppo duro da mangiare, tranne che per *Distichus planus,* a causa della sua macrocefalia. Diversamente i carabidi più grandi possono afferrare il pupario e ingerirlo. È possibile ipotizzare un criterio di scelta dei potenziali carabidi antagonisti di *B. oleae* basato sulle dimensioni.

L'analisi della frequenza di esplorazione ha mostrato che *P. melas* e *C. fuscipes* ingeriscono la prima preda che trovano e si comportano come predatori olfattivo-tattili generalisti. *P. melas, C. fuscipes* e *L. cimmerius* hanno mangiato le pupe.

Ci sono differenze nelle preferenze alimentari tra i maschi e le femmine in *P. melas* e *C. fuscipes,* probabilmente le femmine durante il periodo riproduttivo necessitano di più cibo.

Le analisi del contenuto intestinale al microscopio hanno permesso di identificare due individui adulti nel contenuto intestinale di *C. fuscipes;* le mosche delle olive potrebbero essere state predate già morte o in un momento della notte in cui per le basse temperature erano impossibilitate a volare. Le analisi non hanno evidenziato la predazione su pupa probabilmente perché in genere *P. melas* e *C. fuscipes* non ingeriscono i pupari, ma solo l'interno soffice, come mostrato dalle osservazioni comportamentali. Quindi la predazione su pupe può essere sottostimata dall'analisi microscpica, mentre l'analisi con PCR può supportare questo tipo di ricerca poiché i primers sono specifici per *B. oleae*. Questa è la prima volta che la predazione su *B. oleae* viene studiata usando un approccio molecolare.

Le specie esaminate sono notturne e predano in prevalenza al tramonto. I dati raccolti con le trappole a caduta e i campionamenti esaustivi nei recinti hanno suggerito una densità tale di carabidi da poter influenzare le popolazioni di *B. oleae*.

Gli esperimenti hanno mostrato che *Distichus planus, P. melas* e *P. rufipes* sono in grado di trovare le pupe sotto la superficie del suolo. Inoltre *P. melas* riesce a catturare le larve migranti.

Conclusioni – Questa ricerca ha dimostrato l'esistenza di distinte preferenze alimentari in carabidi predatori in laboratorio. Tra i carabidi, i predatori olfattivo- tattili con abitudini notturne sono i più probabili antagonisti della pupa. Le pupe sono prede commestibili ed abbondanti, possono essere consumate da numerose specie di carabidi e a volte possono essere anche preferite, come mostrano i miei dati. L'analisi sperimentale sottolinea che i carabidi possono essere potenziali predatori della pupa a livello del terreno. I riproduttori autunnali mostrano inoltre una fenologia coincidente e possono ridurre la numerosità delle nuove generazioni di *B. oleae*.

I carabidi sono predatori indigeni che possono controllare la popolazione della mosca delle olive con un minor dispendio economico dei lanci di parassitoidi. I carabidi possono ridurre le popolazioni di *B. oleae,* anche se non sono predatori specializzati della mosca delle olive e non mostrano spiccate preferenze per la pupa, grazie alla loro abbondanza negli uliveti. In ogni modo, quest'ipotesi richiede altre complesse indagini di campo per essere provata. Il ruolo dei coleotteri carabidi come agenti di controllo di *B. oleae* non è ancora stato chiarito per molti aspetti. Ulteriori analisi in campo sono necessarie per investigare l'efficacia dei carabidi nel controllo biologico delle popolazioni di *B. oleae*.

Tale ricerca ha anche potenziali implicazioni sul futuro della gestione uliveti, includendo suggerimenti per le strategie di utilizzo degli insetticidi al fine di promuovere le popolazioni dei predatori del suolo. Il presente studio ha anche aumentato le conoscenze biologiche su comportamento, ecologia e analisi del contenuto intestinale dei carabidi.

Questa ricerca non vuole essere esaustiva, ma fornire un aiuto per comprendere meglio il ruolo dei carabidi come antagonisti del principale fitofago nell'agroecosistema uliveto. Anche se ulteriori indagini di campo e laboratorio saranno necessarie, questo lavoro di tesi fornisce un primo

contributo per risolvere la questione aperta del ruolo dei carabidi nella riduzione della mosca della olive. Dopo questo studio è ragionevole aspettarsi che i carabidi in campo possano ridurre il numero di pupe nel terreno e limitare le nuove generazioni primaverili ed estive e, conseguentemente, quelle autunnali.

Parole chiave: Carabidae, antagonisti naturali, *Bactrocera oleae*, comportamento alimentare, contenuto stomacale, predazione, riduzione del fitofago

ABSTRACT

The olive fly (*Bactrocera oleae*) infests most olive-growing countries of the world. The larvae feed on olive fruits and many mechanisms of control have been developed against this pest, including chemical spraying and biological methods (as parasitoid wasps).

Carabid beetles are found in a variety of olive groves, although their impact on the food web and on olive pests is poorly known. Most olive fly larvae pupate on the ground underneath the trees, where they are vulnerable to many ground predators, perhaps including carabids.

This study examined the existence of carabid predation on *B. oleae* pupal stages by laboratory trials. Research goal is to identify which carabid species are natural olive fly predators, when they prey olive fly pupae, to evaluate carabid beetle role as olive fly pupae predators, and the possibility to employ them as olive fly natural antagonists.

Preexistent data about carabid-coenoses in olive groves, collected by our research group (Brandmayr-Zetto and co-worker) in collaboration with the Research Center for Olive Growing and Olive Oil Industry team (C.R.A. Rende, CS), provided a list of the most abundant carabid species in Calabrian olive groves that was the beginning of this study. The polyphagous and the zoo-spermophagous species, as potential pupae predators, were chosen among these species. Carabids have been reported to consume dipteral pupae. Autumn breeding carabid species and olive fly have a coincident phenology. In literature there were indications of olive fly pupae predation probably operated by carabid beetles. I utilized a combination of feeding laboratory experiments, field sampling, microscopical and molecular analyses.

1- Species that prey olive fly pupae were selected by a preliminary laboratory screening consisting in feeding choice experiments. In order to test prey preference of adult generalist predator carabids on olive fly, *Bactrocera oleae*, I conducted laboratory feeding experiments by using olive fly pupae, adults of fruit flies (*Drosophila*

melanogaster) and pieces of earthworms (*Nicodrilus caliginosus*). In each test the three above mentioned preys were contemporaneously offered to one starved carabid.

2- Further quantitative laboratory experiments were conducted on carabids weighting each prey before and after each test. Feeding preferences were evaluated considering the amount of ingested food (Gram weight) expressed as Electivity Index (EI) and by counting exploration frequencies.
3- Moreover, gut contents of two carabid species (*Pterostichus melas* and *Calathus fuscipes*) sampled in olive groves was analysed using optical microscopy to identify morphologically *B. oleae* remains.
4- Carabid gut contents were also analysed using the polymerase chain reaction (PCR) to put in evidence *B. oleae* predation detecting *B. oleae* DNA. *B. oleae* species-specific PCR primers were designed from not conserved regions based on comparisons of published DNA sequences of *Bactrocera oleae*. Primers specificity was verified using different prey DNAs of various invertebrates (dipterans, chilopods, diplopods, spiders, snails, earthworms, harvestmen, beetles, cockroaches, silverfishes). Then carabids were fed on some pupae for gut content analysis. *B. oleae* primers were used to amplify *B. oleae* DNA from carabid beetle guts.
5- In addition to pitfall traps collected data, we have estimated ground beetle density per square meter in olive grove, in enclosures of 2 m^2 with exhaustive trapping ("*Leerfang*"). To evaluate the effects of carabid predation on olive fly population, it is important to estimate the abundance of predators and preys. Indeed, a predator may reduce prey population only if it is abundant.
6- I verified when carabids are active and when they usually prey during daytime, analysing carabids daily rhythm with a new modern video recording system.
7- The ability of carabids to find *B. oleae* pupae under soil surface was tested in laboratory simulating soil conditions with pupae buried. Moreover I evaluated *P. melas* ability to catch migrating larvae fallen from drupes.

Results - Experiments carried out in laboratory conditions showed that some carabid species, as *Pterostichus melas, Calathus fuscipes, Pseudoophonus rufipes, Laemostenus cimmerius, Distichus planus Brachinus italicus, B. crepitans,* regularly feed on *B. oleae* pupae, while other species (*Carabus coriaceus, Pseudoophonus griseus, Nebria kratteri, Brachinus sclopeta*, *Anchomenus dorsalis*) almost never ingested *B. oleae* pupae. Quantitative feeding test confirmed the preliminary test.

The analysis of EI showed differences between sexes in two species, *P. melas* and *C. fuscipes. P. melas* females showed significant preferences for olive fly pupae, *C. fuscipes* for earthworms, *L. cimmerius* ingested a significant amount of pupae, but preferred earthworms. On

the contrary *C. coriaceus* and *N. kratteri* did not eat pupae. All tested species did not prefer fruit flies. Regarding frequency of explorations, pupae were more preyed by *P. melas* than earthworms and fruit flies, also puparia remains were more explored than fruit flies. Fruit flies were less preyed than earthworms and pupae by *C. fuscipes*. If pupae were ingested at first *P. melas* consumed them in greater quantity than earthworms and *C. fuscipes* consumed more earthworms than pupae. There was concordance between the first explored prey and the first ingested one, for *P. melas* and *C. fuscipes*.

Gut content analysis with microscope revealed a range of prey items for the two species *C. fuscipes* and *P. melas*. The remains belonging to *B. oleae* adults were found in the gut of two *C. fuscipes*; no *B. oleae* pupae remains were found.

Gut content analysis using PCR was applied only to carabids fed in laboratory on pupae. Results are encouraging. Primers were designed in *Bactrocera oleae Transformer* (*tra*) gene implicated in sex determination in *Bactrocera oleae* with six exons and five introns. Primers are specific because there were no amplification products using other invertebrate prey DNAs as template.

Regarding the density of ground beetles in olive grove, 47 individuals belonging to 12 carabid species were found. I can estimate the total density of carabid beetles as 5.9 individuals per m^2. *P. melas* mean density was 0.5 individual/m^2.

Daily rhythm collected data showed that the tested carabid species (*P. melas, C. fuscipes, C. coriaceus, P. rufipes, P. griseus, N. kratteri, L. cimmerius*) are nocturnal and they generally ingested food an hour after the sunset.

Discussion - Not all polyphagous carabid species abundant in olive groves have the same preference for *B. oleae* pupae. *Brachinus sclopeta, Anchomenus dorsalis* do not prefer pupae and do not eat them in feeding experiments. These species have little dimensions and little mandibles too, and may be unable to crush puparia, probably too hard to eat, except for *Distichus planus,* because of its “megacephalic” morphology. Conversely, big carabids can grip puparia and ingest them. It is possible to hypothesise a chosen criterion of potential carabid antagonists of *B. oleae* based on dimension.

Exploration analysis showed that *P.melas* and *C. fuscipes* ingested the first prey that they found and behaved as olfactory-tactile generalist predators. *P. melas, C. fuscipes* and *L. cimmerius* ingested pupae. There are differences in feeding preferences between sexes in *P. melas, C. fuscipes,* probably females during breeding season need more food.

B. oleae adult remains in the gut of *C. fuscipes* may derive form scavenging or the predators captured the olive flies inable to fly at low temperature during the night. No pupae remains were found. Behavioural observations showed that *P. melas* and *C. fuscipes* did not generally ingest puparia, but only the soft internal contents. So pupae predation may be underestimated using

microscopical observations, while molecular analyses are useful and necessary to put in evidence carabid predation on olive fly pupae. PCR analysis may support our research because primers are specific for *B. oleae*. This is the first time that predation on *B. oleae* was studied using a molecular approach.

Carabids species were nocturnal and they may prey pupae prevalently at sunset.

Data collected with pit-fall traps and sampling in enclosures revealed a mean density of 5.9 carabids per square meter.

Experiments conducted on *Distichus planus, P. melas* and *P. rufipes* showed that these species are able to find pupae under the soil surface; *P. melas* is able to capture migrating larvae.

Conclusion - This research demonstrated distinct differences in prey choice by predator carabids in laboratory. Night-active olfactory-tactile polyphagous carabids are the most probable pupae predators among carabid guild. Pupae are available and palatable food, that may be ingested by several carabid species and sometime preferred, as my results shown. Experimental analyses underlined that carabid beetles may be potential predator of olive fly pupae at soil level. Autumn breeding carabids show a coincident phenology with the olive fly and may reduce new *B. oleae* generations.

Carabids are indigenous predators and could control olive fly with a much smaller economic waste than parasitoid introductions. Carabid beetles may reduce *B. oleae* population even if they are not specialized olive fly predators and do not show particular preference for pupae, due to their abundance in olive grove. However, this hypothesis needs other complex field investigations to be proved. The role of carabid beetles as control agent of *B. oleae* has been not yet elucidated for many aspects. Further field analyses are needed for investigating the efficacy of carabid beetles as control agent of *B. oleae* populations.

This research has also potential implications on the future management of olive grove, including suggestions for insecticide use strategies to promote generalist ground predator populations. This study increased biological knowledge of Carabid beetle behaviour, ecology and gut content analyses too.

This research could not be exhaustive, but would only provide an aid to better understand carabid role as pest antagonists in olive grove agro-ecosystem. Even if further laboratory and field experiments will be necessary, this work of thesis gives a first contribution to solve the open question of carabid role in olive fly reduction and, after this study, it is reasonable to expect that carabid in field may reduce olive fly pupae numbers in the soil and may reduce new spring and early summer *B. oleae* generations and, consequently, the autumnal ones.

Key words: Carabidae, natural antagonist, *Bactrocera oleae*, feeding behaviour, gut contents, predation, pest reduction.

1. GENERAL INTRODUCTION

1.1 Adephaga

Beetles are insects having biting mouthparts and front wings modified to form horny covers, or elytra, overlying the membranous rear wings.

The Adephaga Clairville, 1806, (from Greek *adephagos*, that means *'gluttonous'*), is the second largest suborder of highly specialized beetles of the order Coleoptera (Crowson, 1955; Vigna Taglianti, 1993; Brandmayr et al, 2005).

Adults members of the suborder have the following properties:

notopleural sutures visible on prothorax;

six visible abdominal sterna, the first three fused and divided by hind coxae;

metacoxae merged with the metasternum (first abdminal sternitae divided);

five tarsal segments;

wings with trasversal tracery (oblongum);

pygidial defense glands in the adult;

testes tubular, coiled, consisting of a single follicle;

ovaries polytrophic;

larvae have fused labrum and no mandibular molae for liquid-feeding.

Adephagans in addition to ground beetles (Carabidae), include tiger beetles (Cicindelinae) and predacious diving beetles (Gyrinidae and Dytiscidae). The living families with terrestrial members, Carabidae included, are occasionally called Geoadephaga, the remaining aquatic families are Hydradephaga. Phylogenetic relationships within Adephagans have been extensively examined. Adephagans diverged from their sister group, Polyphaga, in the late Permian. The most recent common ancestor of living adephagans probably existed in the early Triassic, around 240 million years ago (Erwin, 1979). The Triassic included both aquatic and terrestrial representatives of the suborder and in the Jurassic trachypachid, carabid, gyrinid, and haliplid-like forms appeared. Carabids emerged in tropical habitat (Erwin, 1976). The Mesozoic period represented a moment of diversification for the family. Few tribes explosively radiated in the Tertiary (e.g., members of the carabid subfamily Harpalinae, Erwin, 1985).

1.2 Carabid Beetles

Carabidae (Latreille, 1810), commonly known as ground beetles, are one of the largest and most successful families of Coleoptera Adephaga with almost 40,000 different species worldwide in 86 tribes, most of which are found in the tropics, about 12,000 in Italy. Carabidae occur in nearly

every terrestrial habitat. This family is fairly uniform from a morphological point of view; even if carabids show great ecophysiological adaptability and life way variability that make them a successful group (Thiele, 1977). A typical ground beetle has an oval form (Casale et al., 1982), prothorax more narrow than elitrae, robust head, filiform antennae, strong mandibles and long legs (Fig. 1.1).

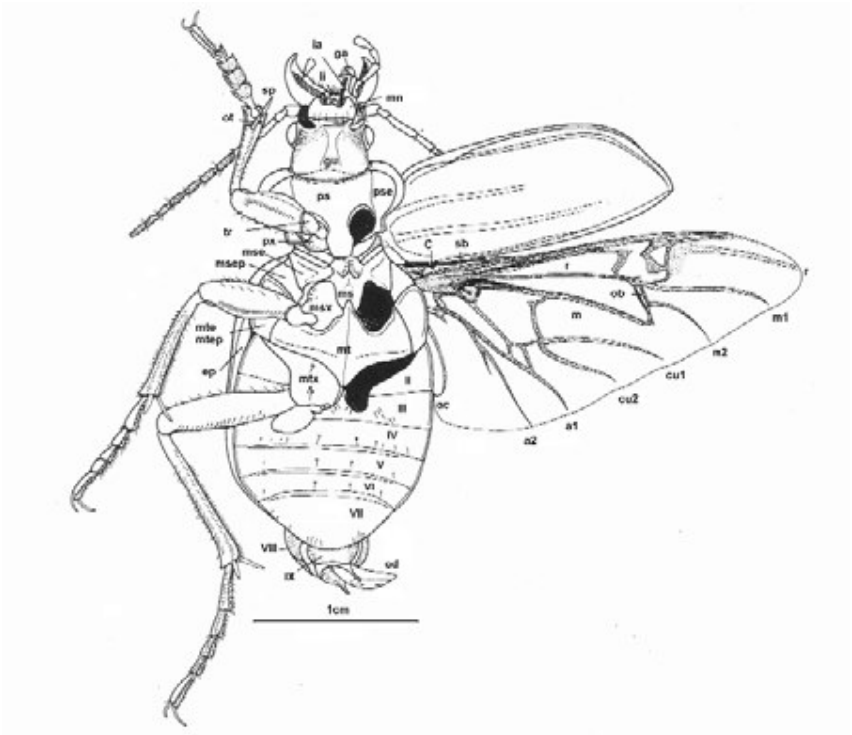


Figure 0-1.1- *Calosoma sycophanta*, an exemplifying carabid beetle

Carabidae are morphologically characterized by protibiae with two apical spurs or one subapical, one cleaning organ (absent only in Paussinae) clipeum and labrum more narrow than the distance between antennae, antennae with 11 items implanted near the base of mandibles and campodeiform larvae. Most species are black or brown coloured, but a few display iridescent and metallic reflections. Body shape and leg morphology are modified for running, climbing, burrowing, digging and swimming (Lövei and Sunderland, 1996; Forsyte, 1981). Carabids are typical ground predators. There are only slight variations of this life form, connected with specialized feeding habits (Brandmayr et al., 2005).

Some carabids present atrophy of the wings and their associated muscles (brachypterous species), while other species are able to fly (macropterous species) and there is also an intermediate situation with dimorphic species including long winged individuals and short winged ones. Adult carabid size varies from 0.7 to 66 mm (Ball and Bousquet, 2001). There is a correlation

between body form and habit (Forsythe, 1986; 1991) i.e. long legs are usually associated with fast running. Principal variations are discussed by Forsythe (1983). Species belonging to genus *Pterostichus* have relatively short limbs and large meso-coxal articulations, so they are horizontal pushing, but not so good at high speed running (Forsythe, 1991). Species belonging to genus *Scarites* and *Distichus*, with short limbs, large meso-coxal articulation, narrow femora and broad, flattened pro-tibia adapted for digging, live in burrow and are good horizontal pushing, but poor runner (Forsythe, 1991). Classification refers to Vigna Taglianti, 1993, Vigna Taglianti, 2004 and Brandmayr, 2005.

1.2.a Breeding and survival

Carabids are holometabolous insects (Baccetti et al., 1991). Some carabids appear to breed in late summer and autumn, and hibernate as larvae through the winter, then, after emerging in spring, aestivate to survive in the hottest period, before they start the egg-laying in autumn. Others (probably the majority) hibernate as adults and reproduce in spring or early summer, after which beetles usually die off and a new generation appears in autumn ready for overwintering again.

So different ground beetle species have been classified as either spring or autumn breeders (Larsson, 1939). Furthermore many species undergo a resting period, called diapause, (overwinter hibernation or summer aestivation), so carabids include species with adult or larval hibernators (Lindroth, 1949), but in many autumn breeding there is a conspicuous part that hibernate also as adults. Many authors gave their contribution to carabid breeding biology (Paarmann, 1970; Thiele, 1977; Boer and Dijk, 1996; Boer and Boer, 1990). Summing up carabids may be divided in three groups:

species with summer larvae including Spring breeders, such as *Pterostichus nigrita, Poecilus cupreus, Calosoma sycophanta,* that lay eggs in spring or early summer, larvae develop quickly (EST), so these species over winter as adults, the new generation may emerge immediately in autumn, or in spring, depending on climatic conditions, characteristics of the habitat and physiology of the species. No spring breeder larvae show a resting period. Adults hibernate in winter (Brandmayr et al., 2005);

species with winter larvae including autumn breeders that lay eggs in late summer or autumn, over winter as larvae, and pupate in spring. Larvae in winter show a termic parapause interrupted by a period of low temperature. Adults of some species, such as *Carabus gigas, Pterostichus melas, Pseudoophonus spp., Calathus spp., Laemostenus spp., Scybalicus oblongiusculus*, do not show a resting period, but other species of autumn breeders, such as *Nebria brevicollis, Carabus coriaceus, Trechus* spp. and other *Nebria* spp., show summer aestivation. Larval development may last until 8-9 month (INV) (Brandmayr et al., 2005);

species with slow development larvae (ANN), larval development in these species may last more than one year, these such as *Abax parallelepipedus* (= *ater*) (Brandmayr et al., 2005). In

general carabids have three larval stages, but few species, e.g. *Harpalus* and *Amara* spp., have only two stages and other symbionts or ectoparasites have more larval stages (Lövei and Sunderland, 1996).

Eggs are usually oviposited singly in the soil, throughout the growing season, depending on the species.

Ground beetles suffer heavy losses in both egg and larval stages, this can reduce the number of individuals that become adults. Predation, dehydration and starvation are the major causes of death for larvae and pupae, while desiccation and moulds are the primary egg killers. Also the parasitism, mostly by nematodes and fungi, and the predation pressure of small mammals, birds, amphibians and reptiles may reduce both population numbers and species diversity. Generally adults reproduce once and then perish, but they can also live more than one season and reproduce again (Lövei and Sunderland, 1996). Even reproduction is an expensive activity that reduced survival in carabid, as shown for *Notiophilus biguttatus* (Ernsting and Isaaks, 1991).

1.2.b Carabid "life form" and diet

Carabid beetles present a variety of different morphologies related to different ways to catch prey (Thiele, 1977; Brandmayr and Zetto Brandmayr, 1980; Forsythe, 1981; Bauer, Kredler, 1991; Zetto Brandmayr et al., 1998b; Zetto Brandmayr and Brandmayr, 1998; Bauer et al., 1998; Brandmayr, 2005) that have been defined "life form" (Sharova, 1981) and are known also as "lebensformen", such as:

- olfactory-tactile predators (OL)
- optical predators (OT)
- spermophagous
    - zoospermophagous (ZSP)
    - exclusive spermophagous (SP)

Even if carabids presumably find their food via random search (Lövei and Sunderland, 1996), specialist feeders tend to use chemical or optical cues.

The olfactory-tactile predators (OL) used chemical cues to find prey and tactile and olfactory senses to recognize it. They are generalist predators usually with nocturnal activity. There are some morphological variations (Thiele, 1977; Sturani, 1962, Assmann et al., 2000) of this life form, as the macrocephaly, the giantism, the cychrization, the procerization, the abacization, the modification of some *Licinus* species to catch snails (Brandmayr and Zetto Brandmayr, 1986) and of *Loricera pilicornis* F. to catch springtails (Bauer, 1986).

The optical predators (OT) or visual hunting carabid beetles (Bauer, 1975, 1979, 1981, 1985), e.g. species of *Cicindela* and *Asaphidion*, have large eyes which protrude significantly more than those of species which hunt using tactile and olfactory senses and mouthparts turned down

(Forsythe, 1991; Wheater, 1989; Brandmayr et al., 2005). These predators have diurnal activity and live in not very grassed habitat (Brandmayr et al., 2005).

Spermophagous have squat, large, never elongate mandibles, robust head, eyes with normal dimension and diurnal or nocturnal activity. Predator behaviour may be regularly present, as in zoospermophagous (ZSP) *Amara* and *Harpalus*, or absent, as in exclusive spermophagous (SP) *Carterus* and *Ditomus* (Brandmayr and Brandmayr Zetto, 1974; Brandmayr Zetto and Brandmayr, 1975; Zetto Brandmayr, 1990; Bertrandi and Zetto Brandmayr, 1991).

In general carabid beetle adults and larvae are mostly carnivorous.

Considering previous synthesis (Sharova, 1981; Zetto Brandmayr et al., 1998), and numerous literature sources, a new version of life forms system was recently developed based on morphology and adaptations of Carabid larvae and a hypothesis of main trends of adaptive radiation was presented (Sharova, 2008). These trends led to the origin of specialistic life forms.

The radiation of zoophagous, the majority of Carabidae, took place in different habitat and led to phytobionts (e.g. *Agra, Dromius*), with running and climbing-types of legs, epigeobionts (e.g. *Drypta, Cychrus*), with well developed organs of sense and legs adapted for walking and running; stratobionts (e.g. *Nebria, Synuchus*), occurring in litter, top layers of soil, caves and burrows; geobionts (e.g. *Carabus, Broscus, Clivina, Scarites, Antia*), with digging legs; ambush-predators (e.g. *Cicindela, Graphipterus*) (Sharova, 2008). A second trend is the transformation from zoophagous to myxophytophages, comprising stratobionts, stratogeobionts and geobionts, with burrowing and cryptic life style (Zabrini and Harpalini larvae,), and to phytophagous, adaptated for feeding both vegetative parts of plants (zabroid) and seeds (ophonoid, ditomoid with a progressive development of parental cares) (Brandmayr and Brandmayr Zetto, 1974). There are also mycetophages, symphyles, ectoparasitoids and aphages (Sharova, 2008).

Most adults are generally olfactory-tactile, omnivorous (feeding on both plants and animals) and polyphagous (being able to use a wide range of foods), feeding on live prey, carrion and plant material (Lindroth, 1992; Ball and Bousquet, 2001), in fact probably carabids also scavenge on the dead remains of insects and other invertebrates if it is not decayed too far or too dried out (Thiele, 1977). Most species prefer a mixed diet of many different invertebrates, and often some vegetable materials. Food is composed by nutrients, toxins, energetic and indigestible components (Toft and Bilde, 2002).

Ordinarily carabids show no specialization on particular group of preys, but in few genera a high degree of specialization occurs (Thiele, 1977). In fact some species are specialist feeders, i.e. *Harpalus rufipes* on seeds, *Ophonus* species exclusively on the seeds of Umbellifers, *Loricera pilicornis* on Collembola and *Abax parallelepipedus* and *Cychrus caraboides* on slugs and snails, and species of the genus *Calosoma* on butterfly caterpillars (Burgess, 1911). Species belonging to the genus *Notiophilus* are specialized on Collembola and can perceive prey movements at a

distance of several centimetres (Schaller, 1950). Many species of the genus *Dyschirius* prey on staphylinids of the genus *Bledius* (Thiele, 1977), *Siagona europaea* is a myrmecophagous carabid beetle (Zetto Brandmayr and Pizzolotto, 1994; Zetto Brandmayr et al. 1998a; Zetto et al., 2007) *Nebria complanata* preys on the anphipods *Talitrus saltator* and *Talorchestia brito* (Thiele, 1977).

A carabid beetle is considered a specialist feeder even if it ingests a fairly narrow range of prey animals such as springtails, aphids and mites, or different species of snails and slugs (oligophagous carabid species) such as carabids of the tribe Cychrini specialized in hunting snails.

Many species consumed plant materials, especially plant seeds, in different quantities (Thiele, 1977; Brandmayr et al., 2005) and in detail ground beetles eat molluscs, ants, hymenopterans, heteropterans, beetles, opilionids, millipedes, collembolans, nematodes and also insect eggs, but little species more than larger (Thiele, 1977; Hengeveld, 1980c; Loughridge and Luff 1983, Pollet and Desender 1987, Sunderland 1975, 1987; Bilde and Toft 1994, Lang and Gsödl 2001; Brandmayr et al., 2005). Earthworms are considered a significant source of food for *Carabus* species by numerous authors (Sturani, 1962; Thiele, 1977; Paill, 2000; Fawki et al., 2005). Species belonging to the genus *Carabus* ingest also slugs, all kind of insects and even vertebrates carrions. Some species eat also plants, pollen and fungal hyphae (Allen, 1979). A few carabids are ectoparasitoids of other insects (brachinine and lebiine carabids) or millipedes (peleciine carabids). Cases of cannibalism are also been reported (Lövei and Sunderland, 1996).

Diet information recorded for about 150 carabid species in North America show that most carabids are opportunists, feeding on 12 available, suitable preys (Allen, 1979). In fact carabids take from restricted ranges of prey the greater component of their food (Toft and Bilde, 2002). These preys provide to the predator the energy sufficient to maintain high fitness and so are called “essential food”, while food items eaten in lower amount are "supplementary food” (Toft and Bilde, 2002). The subfamily Carabini is documented as eating insects and lepidopteran larvae, Pterostichini, Harpalini and Amarini as eating arthropod eggs, larvae and grass, Cichrini and Licinini as snail specialists, some Bembidiini as cabbage root fly egg predators, and Chalenini as eating soft-bodied insects, annelids and molluscs (Allen 1979).

Larvae of carnivorous adults are always carnivorous and share the same feeding guild of the adults (Toft and Bilde, 2002), but in some species larvae may show different diet preferences than adults (Grandi, 1951). Larvae show a restricted food range (Lövei and Sunderland, 1996).

Generally carabid species show differences in prey preferences and ingest a large spectrum of preys. However, the diet composition varies according to the season (Thiele, 1977; Honek et al., 2006). Dawson (1965) found that Diptera are abundant in spring and summer, spider in September, while the presence of mites and Collembola are not influenced by seasons.

Parts of the feeding mechanism reflect the nature of the food of different ground beetles (Evans and Forsythe, 1985). A larger number of sensilla may be related to food specialization, even in larvae (Merivee et al., 2000; 2001; Giglio et al. 2003).

1.2.c Carabid digestion

The digestive apparatus of insects, such as in carabids, consists in three parts: the fore gut, the mid gut (stomach or ventriculus) and the hind gut (Grassé, 1949; Richards and Davies, 1978; Crowson, 1981, Grandi, 1951) (Fig. 1.2). The fore and the hind intestine are ingrowths of the integument and are lined with cuticle. The mid intestine is derived from the mesenteron. In front of the alimentary canal there is the pre-oral food cavity. The fore intestine begins with the pharynx that passes into the oesophagus; the posterior parts of the oesophagus in some species may be expanded to form a crop in which food is stored up. After the crop there is the cardiac sphincter, often modified to form a muscolar proventriculus or guizzard.

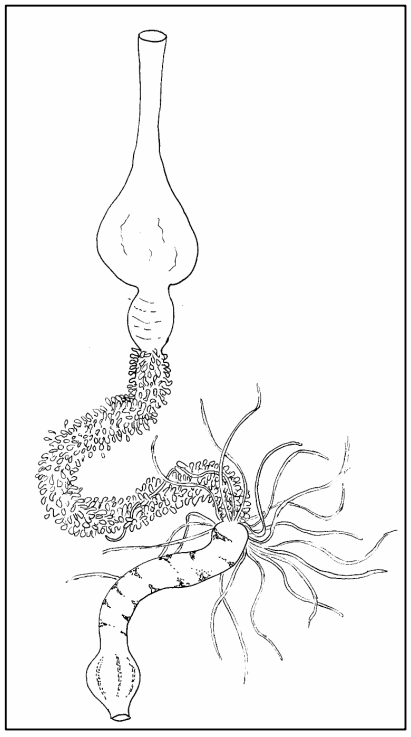


Figure 1.0-2- Digestive apparatus of *P. melas*. Drawed by R. Odoguardi

The proventriculus seams to have different functions, as a sieve, as masticating organ or a pump (Hengeveld, 1980c). The cardiac sphincter connects the fore intestine with the mid intestine. Microvilli and caeca increase the superficial area of the mid gut.

Enzymes (proteases, carboxylases, amylases and polysaccharidases) are synthesized in the mid gut and pass forward to the foregut (Lövei and Sunderland, 1996). The hind intestine includes the ileum, the colon and the rectum and opens exteriorly with the anus. Here the absorption of nutrients takes place. The mid gut is separated from the hind gut by the pyloric sphincter and in this region the Malpighian tubules, organs of excretion, are inserted.

The duration of digestion is function of temperature and quantity of ingested food (Lövei, Sunderland, 1990). Ground beetles are voracious feeders, they may ingest daily a quantity of food equal to their body mass (Thiele, 1977; Luff, 1987; Lövei and Sunderland, 1996). Literature data (Toft and Bilde, 2002) indicate that food composition is important for carabids which can select a diet to supply particular needs (Lövei and Sunderland, 1996).

Carabids may be distinguished according to their manner of food uptake. They may present exclusively extraintestinal digestion, as in the genus *Carabus* and *Calosoma*, in the Cychrini tribe and probably in Cicindelidae family, no trace of extraintestinal digestion, as in Omophroninae, or

intermediate situations with ingestion of fragments of food chewed, but indigested, and liquid derived from extraintestinal digestion in different proportions, as in the majority of Harpalinae, in certain Carabinae (e.g. the genus *Nebria*). The extra-oral digestion involves the ejection of enzyme juices into or on the food outside the insect, followed by the ingestion of predigested material, that is purely liquid food (Crowson, 1981).

The most of carabid larvae use external digestion (Brandmayr Zetto and Brandmayr, 1975) i.e. digestive juices are spat/vomited onto the food and the resulting fluid is then sucked up.

The morphology of the mouthparts is correlated with differences in the nature of the food and the mode of feeding (Crowson, 1981).

There is also a direct correlation between size and quantity of food that a carabid can ingest, in fact a longer beetle is assumed to have a larger gut to fill with prey. Carabid length, between 2.7 and 10 mm in a laboratory study on 35 carabid species, linearly related with the number of consumed cabbage root fly eggs (Finch, 1996). For each 1 mm increase in length up to 10 mm, the beetle consumes an extra 18 eggs (Finch, 1996).

1.2.d Carabid Foraging Behaviour

Carabid beetles exhibit a search behaviour common to invertebrate predators that was intensified when they encounters a prey (Lövei and Sunderland, 1996). There is a good correspondence among the sense used for prey detection, the type of prey and the feeding behaviour in carabid species (Wheater, 1989). Usually adults used their mandibles to kill and cut the prey into small pieces.

Starvation has been found to affect carabid predation activity. Starved *P. melanarius* Illiger were more active in the field than satiated ones (Fournier and Loreau 2001). *Notiophilus biguttatus* Fabricius starved for a long period attacked large preys according with levels of food deprivation (Ernsting and Werf 1988).

Temperature affects carabid prey choice and activity too. At 20°C *Harpalus rufipes* killed most slugs (Nève, 1994) and increases in daily temperatures are correlated with increases in slug predation by *Pterostichus madidus* Fabricius and *Harpalus rufipes* DeGeer (Ayre 2001) .

Pesticides and agronomic practices affect carabid foraging by changing prey availability and by reducing the number of generalist predators (Thorbek and Bilde, 2004, Iannotta et al., 2007b). The use of pesticides (consumption of dimethoate-treated prey) led to significant mortality of *Pterostichus madidus,* Fab., *P. melanarius* and *Nebria brevicollis* Fab. (Mauchline et al., 2004).

1.2.e Carabid as pest antagonist

Insect pests are economically controlled though the utilization of their natural enemies, or “beneficial” organisms such as parasites, predators and diseases (Fleschner, 1959). The predaceous nature of carabids and their prevalence in agro-ecosystems suggest their possible use

as biological control agents (Thiele, 1977; Kromp, 1999). The biological method for insect pest control is based on the knowledge that in nature exists a balance between plant-feeding insects and their enemies. Manipulating environmental factors it is possible to shift this balance towards the favourable situation (Fleschner, 1959). Native parasites and predators have a great economic importance (Fleschner, 1959). Much literature focuses on the ability of carabid beetles to decrease crop pest populations in agricultural or forest habitat. Evidence from field (e.g., Paill, 2000; Winder et al., 2005) and laboratory studies (e.g., Winder et al., 1994; Lang and Gsödl, 2001; Oberholzer and Frank, 2003) indicates that predaceous carabids consume many pest arthropods.

In several studies carabid beetles belonging to tribes Carabini, Pterostichini, Platynini, Loricerini, Bembidiini, played an important role against different pests (Thiele, 1977), as flies (Basedow, 1973; Coaker and Williams, 1963; Thomas et al., 2008), slugs (Mair and Port, 2001a,b), caterpillars and moth pupae (Frank, 1967; Thiele, 1977; Holste, 1915). Moreover some carabids as *Anchomenus dorsalis*, are well kwon to prey aphids (Thiele, 1977; Loughridge and Luff, 1983; Winder et al., 1994; Bilde and Toft, 1994; Lövei and Sunderland, 1996; Kromp, 1999; Kyneb and Toft, 2004). These laboratory and field experiments generally focus on feeding studies with a few carabid species tested with homopteran, lepidopteran, and dipteran pests. *Calosoma sycophanta* is the only carabid species known specialized on a prey and one of the first insect introduced for biological control (Burgess, 1911). *Pterostichus madidus, P. cupreus, P. melanarius* and *Abax parallelepipedus* are predators of winter moth pupae (Frank, 1967). Carabids have also been reported to consume dipteral pupae (Thiele, 1977; Hengeveld, 1980b; Paill, 2000). There were evidence of carabid predation on wheat midge larvae in spring wheat and onion fly pupae in corn fields (Floate et al. 1990, Menalled et al. 1999).

Field and laboratory studies showed that carabids which behave as antagonists are generalist predators not specialized on a particular pest, and this is the main reason for studying ground beetles in agricultural habitats (Allen, 1979). In fact, because of their predatory zoo-polyphagous diet, carabids could also prey olive fly pupae. My research moves to this direction to increase a little our knowledge, considering, according with Lövei and Sunderland (1996) and Toft and Bilde (2002), the notable gaps that the feeding studies present in spite of the wide range of literature and methods.

1.3 Olive groves

Olive groves are the most common agroecosystem in Calabria region, Southern Italy. Olive trees can grow in nutrient-poor, but well-drained soils. Full sun for fruit production and slight winter chill are essential conditions. This plant do not tolerate temperatures below -5°C and get seriously damaged by winter and spring frosts. Hot and dry air, particularly during flowering and fruit setting, may damaged olive trees (Fiorino, 2003). Also, in areas with low air circulation and high humidity,

diseases such as leaf spot appear more easily. In low rainfall areas (200-300 mm), olive yield is satisfactory in soils with good water retaining capacity, unless irrigation is applied. In high rainfall areas (400-600 mm) olive yield is good on condition that adequate drainage is provided. Planting density is proportioned to soil fertility and rainfall. Often trees are planted densely (200-250 trees/ha). The olive fruit is a drupe of nearly 1.5 cm in diameter, which becomes glossy black when ripe, with an extremely lipid-rich pulp and contains a single nut with a hard endocarp (Fig. 1.3). The ripening period extends from October to mid- March (Fiorino, 2003).

Calabrian olive grove is a typical Mediterranean agroecosystem that extend for about 175.000 hectares, and produces a lot of oil (24 % of total Italian production). There are different cultivar, pedo-climatic conditions and different type of agronomical conduction.

Figure 1.0-1- Olive drupes

Many arthropods live in olive grove. Carabid beetles are abundant in arable habitats all over the world (Gobbi e Fontaneto, 2005). Several studies have monitored the presence of predator insects in olive groves (Petacchi, and Minnocci, 1994; Belcari, and Dagnino, 1995; Morris and Campos, 1999), discovering between them also carabid species (Morris et al., 1999; Iannotta et al., 2007a). Few carabid species living in arable lands have specialized feeding habits. Most carabid species are zoo-polyphagous and take a number of different preys, ingesting eggs and larvae of Diptera and Lepidoptera, Coleoptera, aphids, spiders, Collembola and sometime flying preys (Hengeveld, 1980c; Paill, 2000; Toft and Bilde, 2002). In carabid fauna of calabrian olive grove, analysed by means of pitfall traps, macropterous species are dominant on brachypetous and dimorphic ones. Polyphagous predators are the most abundant (Pterostichinae, Nebriinae and Brachininae) followed by zoospermophagous (Harpalinae and Amarinae), spermophagous species (the genus *Ophonus* prevails) and few specialized predators as *Leistus* and *Notiophilus* that catch collembola, the helicofagous *Licinus silphoides* and the myrmecophagous *Siagona europaea*.

Ecological attributes of carabid assemblages give information about their role in the agroecosystem (Gobbi e Fontaneto, 2005). Olive groves, as other rural habitats (Gobbi e Fontaneto, 2005), are rich of eurytopic elements, well adapted to live notwithstanding high human impact (Brandmayr and Iannotta, unpublished data). In fact, frequency of predators or phytophagous species may be sensitive to human disturbance (Gobbi e Fontaneto, 2005). The dominance structure shows that nine species constitute the 92% of total captured ground beetles (Brandmayr and Iannotta, unpublished data).

The most abundant species were *Calathus fuscipes* (53.22 %), *Pterostichus melas* (17 %) and *Pseudophonus rufipes* (5.17 %) olfactory-tactile predators (Brandmayr and Iannotta, unpublished data). Other abundant carabids are *Calathus cinctus,* generalist predator; *Harpalus smaragdinus,* zoospemophagous abundant on sandy soil; *Amara aenea,* steppe element, zoospemophagous, xerophilic ed heliophilic; *Harpalus distinguendus*, steppe element, zoospemophagous; *Laemostenus cimmerius,* zoopolyphagous (Brandmayr and Iannotta, unpublished data).

1.3.a Olive Pests

The major insect pests of olive trees are the olive fruit fly *Bactrocera oleae* (Rossi 1790) (Diptera: Tephritidae), the olive-kernel borer or olive moth *Prays oleae* (Bernard 1788) (Lepidoptera: Yponomeutidae) and the black scale *Saissetia oleae* (Olivier 1791) (Rhynchota: Coccidae). Although *B. oleae* is considered the most serious threat, the other pests are widely distributed in the Mediterranean region and occur on olives at population densities causing important economic losses (Fiorino, 2003).

Of the less important insect pests, some occur in particular areas at population levels that cause serious damage, e.g. *Zeuzera pyrina* (Linnaeus 1761) and *Palpita vitrealis* (Rossi 1794) belonging to Lepidoptera, *Euphyllura olivine* Costa and *Aspidiotus nerii* Bouché 1833 belonging to Rhynchota*, Resseliella oleisuga* (Targioni-Tozzetti 1887)*, Otiorrhynchus cribricollis* Gyllenhal, 1834 and *Phloeotribus scarabaeoides* (Bernard 1788) belonging to Coleoptera. Others occur only occasionally, e.g. *Parlatoria oleae* belonging to Rhynchota*, Leucaspis riccae* Targioni Tozzetti 1881, *Philippia follicularis* (Targioni Tozzetti 1867). Moreover there are also some cryptogamic and bacterial diseases. Olive leaf spot (*Pseudocercospora sp.*) and olive knot (*Pseudomonas sp.*) are two very important diseases of olive trees. Spot develops with high humidity and rain, so cultural practices such as selective pruning are advisable to keep trees well aerated (Iannotta, 2001; Fiorino, 2003).

1.3.b The Olive fly *Bactrocera* (*Dacus*) *oleae* (Rossi 1790)

The olive fruit fly *Bactrocera oleae* (Rossi 1790) (Diptera: Tephritidae) (Fig. 1.4) from the tribe Dacini, is a key pest of olive groves in many regions of the world and a serious threat to the economic well-being of the olive industry.

Many literature data are available about the biology of *B. oleae* (Delrio and Cavalloro, 1977; Dominici et al., 1986; Delrio and Prota, 1989; Tremblay, 1995; Fiorino, 2003; Dimou et al, 2003). In the Mediterranean region *B. oleae* has occurred for over 2000 years, it can be found in all Mediterranean olive-growing countries, e.g. Italy, Spain, Greece, southern France, Africa, except for western Africa, Canary Islands, India; western Asia and it has recently invaded California (Zalom et al., 2003), where the olive is an introduced species, spread rapidly throughout the state.

Figure 1. 2- *Bactrocera oleae* female

The body is predominantly dark fuscous (length 6.5-7 mm), the head is larger than long, eyes are round, the torax in general is black with a silvery pubescent dorsal surface stripped with three narrow parallel black lines, femora are slender and without ventral spines, the abdomen is ovate, arched in lateral view, the abdominal tergites are separate and yellow to orange brown, or predominantly black (Tremblay, 1995). Olive fruit flies may be distinguished from related fruit flies by the presence of black spots on the wing. Females can be distinguished from males by the presence of an ovipositor. Males present a pecten of dark bristles on tergite 3 (Tremblay, 1995).

The life cycle of the olive fruit fly is closely linked to the seasonal development of *Olea europea* L. and to the local climate. Olive fly is able to reproduce and develop throughout all the year, provided that temperature and humidity are favourable and host fruits are available (Zalom et al., 2003). Under summer conditions, a preoviposition period of six to ten days elapses before mating. Courtship and mating occur at dusk during late summer. Male produces an auditory signal during courtship, while female produces a multicomponent pheromone that is a relatively long-range attractant for male. The major component of the female pheromone of *B. oleae* is racemic 1,7 dioxaspiro[5.5]undecane, which is accompanied by low levels (about 3%) of hydroxyl derivatives (Hungerford et al., 1998).

Fertilized females fly within the canopy, since they find olive fruit. In June females deposit eggs in early maturing olive fruits with the ovipositor. Green olive fruits shown a higher oviposition rate that red blush mature fruits (Yokoyama and Miller, 2004). The female usually deposits one egg per olive fruit, however multiple eggs may be laid in the same olive drupe. One female may lay about 200 to 250 in a lifetime and prefer large-fruited varieties to smaller-fruited varieties for egg laying.

Figure1.5- *Bactrocera oleae* larva

Eggs are laid directly into the host fruit; hatch in 2 to 3 days and the larvae (maggots) (Fig. 1.5) feed and develop inside the drupe destroying the pulp by tunnelling and causing serious damages to quality and quantity of olive and oil production (Michelakis and Neuenschwander, 1983; Richard and Rice, 2000; Iannotta, 2003). Feeding damage can cause the entry of secondary bacteria and fungi and the premature fruit drop. The larval stage is spent entirely within the fruit. Larvae develop in about 20 days during summer. Larvae that develop during summer may pupate in the fruit and emerge later in the season. Mature larvae produced during late summer fall and may complete pupal stage in the soil,

as in most tephritid species. The early olive fly generation pupate into the drupes, while the latter into the soil, this change is not influenced by photoperiod, but it is probably due to variation in chemical drupes composition (Cavallaro and Delrio, 1975). Larvae begin to colonize soil in October, pupae (Fig. 1.6) have a peak of abundance in November, and their number decrease until Febrary (Liaropoulos et al., 1978, Cavallaro and Delrio, 1975). Multiple generations occur throughout summer and fall. However, some maggots overwinter in fruit on trees and pupate in spring.

Figure 1.6- *Bactrocera oleae* pupae

The activity of larvae leaving the fruits has a circadian rhythm influenced by temperatures and photoperiods.

The distance which the larvae of *B. oleae* penetrate the ground varies in comparison with the soil and the amount of moisture which it contains. Under natural condition the majority of the larvae of *B. oleae* pupate in the top of 3 cm (Cavallaro e Delrio, 1975; Dimou, 2003), but the mean depth measured by Dimou, 2003, was 1.16 cm. Cavallaro and Derio (1975) and Liaropoulos et al. (1978) investigated the horizontal and vertical distribution of the pupae in the ground discovering the highest density in the south and west directions under trees. Pupae number under trees increases from trunk to the external part (Cavallaro e Delrio, 1975; Liaropoulos et al., 1978).

Pupal development requires 8 to 10 days during summer but may take as long as 6 months in winter. The insect spends the winter in the pupal stage few cm below the soil. Duration of the life cycle varies from one month in summer, to six or seven months (Tremblay, 1995).

Adult flies emerge from March to May and attack olives from the previous season remained on trees. During early summer, as day length and temperatures increase, females enter a state of reproductive diapause, in which few or no eggs are produced. However adult flies remain active and they may disperse to new locations. In fact *B. oleae* has a long-distance dispersal power, even 10 km of displacements although movements are of short range in favourable habitats (Tremblay, 1995). By late June to the beginning of July the new olive crop develops and attracts females that begin to produce eggs again. Adults feed on nectar, honey dew and other opportunistic sources of liquid food.

Infestations may begin in June-July, but after the first generation development, the population decreases, due to high summer temperatures (exceeding 33°C) and low relative humidity. Then population gradually increases to a maximum by September-October. Infestation lasts from September until November-December. Severe olive fruit fly infestations are generally associated with significant losses (Tremblay, 1995).

Where climate is favourable up to three generations may be completed during the year. Adults are able to live for several months. Individuals that emerge in autumn show the maximum

longevity. Olive fly can survive for a short time at temperatures slightly below 0°C, and only some individuals can tolerate temperatures from 0 to 5°C. Temperature and light intensity are important factors for pupae and adults (Delrio and Cavalloro, 1977; Delrio and Prota, 1989; Koveos, 2001; Yokoyama and Miller, 2004; Raspi et al, 2005). Long term studies on the population dynamics of *B. oleae* (Delrio and Cavalloro, 1977; Delrio, 1978; 1979; Delrio and Prota, 1989) underline the fundamental influence of the fruiting cycle on the regulation of the populations, olive production is the most important single factors determining adults numbers.

B. oleae control strategies include insecticides, bait sprays, use of sterile males, semiochemical based products (Montiel, 2002), trapping of adult flies (Katsoyannos and Kouloussis, 2001), harvest timing, biological control and fruit sanitation after harvest. Olive fly control has been based mostly on bait sprays with organophosphate insecticides (usually dimethoate and fenthion). A Cellular Automata model was also developed to simulating life and reproduction cycles of olive fruit flies (Pommois et al., 2006).

Survival of pupae was due to abiotic and biotic causes, among those in the latter case the predators were most important (Cavallaro and Delrio, 1975).

Among specific olive fly predators and parasitoids the commonest is *Psyttalia* (*Opius*) *concolor* (Szépligeti, 1910) (Canale and Loni, 2006) a Braconid larval parasite, utilised in biological control programmes (Raspi, 1995). There are other Braconid larval parasites as *Opius lonsburyi, Opius africanus, Opius dacicida* too. Nevertheless, in addition to parasitoids, polyphagous predators as ants, carabids, Dermaptera, Myriapoda and Staphylinidae can contribute to the reduction of olive fly population (Neunschwander et al., 1983). Ants are considered the primary olive fly pupae predators, but also mites are found in the pupal samples (Bateman, 1976).

1.3.c Carabids/olive fly interaction

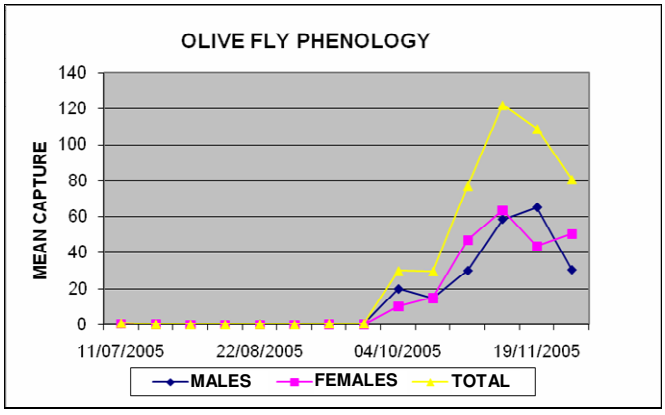
Until now the predator activity of carabid beetles on olive pests have not been entirely studied. In fact there are insufficient data regarding not only carabid predation on olive fly pupae, but also carabidocoenosis in olive grove. Moreover, the value of Diptera larvae and pupae as carabid prey has not been systematically studied (Toft and Bilde, 2002).

Larvae and pupae of *B. oleae* are vulnerable to many predators as birds, arthropods, especially ants and ground beetles, and others organisms as nematodes, pathogenic bacteria, fungi (Cavallaro and Delrio, 1975; Orsini, 2006).

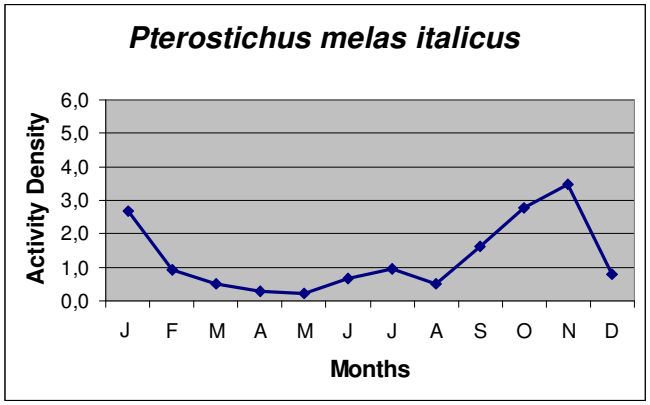
Delrio and Cavalloro (1977) reported centipedes, ants, carabids, and birds as important predators of olive fly pupae and Katsoyannos (1992) indicated carabids ants and earwings as soil predators of *B. oleae* pupae. Bigler et al. (1986) in western Crete found that birds were responsible for 70.2% of pupal predation, with the arthropods (mainly ants, with some carabids and others) for 29.8% . Liaropoulos et al. (1979) in Greece suggested that the influence of arthropods on pupae in the soil is very low, while bird predation could be of certain importance. Delrio and Cavalloro

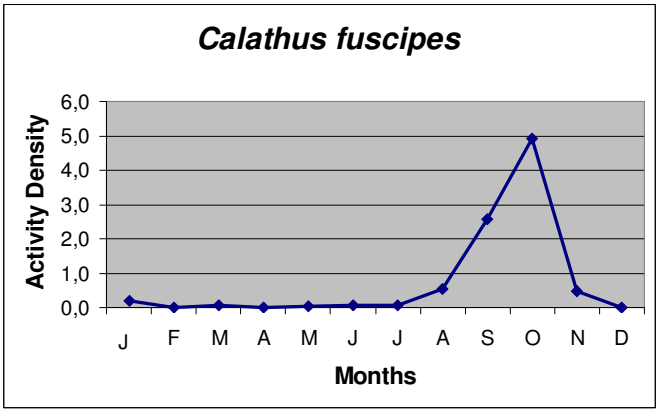
(1977), that did an experiment on olive fly pupae in Italy, also deduced that ants, chilopods, birds and carabids may be important predators of olive fly pupae and O'Neal et al. (2005) affirmed that carabids have potential to assist in controlling olive fly populations at the pupal stage. In literature there are only few indications of olive fly pupae predation probably operated by carabid beetles (Nuenschwander, et al., 1983; Delrio and Cavalloro,1977; Bigler et al.,1986). Larvae and adults of *Poecilus cupreus* (L.), *Pterostichus creticus* and *Carabus banoni* (Dejean) are indicated as olive fly larval-pupal predators, but laboratory feeding trials have been executed without alternative prey, in Crete (Nuenschwander et al., 1983).

Natural enemies living in olive grove, including, ants, chilopods, earwigs (Dermaptera), spiders, beetles and particularly carabids, often reach the highest densities between June and October (Morris et al., 1999; Morris and Campos, 1999). Autumn breeding carabid species show a perfect coincident phenology with *B. oleae* live cycle, in fact the peak of seasonal activity of these carabids takes place when there is the greatest abundance of olive fly pupae in the soil (Grafic 1.1; 1.2 and 1.3).



Grafic 1.1- Olive fly phenology (CRA data)





Grafic1.2- *Pterostichus melas* phenology Grafic 1.3- *Calathus fuscipes* phenology

Summing up, in literature there are only few and incomplete information regarding the potential of polyphagous carabids to assist in controlling olive fly populations at the pupal stage, so my research investigates in this direction and tries to fill, even if partially, this gap.

According to literature (Sunderland, 1987; Wratten, 1987; Lövei and Sunderland, 1996), the effects of a natural antagonist on a pests may be studied as follow:

1- Evaluate dynamics and correlating predator and pest density

2- Obtain direct evidence of trophic link between the prey and the predator

3- Experimentally manipulate predator number and its effects on prey density

4- Integrate the above information to quantify the effect of predator on prey.

This research is extensive, complex, ambitious, to be completely developed needs many researchers and need more than three years of study. This work of thesis has been mainly a laboratory study and the point 2 has been developed in laboratory. This will be the beginning of a new line of investigation with future advantageous applications and it is the first study in Italy about the interaction between carabids and olive fly.

RESEARCH GOAL

This study examine the existence of carabid predation on *B. oleae*, especially on pupal stages. Research goal is to identify which carabid species are natural olive fly predators and when they prey olive fly pupae, to evaluate carabid beetle role as olive fly pupae predators, and the possibility to employed them as olive fly natural antagonists. During this study the major quantity of information about carabid biology (e.g. food preferences, daily rhythm) and relation to their environment (e.g. density, alternative prey) have been experimentally collected to investigate possible implication with ground beetles role as olive fly antagonist.

2. INTRODUCTION TO THE PHASES OF THE STUDY

2.1 Preliminary test

A necessary first step is to establish which carabid species prey on the target pest, *Bactrocera oleae*.

Other authors carried out laboratory feeding choice tests offering different prey items to a predator and recording preferences (Fawky et al., 2003; Oberholzer and Frank, 2003; Prasad and Snyder, 2004).

Preexistent data about carabid-coenoses in olive groves, collected by our research group (Brandmayr and co-worker) in collaboration with C.R.A. Research Center for Olive Growing and Olive Oil Industry team (Rende, CS), provided a list of the most abundant carabid species in Calabrian olive groves that was the beginning of this study (Brandmayr and Iannotta, unpublished data). The polyphagous and the zoo-spermophagous species, potential pupae predators, except spermophagous ones, was chosen among these species. Species that prey olive fly pupae were selected by a preliminary laboratory screening consisting in feeding choice experiments with alternative preys. It is important to offer alternative preys in laboratory experiments (Oberholzer and Frank, 2003).

Preexistent laboratory studies conducted by Neuenschwander et al. (1983), without alternative prey, indicated some carabid specie as *B. oleae* predators. However, very low numbers of individuals was tested for each species. In literature there were nothing else regarding the efficacy of carabids against olive fly.

2.2 Quantitative test

Further experimental, quantitative and careful data are necessary to have other information on carabids/olive fly pupae interaction. This second laboratory phase of the study is finalized to verify direct predation on olive fly pupae by species of ground beetles abundant in calabrian olive groves and their possible role as olive fly antagonists. Feeding choice experiment with alternative preys conducted in petri dish arenas are common in carabid studies (Bilde and Toft, 1994; Oberholzer and Frank, 2003; Prasad and Snyder, 2004).

Some adults of carabid beetles may consume at least their own body weight in food daily, but there were differences between species, *Pterostichus vulgaris* may ingest more than three times its own weight daily (Thiele, 1977). Partially phytophagous *Amara* and *Harpalus* species ate considerably less than polyphagous species. *Carabus banoni, Poecilus cupreus* and *Pterostichus creticus* were able to destroy up to 26 puparia in three days (Neuenschwander et al, 1983).

2.3 Microscopical gut content analysis

The classical method to study diet composition in the field is the microscopical analysis of the contents of the digestive tract. Microscopical gut content analysis was performed by many authors to study carabid diet (Davies, 1953; Sunderland, 1975; Thiele, 1977; Hengeveld, 1980a, 1981; Forsythe,1991; Zetto Brandmayr et al., 1998a). Nevertheless, crop analysis is impossible in species with extraintestinal digestion, because of the absence of recognizable solid fragments in their gut-contents (Crowson, 1981; Toft,and Bilde 2002), in fact our knowledge about these species derived only from observations. Indigested musculature of the prey may be present in the crop of predator with negligible preoral digestion. Digestive tract content analyses were mainly used to find pests in the gut of predators collected in cultivated fields.

To understand the effective role that native natural enemies of *B. oleae* might play in pests predation in field, could give us important information, also about soil food webs.

Direct observations in field of feeding behaviour are an important method to investigate carabid diet, but for most carabid species direct observations are too rare. Microscopic identification of prey remains or direct observations of predation events are traditional approaches to analyse predation under field conditions, but these techniques are time-consuming and of limited use in such instances at present. In fact it is impossible to identify liquid food or soft-body prey using dissections.

Modern techniques include stable isotope analysis, fatty acid spectra analysis (Ruess *et al*. 2004; Albers *et al*. 2006), prey proteins detection using polyclonal or monoclonal antibodies (Frank, 1967; Sunderland, 1988, 1996; Pierce & Boyle, 1991; Symondson and Liddell, 1996; Symondson, 2002), electrophoretic detection of prey isozymes, radioactive tracer tests (Frank, 1967), chromatographic detection of prey pigments. Diet and food preferences may be studied also using radioactive tracers, isotope-labeled prey technique, electrophoresis and various serological technique (Lövei and Sunderland, 1996).

2.4 Molecular gut content analysis

Recently molecular markers have also been used to study invertebrate predation in the field (Agustí et al., 2003; Hoogendoorn & Heimpel, 2003; Greenstone and Shufran, 2003; Dodd, 2004; Kaspar et al., 2004; Harper et al., 2005; Ma et al., 2005; Campobasso et al. 2005; Zhang et al., 2007a; 2007b).

Molecular prey identification in the gut of a predator is an effective approach to quantifying predator–prey interactions (Wallace 2004; Foltan et al., 2005; Pons, 2006). Molecular techniques enable identification of species-specific trophic relationships among soil invertebrates (Juen and Traugott 2005, 2007) and bring to detection of prey at high sensitivity and specificity (Symondson 2002).

PCR molecular gut content analysis is a well established technique. An advantage is that in insects an assortment of candidate target regions has already been sequenced, moreover,

once prey species-specific primers have been designed and published, they can be produced cheaply and employed in reproducible protocols.

The purpose of this part of my research was to develop and test a DNA-based identification method to put in evidence carabid predation on *B. oleae*.

2.5 Carabid daily rhythm

The aim of this part of the study is to discover when carabids usually prey during daytime. A work on the possible role of carabids as pest antagonists, must takes account of their pattern of activity. Carabids may prey olive fly pupae or fallen larvae too. Temperatures and photoperiods influence the activity of olive fly larvae in leaving the fruits. In open field larvae leave the fruits with a frequency which oscillates daily following the light-dark cycle. The frequency is significantly higher in the section of the tree facing South and West (Ricci et al. 19;). *B. oleae* larvae falls during the whole day, night included; in detail, with observed temperature in the early morning below 0° C, the peak in the frequency is always in the late morning, with higher temperature the peak in the frequency advanced at the sunrise. The frequency of falls increases with the temperature (Ricci et al. 19; Tremblay, 1995).

Moreover this study of carabid daily rhythms increases our knowledge about biology and relations with the environment of ground beetles.

According to literature, the rhythmicity of carabid beetles seems to be a complicated phenomenon, depending on many different factors. A dominance of nocturnal activity seems to exist in autumn breeders (e.g. Thiele and Weber 1968, Luff, 1973; Kegel 1990), big sized species (Luff 1978,), south palaearctic species (Thiele and Weber 1968), and species typical for forests (e.g. Thiele, 1968; Gruschwitz, 1983). On the other hand, Luff (1978) observed an intensive nocturnal activity in a strawberry field. The night activity of carabids seems to be influenced mainly by the “lightness” factor, whereas in day activity the moisture plays an important role too (Thiele, 1968; Thiele and Weber, 1968; Kegel, 1990).

Activity rhythm has been studied in field using pitfall traps, that were emptied at regular intervals of time, (Thiele, 1977; Luff, 1978; Alicata et al., 1980; Atienzal and Farinós, 1996) and in laboratory with different methodology, stabilimeters, ultrasound recorders, infrared recorders, radar monitoring (Feng et al., 2007) and tracking methods (Chapman, et al., 2004; Szyszko et al., 2005), microwave radar based on the Doppler effect (Pasquali and Renzi, 2005; Pasquali et al., 2006), or attogramm (Thiele, 1977).

Several studies deal with the daily activity of carabid beetles in Middle Europe in different biotopes (e.g. Williams, 1959; Thiele, 1967; Thiele and Weber, 1968; Luff, 1978; Gruschwitz, 1983; Brunsting, 1983; Kegel, 1990; Chapman et al., 1999; 2004) According to the majority of these studies, carabid beetles living in forests are ascertained to be active mainly in the night (e.g. Thiele and Weber, 1968; Gruschwitz, 1983) and hungry individuals may be more active because of searching for prey (e.g. Fournier and Loreau, 2002). Therefore, higher activity may indicate worse feeding conditions in the habitat. Luff (1978) puts in evidence a relationship

between body size and nocturnal activity, underlining that larger carabids are prevalently nocturnal.

2.6 Carabid density in olive grove

Cultivated lands give hospitality to a typical carabid fauna (Kromp, 1999). Carabids are often the numerically dominant arthropods in the soil (Lövei and Sunderland, 1996; Kromp 1999), besides, ground beetles constitute an important component of the litter fauna in agro-ecosystems and are very common in olive groves (Iannotta et al., 2007a). Even so high density not always corresponds to numerical dominance (Lövei and Sunderland, 1996). Collected data show a density from <1 to >1000 individuals per square meter, with enormous fluctuation in time and space (Lövei and Sunderland, 1996). In annual crop total adult carabid averaged 32 per square meter, ranging from 1 to 96. Much higher densities were found in filed boundaries (233 per square meter) and lower in forest (2 per square meter) (Lövei and Sunderland, 1996).

Knowing the effective density of carabids in field may give a lot of information about ground beetles biology, may be used to reproduce the same conditions in the laboratory and to evaluate the influence of carabid guild in agro-ecosystem.

A high density of a carabid species indicates good conditions for reproduction and survival. Abundance of beetles was strongly correlated with habitat structural complexity (Atienzal and Farinós, 1996). To evaluate the effects of carabid predation on olive fly population it is important to estimate the abundance of predators and preys. Indeed, a predator may reduce prey population only if it is abundant (Oberholzer and Frank, 2003).

In addition to pitfall trap collected data, we have estimated ground beetle density per square meter (Sotherton, 1984; 1985) in olive grove, in enclosures of 2 m^2 with exhaustive trapping ("*Leerfang*"). This method is considered to provide reliable density estimates for carabids that are active on the ground (Kromp, 1999).

2.7 Simulation of carabid predation on *B. oleae*

B. oleae pupates under the soil surface, where soil is easily to larvae penetration. Dimou et al. (2003) tested 96 larvae and discovered that the majority of them pupated in the top 3 cm and the mean depth of all units was 1.16 cm. The depth of pupation depends on soil type, moisture, temperature–soil type interaction and soil type–moisture interaction. Larvae pupated at a greater depth in limestone than in the alluvial deposits and flysch soil (Dimou et al., 2003). I verified the capacity of carabids to prey on buried olive fly pupae and to catch fallen larvae in laboratory experiments.

3. MATERIALS AND METHODS

3.1 Sampled olive grove

The sampled sites were Calabrian olive groves of Terranova da Sibari, Mirto-Crosia, Arcavacata and Piana della Torre (Trebisacce, CS) from October to November 2006, 2007 and 2008. In the selected olive groves the soil cover was low. *Nebria kratteri* was collected in a beechwood near Orsomarso in december, 2007. Tested species were collected using bait traps for the capture of live carabids (Zetto Brandmayr et al., 2007). The bait trap consists of standard measuring cups, with upper diameter of 6 cm and depth 17 cm, provided by attractive bait composed by vinegar and fruit juice in equal parts (Fig. 3.1). Traps were emptied one way at week. This sampling method consents to collect data concerning the presence of carabids and their soil surface activity, relevant in carabid predation studies. Piana della Torre is an alluvial plain of rivers Ferro and Straface, composed by coarse sediments with dimensional range from moderately to thin. The olive grove of Piana della Torre (Fig. 3.2) is located near Trebisacce (Calabria, South Italy) on an area of about 1 ha, at 30 m a.s.l. and it contained about 300 olive tree of 3-4 m in height. The soil is moderately calcareous (5-10% CaCO3), not-salt, low content of organic materials (0,7-1,5%), reaction alkaline (7,9-8,4 pH), moderate capacity to assume the nutrients and make them available to plants in the surface horizons (ARSSA, 2003). The site was not chemically treated during the experimental period.

Figure 3.1- Bait trap

Figure 3.2- Olive grove of Piana della Torre

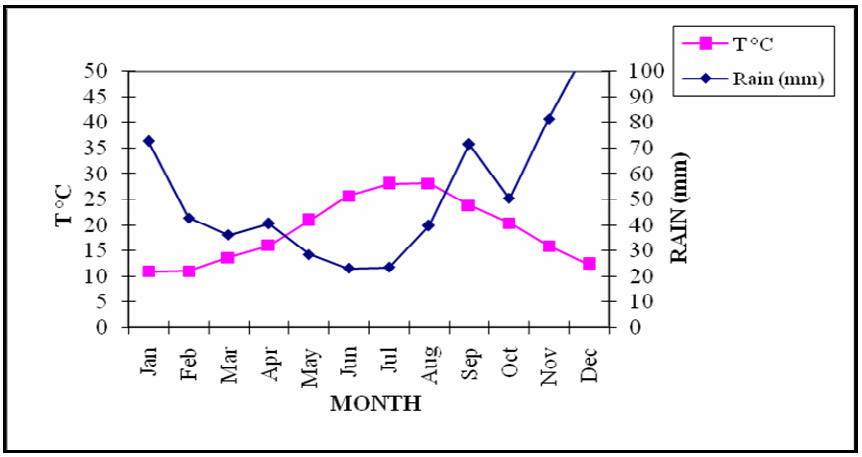
The olive grove of Terranova is positioned on the hills of the Crati valley, at about 300 m a.s.l.. The soil is formed by pleistocenic silty claies, characterized by alternation of rehydratation and desiccation, with mean content of organic materials and reaction alkaline (8-7,7 pH).

The olive grove of Mirto is in plain of the river Trionfo; the soil is formed by alluvial deposits with texture muddy, reaction alkaline (7.8 pH), moderate contents of limestone and high content of organic, scarcely humic materials. We used 10 bait traps per site.

Figure 3.3 -Olive grove of Terranova.

3.2 Laboratory rearing conditions

The carabid beetles were housed in terrains in the laboratory in a climatic chamber at day/night T 20°C/15°C, under L/D: 11/13 photoperiod. These conditions are similar to mean autumnal ones (September, October and November 1999-2005) recorded by the thermo-pluviometric station of Mirto (Grafic 3.1).



Grafic 3.1- Climogram recorded by the thermopluviometric station of Mirto during the period 1999-2005

The bottom of the terrains was covered with soil taken from the sampled olive groves and form the Botanic Garden of the University of Calabria, humidified with regular additions of water. Stones and plastic refuges were provided.

Polyphagous carabids were fed on veal meat, zoospermophagous ones on wheat seeds. Before experiments carabids had passed in the climatic cell a period of acclimatization of nearly a month.

3.2.a Predator carabid beetles studied

Pterostichus melas, Calathus fuscipes, C. cinctus, Laemostenus cimmerius, Carabus coriaceus were autumn breeding species, zoo-polyphagous predators both as adults and larvae (Magistretti, 1965; Brandmayr et al., 2005) having olfactory-tactile searching strategies (Brandmayr and Zetto Brandmayr, 1980). *Pseudoophonus rufipes, P. griseus* are zoospermaphagous species, *Brachinus sclopeta*, *B. italicus, B. crepitans, Anchomenus dorsalis, Distichus planus* are spring breeders zoo-polyphagous predators (Magistretti, 1965; Brandmayr et al., 2005). *Nebria kratteri,* is a species very rare in olive groves, used as comparison.

Calathus (Calathus) fuscipes (Goeze, 1777)

Corotype: Euro-Mediterranean

Distribution: Europe, Great Britain and isles, Caucasus, Asia minor, Northern Iran, Syria, Palestine, Morocco, Algeria, Tunisia, Cyrene (Magistretti, 1965).

Calathus fuscipes is a medium-sized, 9-14 mm, quite fat carabid of oval body-shape (Trautner and Geigenmüller, 1987). The typical form does not exist in Italy, where the sub-species *C. fuscipes graecus* Dejean, 1831 (= latus Serville, 1821) is present (Magistretti, 1965).

Ecological notes: generalist predator, steppe element of open formation, present in glade and in anthropic habitats, (Vigna Taglianti, 1997; Brandmayr et al., 2005). Eurytopic of open formations (Lindroth; 1945), absent in high vegetable covering and on sandy soils, frequent in cultivated lands (Thiele, 1977), but also in grasses and grazing lands of Central Europe. Autumnal breeding species with winter larvae. Dimorphic species (den Boer, 1977). *Calathus fuscipes graecus* is the most common species in olive groves.

Calathus (Neocalathus) cinctus (Motschulsky, 1850)

Corotype: W-Paleartic

Distribution: Europe, Great Britain and isles included, Caucasus, Asia minor, Syria, Palestine, North Africa, except Egypt, Italy (Magistretti, 1965).

Ecological notes: autumn breeding species, generalist predator, prefers clayey moist soils (Magistretti, 1965; Brandmayr et al., 2005).

Carabus (Procrustes) coriaceus (Linneo 1758)

Corotype: Euro-Anatolic

Distribution: Asia Minor and Europe, except Great Britain, Ireland and Iberic peninsula. In Italy from Alpi Cozie to Giulie, in Padana plain and Appennino, except Aspromonte (Magistretti, 1965).

The subspecies *C. coriaceus mediterraneus* Born, 1906, 32-40 mm, black and large, with irregularly reticulated elytral sculpture, is present in Calabrian olive groves. *Carabus coriaceus mediterraneus* has a trans-Adriatic distribution and is widespread in the Balkan Islands, Corfù, Italy and Albania (Magistretti, 1965).

Ecological notes: brachipterous, olfactory-tactile zoo-polyphagous predator with external digestion, autumn breeding species, (Brandmayr et al., 2005). Ovideposition lasts 14-20 days and the egg is placed in a single cell underground. Embryonic development lasts 14 days; larval development 50-60 days; pupal 14-15 days (Sturani, 1962). For defence this species can spray acid fluids from its anal glands and salivate digestive ferments. Wings are reduced (Trautner and Geigenmüller, 1987). Eurytopic, this species prefers soils with good water retention and is easily found in mountain pinewood, thermophilic beechwood.

Brevimandibularis *Carabus* species ingest earthworms, slugs, snails and arthropods and are less specialized than longimandibularis ones, that ingest snails.

Pseudoophonus (Pseudoophonus) rufipes (De Geer, 1774)

Corotype**:** Paleartic (Oloartic).

Distribution: Europe, included the British Isles, Caucasus, Siberia, Japan, Turkestan, Asia Minor, Northern Iran, Morocco, Algeria, Azores, Madeira, Italy (Magistretti, 1965).

Ecological notes: steppe element, linked to moist clayey soil, zoospermophagous with opportunistic diet, (Brandmayr et al., 2005); it ingests different seeds and presents predatory activity on insects and little invertebrates (Luff, 1987). Autumn breeders with winter larvae, macropterous, eurytopic, abundant in open habitats, arable fields, cultivated lands and grasses, present also in urban zones (Brandmayr et al, 2005).

Pseudoophonus (Pseudoophonus) griseus (Panzer, 1796)

Corotype: Palaearctic

Distribution: Europe, except on the British Isles, Caucasus, Siberia, Turkestan, China, Japan, Asia Minor, Northern Iran, Morocco, Tunisia, Azores, Italy, included Sicily and Sardinia, rare on the Alpine Chain (Magistretti, 1965).

Ecological notes: it is found mainly on dry, sandy or gravely soils, both in plains and in mountains.

It is possible to distinguish *P. rufipes* from *P. griseus* observing sides of pronotum, that are concave towards the base, hind-angles sharp in *P. rufipes* while are almost straight toward base, hind-angles blunted in *P. griseus* (Trautner and Geigenmüller, 1987).

Pterostichus (Feronidius) melas (Creutzer, 1799)

Corotype: Eurapean

Distribution: Middle and South-Eastern Europe and Caucasus, Italy, except Alps, Sicily, Elba and Giglio islands.

Species belonging to the genus *Pterostichus* are medium-large beetles. The subspecies *Pterostichus melas italicus* (Dejean, 1828), is an endemic species in Italy, absent in Eastern Veneto, Venezia Giulia and Sicily (Magistretti, 1965).

Ecological notes: brachypterous, thermophilic, eurytopic, lives both in flat and mountainous areas, until 2000 m, but prefers open habitats. Abundant on clayey soil, frequent in open habitats and wood. In a previous study (Brandmayr, Iannotta et al., unpublished data) *P. melas italicus* was found to be an abundant species in the study area of Terranova.

Laemostenus (Pristonychus) cimmerius (Fischer von Waldheim, 1823)

Corotype: Oriental-Nordic-Mediterranean

Distribution: Dalmatia, Greece, Albania, Italy. Probably also in Caucasus and Crimea (Magistretti, 1965).

Laemostenus cimmerius cimmerius (Fischer von Waldheim, 1823) is an endemic autumn breeding species present only in Southern Italy.

Ecological notes: species which lives at the entrance of the caves, in low light conditions, in humid and shady habitats (Trautner and Geigenmüller, 1987). Adults show a thermal parapausa. Larvae are present in winter. Usually tarsi are pubescent on the dorsal side.

Brachinus (Brachinoaptinus) italicus (Dejean, 1831)

Corotype: Sicily-Appenninic

Distribution: endemic Italian species, present in central and southern Italy, Sicily, Tuscan, Umbria, Lazio, Campania, Basilicata, Calabria (Magistretti, 1965).

Ecological notes: lives both in mountains and in plains.

Brachinus (Brachinus) crepitans (Linnaeus 1758)

Corotype: West-Paleartic

Distribution: Middel and southern Europe, Great Britain and Ireland, Caucasus, Western Turkestan, Siberia, Asia Minor, Morocco, Syria, Iran, Algeria, Tunisia, Italy, except isles and Alpine chain (Magistretti, 1965).

Ecological notes: live on moist soils, both in plain and mountains

Brachinus (Brachynidius) sclopeta (Fabricius, 1792)

Corotype: Euro-anatolic-maghrebic

Distribution: Middle and southern Europe, Caucasus, Asia Minor, Morocco, Algeria, Tunisia, Italy, except Alpine chain and isles (Magistretti, 1965).

Ecological notes: lives both in plains and in mountains on moist soils.

“Bombardier beetle” head and pronotum red, elytrae blue. They are often present in open land and clayey soil (Trautner and Geigenmüller, 1987).

Distichus planus (Bonelli, 1813)

Corotype: African-Indian-Mediterranean

Distribution: Meidterranean bacin, Caspio, eastern Africa, northen India. Italy: Sicily, Sardinia, Corsica, from Calabria to Tuscany (Magistretti, 1965).

Ecological notes: lives on alluvial deposits, near lakes, rivers, ponds and sandy beach near salt marshes, brachipterous, spring breeder.

Anchomenus (Anchomenus) dorsalis (Pontoppidan, 1763)

Corotype: western Paleartic

Distribution: Europe, Great Britain Ireland included, Caucasus, Siberia, Turkestan, Asia Minor, Morocco, Italy (Magistretti, 1965).

Ecological notes: lives in plains and in mountains until 2000 m, on moist soils; forms colony with *Brachinus sclopeta*. Olfactory tactile predator (Magistretti, 1965; Brandmayr et al., 2005).

Nebria (Nebria) kratteri (Dejean & Boisduval, 1830)
Corotype: South-Eastern -European
Distribution: South paleo-Aegean, trans-Ionic species.
Southern Albania, Greece, Central and Southern Italy (from Lazio and Abruzzo to Aspromonte) (Magistretti, 1965).
Ecological notes: typical wood-species, live in humid habitats, common in cultivated fields and forests in Europe. Autumnal breeder with winter larvae that burrow in the soil (Trautner and Geigenmüller, 1987).

3.3 Prey used and food preparation

Adult wild-type fruit flies *Drosophila melanogaster* (Meigen, 1830), earthworms *Nicodrilus caliginosus* Savigny, 1826, and pupae of *Bactrocera oleae* were used as preys. These prey items may be natural prey for carabid beetles in olive grove and are commonly used in choice feeding experiments (Toft, 1995; Mair and Port, 2001a,b).

Earthworms were collected in sampling olive groves and in the Botanic Garden of the UNICAL and housed in a terrain in laboratory. Before feeding preference tests earthworms were frozen and cut in minute pieces.

Pupae of *B. oleae* were obtained from infested olives collected in study areas and placed on a wire screen in the laboratory. Mature larvae left olive drupes falling through the screen in a plastic box where each morning I collected pupae and stored them at -20°C.

Fruit flies were obtained from laboratory culture (recipe of fruit flies breeding substrate by the Agrarian Entomology Institute of Milan, Italy). Fruit fly is commonly used in laboratory feeding experiments and is known as a valuable food source (Allen, 1989; Toft, 1995; Barker, 1998). All preys were killed by freezing to conserve their nutritional quality aspects (Bilde and Toft, 1994), and then offered in the experiments on a piece of tinfoil to reduce dehydration.

3.4 Preliminary tests

Brachinus sclopeta, B. italicus, B. crepitans, Anchomenus dorsalis, Pterostichus melas, Calathus fuscipes, C. cinctus, Laemostenus cimmerius were collected from mid-October to mid-December 2006 using bait traps in the olive groves of Mirto, Terranova da Sibari and Arcavacata; *Carabus coriaceus, Pseudoophonus rufipes, P. griseus, Laemostenus cimmerius* from mid-October to mid-December 2007 in olive groves of Terranova da Sibari, Calabria, South Italy. *Distichus planus* was collected in may in the olive grove of Piana della Torre di Trebisacce, Calabria, South Italy. *C. coriaceus* larvae were obtained in laboratory rearing. All larvae were tested at the first instar. Each carabid was tested individually in a petri dish with moist filter paper and about equal quantities of the three different prey types (Fig. 3.4). To avoid that thigmotactic behaviour by predator could increased encounters between beetle and prey

foods, preys were placed a few distant from the dish edge. Each carabid must make a choice among different prey items. Carabids were starved two days before the feeding test, as in Fawky and Toft (2005).

Figure 3.4-Feeding choice experiment

Larvae were tested in petri disches of 9 cm of diameter with moist soil, being larvae more sensitive than adults. Each individuals were tested once and the test lasted one night, when carabids are more active (Fig. 3.5).

For each species (*Brachinus italicus, B. sclopeta, B. crepitans, Anchomenus dorsalis, Carabus coriaceus, Pseudoophonus rufipes, Pseudoophonus griseus, Pterostichus melas, Calathus fuscipes, Distichus planus, Laemostenus cimmerius*) at least 30 individuals was tested. Species of little dimension (*Brachinus sclopeta*, *B. italicus, B. crepitans, Anchomenus dorsalis, Distichus planus*) were tested in petri dishes of 9 cm of diameter, with 2 pupae, 5 fruit flies and a proportioned piece of earthworm. Species of larger dimension (*Carabus coriaceus, Pseudoophonus rufipes, Pterostichus melas, Calathus fuscipes, Laemostenus cimmerius*) were tested in petri dishes of 15 cm of diameter, with 4 pupae, 8 fruit flies and a proportioned piece of earthworm. The number of prey items offered was proportioned to carabid dimension. Food remains were recorded the next morning.

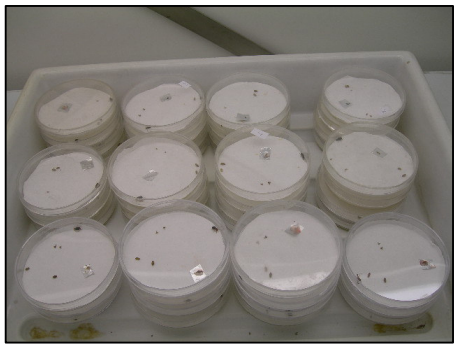


Figure 3.5- Preliminary feeding tests in petri dishes.

Data were analysed with the chi-square test, and the number of prey items was not considered.

3.5 Quantitative tests

A quantitative analysis of the ingested food was performed. Species tested in this phase were *P. melas* (30), *C. fuscipes* (30), *L. cimmerius* (19), *C. coriaceus* (12), which ingested pupae during the preliminary feeding experiments, and *Nebria kratteri* (10) tested to constitute a comparison. Carabids are not equally abundant in olive groves. Half of the quantitative tests were made with males.

3.5.b Experimental conditions

Carabid beetles were not fed two days before experiments. The food search motivation affects carabids in their prey choice and activity, as showed by Fournier and Loreau (2001). In fact, satiated predators were expected to be more selective in their choice of prey than hungry ones (Bilde and Toft, 1994). In preference experiments carabids were kept individually in petri dishes with filter paper on the bottom (height 3 cm, diameter 15 cm) (Bilde and Toft, 1994; Mauchline et al., 2004). All experiments were performed at 20° C.

Bactrocera oleae pupae, fruit flies and earthworm pieces were positioned in petri dishes. Carabid tested specimen had made a choice among the three offered prey types. To avoid

biases due to prey position, they were positioned in different combination of places, equidistant among them and from the tested carabid.

Each beetle was tested once. Each test lasted 2 hours. Preys and carabid predators were weighed before and after the test, with a precision balance 10^{-4} grams (Adventure Pro AS64, Ohaus Corporation), to measure weight variations and the amount of consumed food. In each test were approximately used the same weight of different preys (0.0300 g of weight, except for the large *C. coriaceus,* 0.0700 g). The experimental procedure was similar to study carried out to determine feeding preferences by Bilde and Toft (1994, 1997b), Mundy et al. (2000), Langs and Gsödl (2001), Oberholzer and Frank (2002), Mauchline et al. (2004). The test started when specimen was placed inside the petri dish.

3.5.c Behavioural analysis

Feeding behaviour was divided in typical consequential events (search, exploration of prey, ingestion, cleaning movements). We consider only search and cleaning movements related with feeding behaviour. Frequency and duration of events was registered.

Mean latency of the first exploration (mean elapsed time from the beginning of the experiment and the first food exploration) and then mean latency of the first food ingestion (mean elapsed time from the beginning of the experiment and the first food ingestion) were recorded.

During each test, food preferences, order of preference, total exploration frequency (number of explorations for type of food) for earthworms, pupae and fruit flies were recorded. These frequencies was divided in three groups: (i) exploration followed by food refusal, when the carabid explored prey but did not eat it, (ii) exploration followed by eating behaviour, when the carabid explored prey and ate it, and (iii) exploration after eating behaviour, when the carabid after having eaten a prey, explored the remains of the same prey, that were wings and legs of fruit flies, puparia of *B. oleae* and earth contained into earthworms, or the place where it was in. The statistical analysis of exploration frequencies was performed with the Yates' chi-square test. Behavioural analysis was preformed only for *P. melas* and *C. fuscipes*.

3.5.d Food preferences

Data feeding preferences were quantified using the Electivity Index (Ivlev, 1961):

$$E = \frac{(r_i - p_i)}{(r_i + p_i)}$$

where: r_i is the proportion of ingested species, p_i is relative abundance of each prey item available. Electivity Index ranges from -1 to 1. A value of 1 indicates preference, zero indicates that prey was consumed proportionally to availability and -1 means total avoidance.

The Electivity Index of each prey was confronted and analysed by species and sexes using t-test with sig. 2-tailed. Using one-way ANOVA was verified if the first ingested prey is also consumed in more quantities. Concordances between the first explored and the first ingested prey were valued with Cohen's Kappa test. Statistical analyses were performed using SPSS 15.0 th Edition.

3.6 Microscopical gut content analysis

First of all I prepared some slides of *B. oleae* puparium, larvae and adults (males and females) as references and comparisons. Olive flies were boiled in KOH (40%) to remove organic portion. Then adults, puparium and larvae were washed in distilled water for 30 sec., in 50% acetone (30 sec), again in distilled water (60 sec), in 70% ethanol (60 sec), in absolute ethanol (120 sec), in xylol (60 sec). The puparium was cut and stretched on a slide, closed with Canadian balm and observed using a professional microscope (Zeiss Axioscop). Adult olive flies were disarticulated and different body parts were used to prepare slides. Diagnostic structures of *B. oleae* was observed using the microscope. These structures might be recognized inside the gut of a carabid predator. Furthermore, five carabids for species were starved for three days and then alimented with *B. oleae* pupae *ad libitum*. Their gut contents were analysed.

73 specimens of *P. melas* and 75 of *C. fuscipes,* collected in the olive groves of Mirto and Arcavacta in autumn of 2005 and 2007 and stored in 60 % alcohol were dissected, gut contents were removed and then mounted on not permanent light microscope slides. Prey remains were visible under the Axioscop Zeiss microscope. Diagnostic characters used to recognize different prey taxa can be found in Hengeveld, 1980a; 1991; Sunderland, 1975. Specific structures of the prey must be found in the predator digestive tract. Cuticle, antennae, typical mouth-parts, compound eye, wings and legs are important to identify prey taxa. The structure of the compound eye, antennae, legs, and wings allowed family-level identification of Diptera, while sclerotized remnants of cuticle, claws, mouthparts, and legs allowed family-level identification of the larvae of flies, beetles, springtails, and other arthropods (Pollet and Desender 1987). Lumbricidae can be identified by the presence of chetae. A photographic archive with brightfield Illumination digital image of analysed fragments was created.

3.7 Molecular gut content analysis

3.7.a Primer design

Specific PCR primers were designed using **Primer 3** (http://fokker.wi.mit.edu/primer3/input.htm) from not conserved regions based on comparisons of published DNA sequences of *Bactrocera oleae* (Genbank accession).

The target regions selected to design our primers was the *Bactrocera oleae Transformer (Botra) gene* (Lagos et al., 2005; 2007). This gene is present in *C. capitata* (Pane et al., 2002; 2005) and it has a rapid rate of divergence even within the genus *Drosophila* (Kulathinal *et al*. 2003). *Botra gene* is the second gene of a regulatory cascade based on RNA splicing that determines sex in *Bactrocera oleae.* Splicing of *Botra* transcripts is regulated by the master gene *Sex lethal* and *tra* itself regulates splicing of the transcriptional regulator *doublesex* (*dsx*). Transcripts are spliced in a sex-specific manner so that only females encode a functional polypeptide of 422 amino acids, whereas males encode presumably non-functional peptide(s).

B. oleae tra gene expands in a chromosomal region 3.6 kb long and consists in six exons and five introns.

A selected portion of the intron 2 of the nuclear *Bactrocera oleae* tra gene for female-specific transformer protein, (3635 bp in length, LOCUS AJ715414) was amplified by polymerase chain reaction (PCR). Later on, selected primers were evaluated in the laboratory. Specificity was verified using other invertebrate prey DNAs.

3.7.b Insects and primary predation feeding trial

P. melas and *C. fuscipes* were used as the model of predator to test for the presence of *B. oleae* in their gut. *Pterostichus melas* and *Calathus fuscipes* were divided in two groups of five beetles for each species, one group was starved for a week, and the other group was fed on *Bactrocera oleae* pupae *ad libitum.* Carabids were fed on pupae for gut content analyses. After the feeding period the beetles were moved to clean test tubes and were killed by freezing at -80 °C immediately (0 h) after feeding. Following starvation period, carabid beetles were killed and frozen for use as starved controls that were assayed by primers for each predator species. In fact these carabids had not been in contact with the target prey. All beetle and invertebrate prey samples, including starved controls, were analysed using PCR.

3.7.c Species specificity

The specificity of the primer was tested against 16 other potential prey soil-dwelling invertebrates that generalist predator carabids may feed on: Lumbricidae (1), Araneae (1 spider and 1 harvestman species), Chilopoda (1 *Scolopendra* sp), Diplopoda julidea (1), Diptera of the family Tephritidae, (1, *Ceratitis capitata*), fruit flies (1, *Drosophila melanogaster*) and Calliphoridae (1, *Lucilia casear*), as well as Coleoptera of the families Silphidae (1), and Nitidulidae (2, *Carpophylus sp.*), silverfishes (1), caterpillars (1), slugs (1), snails (1, *Rumina decollata*), cockroaches (1), (numbers of species are given in parentheses).

Fruit flies derived from my laboratory rearing, spiders, earthworms, diplopods, nitidulidea, silverfishes, *Scolopendra* sp., caterpillars, slugs, snails, cockroaches, harvestmen and *Ceratitis capitata* were collected in the olive grove of Piana della Torre, *Lucilia casear* and carrion beetles from a pig carrion, used for an other entomological research project, in the Botanic Garden of UNICAL to increase prey variability. These preys were frozen at -80°C.

3.7.d DNA Protocols

DNA was extracted individually for each specimen using the commercial QIAGEN DNeasy Tissue Kit. PCR amplifications were carried out in a final volume of 25 µl containing 16.75 µl H_2O; 5 µl Buffer (Promega, Milan, Italy); 0.5 µl dNTPs (10 mM; Promega); 0.75 µl each primer (10 µM; Invitrogen, Milan, Italy); 0.25 µl *Taq*DNA (5 u/µl, Promega), and 1 µl of template DNA (20-50 ng DNA). The reaction probe was one step of initial denaturation at 94°C for 3 min followed by 40 cycles of denaturation at 94° C for 50 sec, annealing at 55°C for 50 sec, and extension at 72°C for 1 min. A final extension step of 72°C for 5 min was also added. PCR products were electrophoresed on 1% denaturing polyacrylamide gels and visualized by UV.

Sequencing reactions were carried out by the bmr-genomics (Padova, Italy) in both directions to increase accuracy.

Two extractions of different individuals were tested for each prey species, five for olive fly pupae. DNA was singly extracted from each carabid beetle. For each species I tested 5 starved individuals and 5 fed on *B. oleae*. PCR analysis was carried out on total DNA extracted from *Pterostichus melas* and *C. fuscipes* gut samples. All PCR products were visualized on ethidium bromide stained 1% agarose gels. DNA of the prey was run with each PCR assay to test for false negative amplification. In addition, a distilled water sample, together with other PCR reagents, was included to check for contamination during PCR.

3.8 Carabid daily rhythms

Specimens were caught in the field with bait traps (Zetto Brandmayr et al. 2007) near Terranova di Sibari (CS), Italy, in the period between 13 October 2006 and 10 November 2006. Carabids were transported back to the laboratory and placed in terrains in the refrigerator in order to detect daily activity rhythms and feeding periods. The study comprises the data with the sunset being at 17:30. The photoperiod of the climatic chamber was 07:10 - 18:30 light and 18:30 - 07:10 dark. Specimens had food *ad libitum* for the experimental period. Registration of activity lasted five days. I verified when carabids were active and when predation was higher, analysing carabid daily rhythms with a new modern video recording system, Sony Network Camera (IPELA SNC-RZ25N/RZ25P) (Fig. 3.6), utilized for the first time. Subsequently the number of active beetles was manually recorded from registrations. Carabids during the dark periods were illuminated by red light led that apparently did not have any influence on the beetles. Drees et al. (2008) defined red light condition a proved valuable tool by which to observe carabid beetle movement patterns. The monitoring system that we used does not affect the activity of carabids and the recording technique is not intrusive. The monitoring was continuous and automatic, the output was easy to analyse.

Figure 3.6- Experimental video recording system

The daily rhythm of the following species was analysed: *P. melas* (35), *C. fuscipes* (20), *C. coriaceus* (21), *P. rufipes* (12), *P. griseus* (8), *N. kratteri* (11), *L. cimmerius* (19). Carabids are not equally abundant in olive groves.

3.9 Carabid density in olive grove

The study was carried out in the olive grove of Piana della Torre and was a methodological attempt to use the exhaustive sampling method to measure the density of carabids. Kromp (1999) recommended pitfall trapping or fenced pitfall trapping according to the type of study. To estimate the density of carabid beetles in an olive grove, four little enclosures (enclosures A, B, C, D) including an area of 2 m^2 were constructed in the period between October 26 and November 25, 2007. Each enclosure was made with four wooden poles, encircled by plexiglass sheet, all embedded for 15 cm in the ground, and 35 cm outside, in order to prevent carabids to escape (Fig. 3.7). To prevent carabid climbing, poles have been placed outside the plexiglass sheet. Five bait traps were placed in each enclosure, four placed along the perimeter and the remaining one in the middle. In addition other four pitfall traps were placed around each enclosure. Traps were maintained active and checked periodically for 15 days, until all carabids present in the bounded area were caught. Combining information of carabid presences inside and outside the enclosures gave us an ideas of real availability of carabids and soil invertebrates. The observation of the beetles was carried out in the period of two week. Each carabid beetle was counted and determined.

Figure 3.7-Enclosure in olive grove.

3.10 Simulation of carabid predation on *B. oleae* pupae and larvae

To verify the capacity of carabid predation on *B. oleae* pupae a simple experiment was performed. Considering a mean density of pupae in the soil of 200 per square meter (Liaropulos et al., 1978), in a big terrain (40 x 30 x 20 cm) covered with 5 cm of moist soil, 20 pupae were buried into the soil at 2.5 cm depth, where the 80% of *B. oleae* larvae pupate (Tremblay, 1995). The soil was sampled in the Botanic Garden of UNICAL. The equidistance of the pupae was maintained. Ten two-days starved carabids for each species (*D. planus, P. melas, P.rufipes*) were placed into the terrain where they allowed to search pupae for a week. Then soil was carefully sieved and the number of survived pupae was noted. To verify direct predation on fallen larvae, infested olive drupes were placed on a sieve upon a terrain with humid fine soil containing ten *P. melas* collected in the olive grove of Piana della Torre (Fig. 3.8). Carabid behaviour was videotaped for two weeks. The number of captured larvae out of fallen ones were noted.

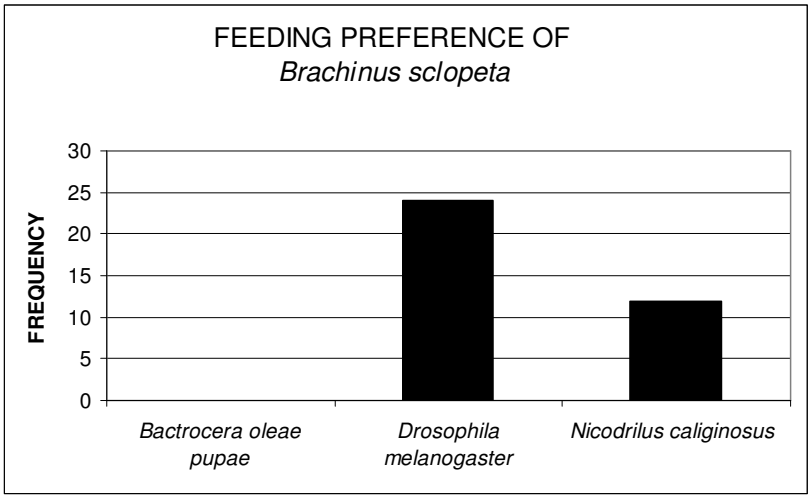
Figure 3.8- Experimental apparatus to verify *P. melas* predation on fallen olive fly larvae.

4. RESULTS

4.1 Preliminary test

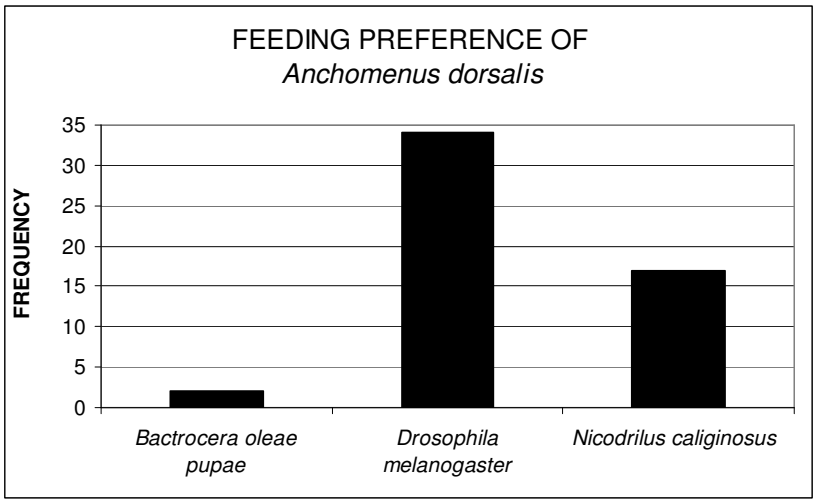
Feeding preliminary experiments carried out in laboratory conditions showed that carabid species *Pterostichus melas, Calathus fuscipes, Pseudoophonus rufipes, Laemostenus cimmerius, Distichus planus, Brachinus italicus, B. crepitans* regularly feed on *B. oleae* pupae, while other species (*Carabus coriaceus, Pseudoophonus griseus, Calathus cinctus, Brachinus sclopeta*, *Anchomenus dorsalis*) almost never ingested *B. oleae* pupae. The following graphs show the feeding preferences of tested carabid species.

B. sclopeta preferred fruit flies (2 df; chi-square$_{\text{fruit flies}}$ = 11.02; $p < 0.001$), ingested earthworms (2 df; chi-square$_{\text{earthworms}}$ = 0.02; $p > 0.05$) and refused pupae (2 df; chi-square$_{\text{pupae}}$ = 13.02; $p < 0.001$) (Grafic 4.1).



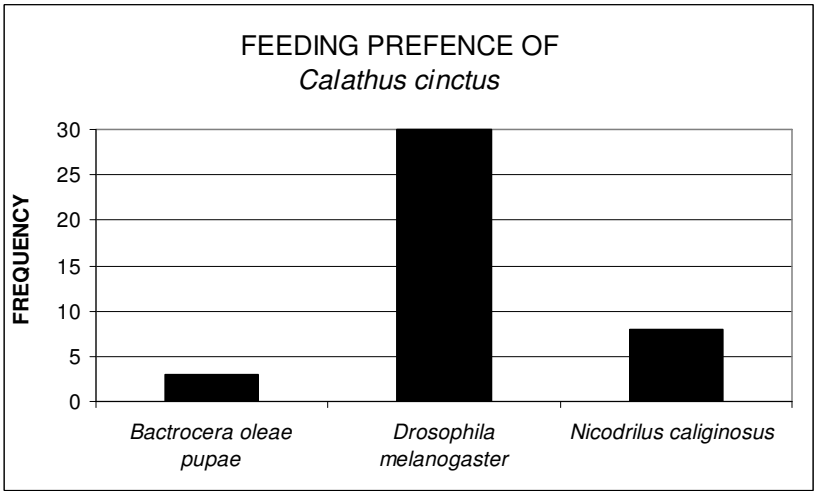
Grafic 4.1- *B. sclopeta* frequency of ingestion for each prey item.

A. dorsalis ingested all prey types, but fruit flies were preferred (2 df; chi-square$_{\text{fruit flies}}$= 14.19; p<0.001) earthworms were ingested (2 df; chi-square$_{\text{earthworms}}$= 0.08; p>0.05) and pupae were refused (2 df; chi-square$_{\text{pupae}}$ = 14.79; p<0.001) (Grafic 4.2).



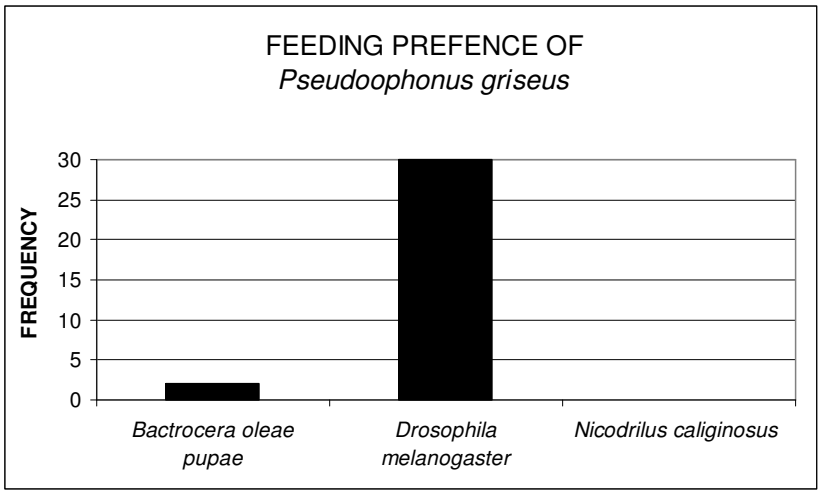
Grafic 4.2- *Anchomenus dorsalis* frequency of ingestion for each prey item.

C. cinctus ingested fruit flies with the highest frequency (2 df; chi-square$_{\text{fruit flies}}$= 18.34; p<0.001), ingested earthworms (2 df; chi-square$_{\text{earthworms}}$= 2.79; p>0.05) and it showed no preference for pupae (2 df; chi-square$_{\text{pupae}}$= 9.12 p<0.025) (Graphic 4.3).



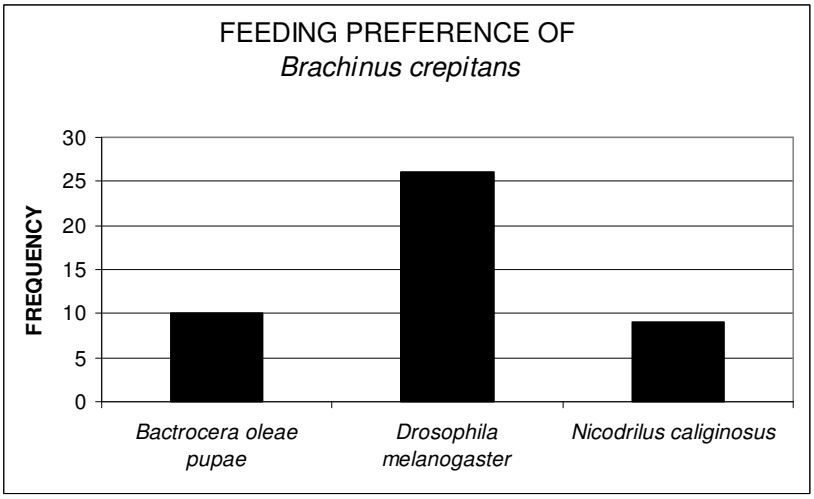
Grafic 4.3- *Calathus cinctus* frequency of ingestion for each prey item.

P. griseus ingested fruit flies with the highest frequency (2 df; chi-square$_{\text{fruit flies}}$ = 11.69; p < 0.001) and it showed no preference for pupae (2 df; chi-square$_{\text{pupae}}$ = 7.88 p < 0.05) and earthworms (2 df; chi-square$_{\text{earthworms}}$ = 33.25; p < 0.001) (Grafic 4.4).



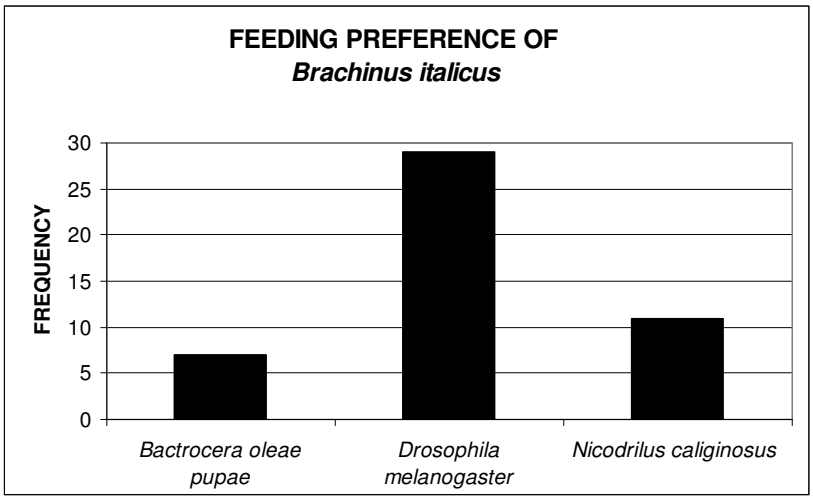
Grafic 4.4- *P. griseus* frequency of ingestion for each prey item.

B. crepitans ingested all prey types, but fruit flies were ingested with the highest frequency (2 df; chi-square$_{\text{fruit flies}}$= 5.64; p<0.01), while there were no differences between observed and expected frequency of ingestion for earthworms (2 df; chi-square$_{\text{earthworms}}$= 3.52; p>0.05) and pupae (2 df; chi-square$_{\text{pupae}}$= 2.64; p>0.05) (Grafic 4.5).



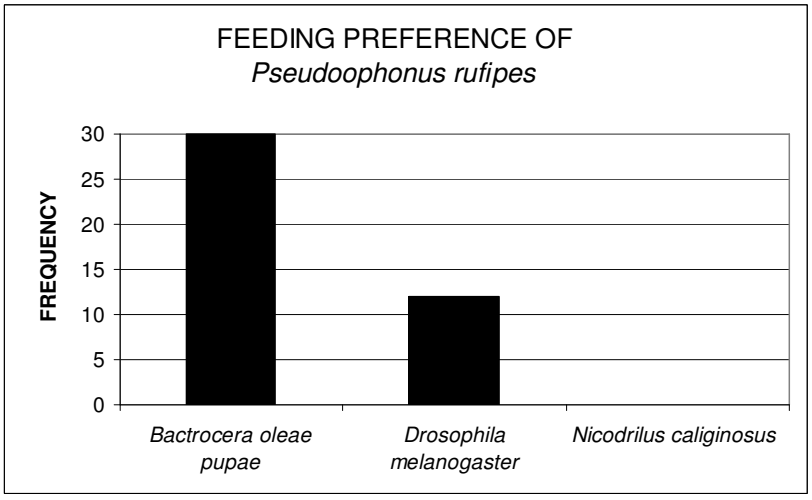
Grafic 4.5- *B. crepitans* frequency of ingestion for each prey item.

B. italicus ingested all prey types, but fruit flies were preferred (2 df; chi-square$_{\text{fruit flies}}$= 10.51; p<0.01), earthworms (2 df; chi-square$_{\text{earthworms}}$= 1.70; p>0.05) and pupae were ingested (2 df; chi-square$_{\text{pupae}}$= 5.36; p>0.05) (Grafic 4.6).



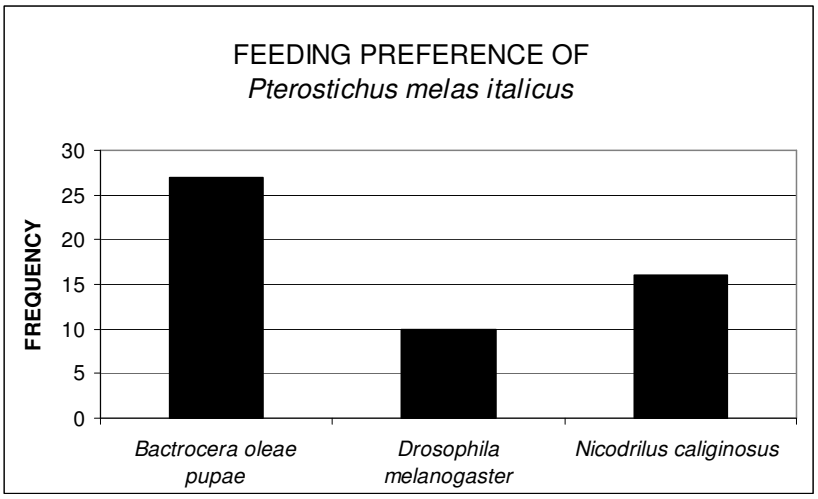
Grafic 4.6–*Brachinus italicus* frequency of ingestion for each prey item.

P. rufipes preferred pupae (2 df; chi-square$_{\text{pupae}}$= 17.16; p<0.001), ingested fruit flies (2 df; chi-square$_{\text{fruit flies}}$= 0.45; p>0.05), while they refused earthworms (2 df; chi-square$_{\text{earthworms}}$= 15.02; p<0.001) (Grafic 4.7).



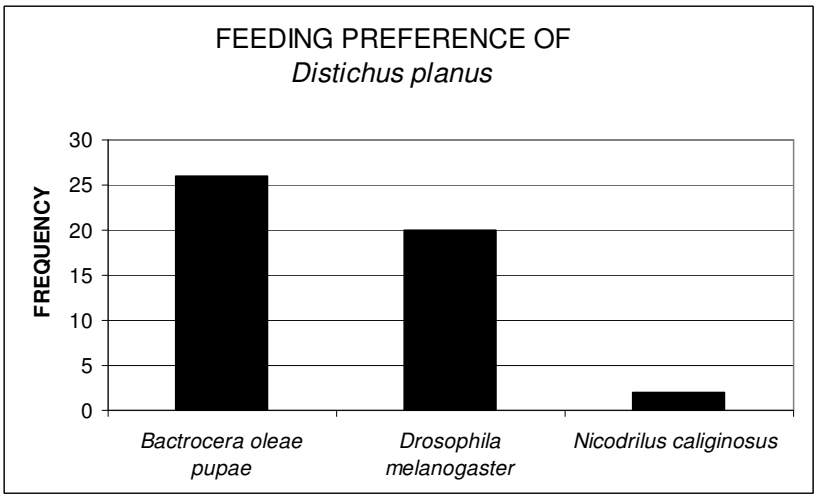
Grafic 4.7- *Pseudoophonus rufipes* frequency of ingestion for each prey item.

P. melas ingested all prey types, pupae (2 df; chi-square$_{pupae}$= 4.42; p>0.05), fruit flies (2 df; chi-square$_{fruit\ flies}$= 3.78; p>0.05) and earthworms (2 df; chi-square$_{earthworms}$= 0.27; p>0.05). No food seems to be preferred (Grafic 4.8).



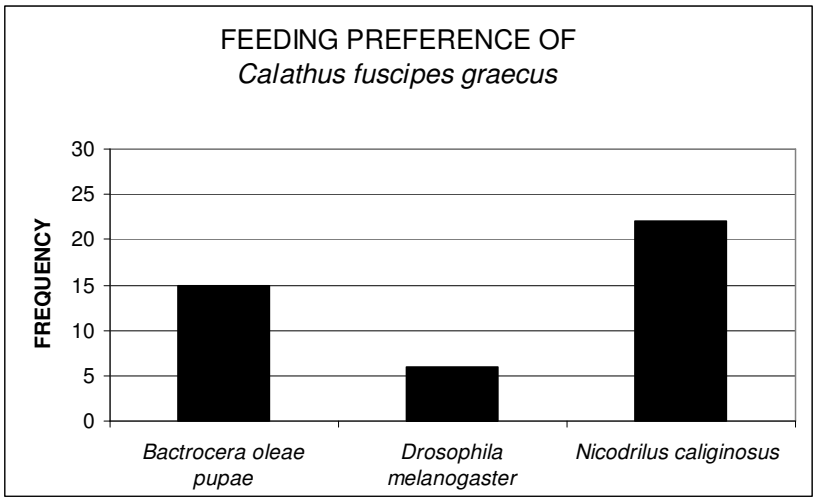
Grafic 4.8- *Pterostichus melas italicus* frequency of ingestion for each prey item.

D. planus ingested pupae (2 df; chi-square$_{pupae}$= 5.64; p>0.05) and fruit flies (2 df; chi-square$_{fruit\ flies}$= 0.77; p>0.05), while earthworms are ingested with lowest frequency (2 df; chi-square$_{earthworms}$= 13.14; p<0.001) (Grafic 4.9).



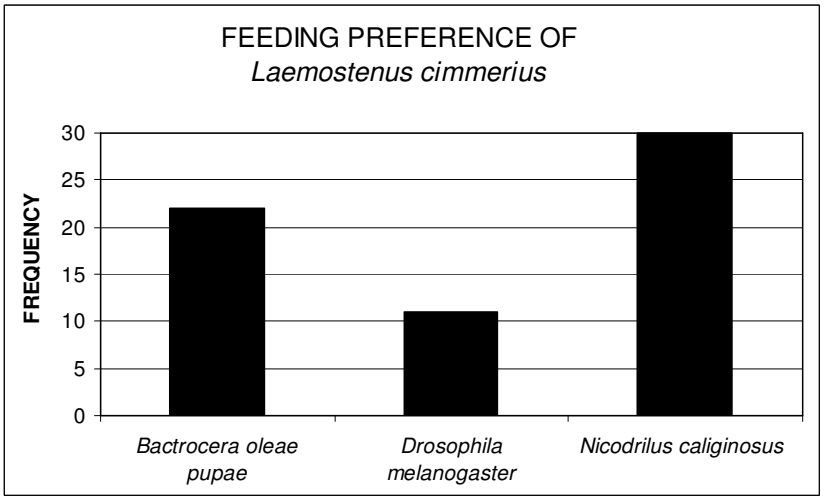
Grafic 4.9- *Distichus planus* frequency of ingestion for each prey item.

C. fuscipes ingested all prey types, pupae (2 df; chi-square$_{pupae}$= 0.002; p>0.05), fruit flies (2 df; chi-square$_{fruit\ flies}$= 5.44; p>0.05) and earthworms (2 df; chi-square$_{earthworms}$= 3.58; p>0.05). No food seems to be preferred (Grafic 4.10).



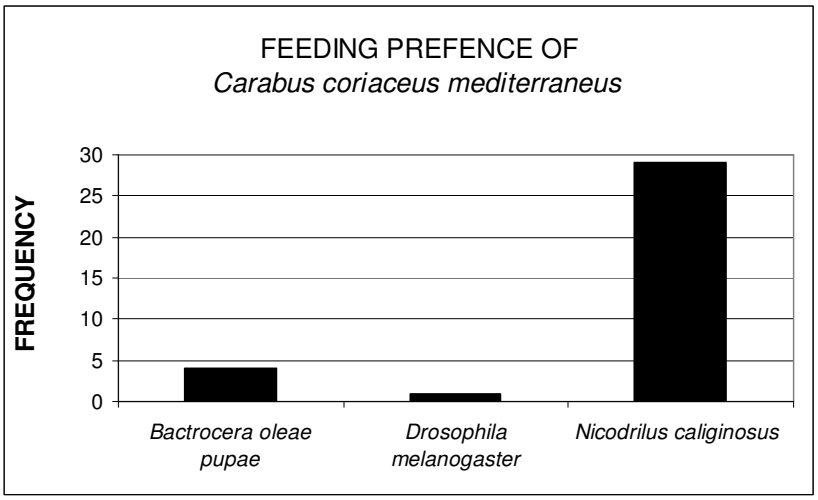
Grafic 4.10-*Calathus fuscipes graecus* frequency of ingestion for each prey item.

L. cimmerius ingested all prey types, it showed preference for no prey (2 df; chi-square$_{pupae}$= 0.012; p>0.05; chi-square$_{fruit\ flies}$= 5.25; p>0.05; chi-square$_{earthworms}$= 3,44; p>0.05) and behaved as a generalist predator (Grafic 4.11).



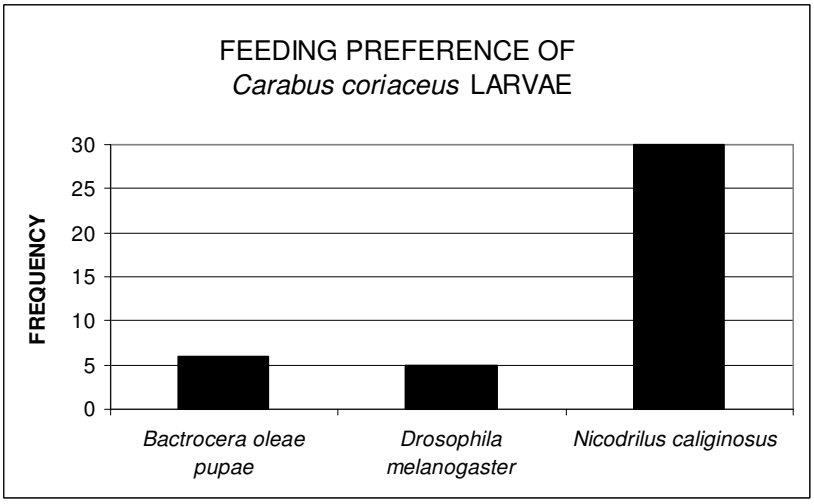
Grafic 4.11- *Laemostenus cimmerius* frequency of ingestion for each prey item.

C. coriaceus ingested earthworms with higher frequency (2 df; hi-square$_{earthworms}$= 26.02; p<0.001), pupae with observed frequency no different from expected one (2 df; chi-square$_{pupae}$= 5.41 p>0.05) and it showed no preference for fruit flies (2 df, chi-square$_{fruit\ flies}$= 10.35; p<0.01) (Grafic 4.12).



Grafic 4.12- *Carabus coriaceus mediterraneus* frequency of ingestion for each prey item.

C. coriaceus larvae ingested earthworms with higher frequency (2 df; hi-square$_{earthworms}$= 18.33; p<0.001), than pupae (2 df; chi-square$_{pupae}$= 4.88 p>0.05) and fruit flies (2 df, chi-square$_{fruit\ flies}$= 6.15; p>0.05) were ingested with observed frequency which are not different from the expected ones (Grafic 4.13).



Grafic 4.13- *Carabus coriaceus mediterraneus* larvae frequency of ingestion for each prey item.

Adults and larvae showed similar preference for earthworms. *Carabus coriaceus* adults preferred earthworms, but some individuals may eat pupae and fruit flies in low quantity. *Brachinus sclopeta, B. italicus, B. crepitans, Anchomenus dorsalis, P. griseus, C. cinctus* preferred fruit flies. *Calathus fuscipes* and *Laemostenus cimmerius* ingested all prey types, earthworms, pupae and fruit flies, and seem to prefer earthworms. *Pterostichus melas, Distichus planus* and *Pseudoophonus rufipes* preferred pupae.

4.2 Quantitative tests

4.2.a Behavioural analysis

After the introduction into the experimental petri dishes, the carabids exhibited feeding behaviour with a typical repertoire of consequential events. Feeding behaviour is very similar in all the tested species, but it was analysed in detail only for *P. melas* and *C. fuscipes*.

During the experiments, *Pterostichus melas* moved in the petri dish touching the soil with antennae and palps (search phase: observed frequency, F_o = 186; mean, ($\bar{x}$ = 2.02 ± 0.15 min), and sometimes remained motionless inside the arena. When it found a prey, it explored a few seconds with its antennae, palps and mandibles (F_o = 367; $\bar{x}$ = 0.35 $\pm$ 0.02 min). The ingestion phase (F_o = 138; x = 4.50 ± 0.46 min) had a different mean duration for different prey eaten in the same quantity; *P. melas* employed more time for fruit flies and less for pupae and earthworms. After prey ingestion, the predator often spent time in cleaning operations (F_o = 53; $\bar{x}$ = 3.22 ± 0.32 min). Exploration and also ingestion, may be interrupted if carabid rejected its prey.

Pterostichus melas total exploration frequency was F_o= 122 for earthworms, F_o= 143 for pupae and F_o= 102 for fruit flies. By comparing the total frequency of exploration among the three preys, there were no significant differences. So no food was explored more than others. Explorations followed by food refusal were F_o= 15 for pupae, F_o= 43 for the earthworms and F_o= 53 for fruit flies. By comparing the frequencies of explorations followed by rejection of prey, there was a highly significant difference (2df; chi-square= 20.97; p<0.01) among earthworms, fruit flies and pupae; these latter were less refused than earthworms (1df; chi-square$_{pupae\text{-}earthworms}$= 13.53; p<0.01) and fruit flies (1df; chi-square$_{pupae\text{-}fruit\ flies}$= 20.13; p<0.01). There are no significant differences between earthworms and fruit flies. Explorations followed by eating behaviour were F_o= 74 for pupae, F_o= 44 for earthworms and F_o= 28 for fruit flies. Comparing the frequencies of exploration followed by predation, we found a highly significant difference (2df; chi-square= 22.41; p<0.01) among preys, with pupae more preyed than earthworms (1df; chi-square$_{pupae\text{-}earthworms}$= 7.13; p<0.01) and fruit flies (1df; chi-square$_{pupae\text{-}fruit\ flies}$= 20.75; p<0.01). Explorations after eating behaviour were F_o = 54 for pupae, F_o= 35 for earthworm and F_o= 21 for fruit flies (1df; chi-square$_{pupae\text{-}fruit\ flies}$= 20.75; p<0.01). There was no significant difference between earthworms and fruit flies. By comparing the frequencies of exploration on the remains of preys (that were wings and legs of fruit flies, puparia of *B. oleae* and earth contained into earthworms), there was a highly significant difference among preys (2df; chi-square= 14.96; p<0.01), with puparia remains significantly more explored than fruit flies (1df; chi-square$_{pupae\text{-}fruit}$

$_{flies}$= 14.53; p<0.01). There are no significant differences between earthworms and fruit flies and between earthworms and pupae.

During the experiments, *C. fuscipes* was active (search phase: F_o= 102; $\bar{x}$= 1.19 ± 0.15 min) and crossed the central part of the arena, moving away from the rim of the arena, more often than *P. melas*. When a prey was found, *C. fuscipes* explored for 0.47 ± 0.03 minutes (F_o= 300). The ingestion phase had a mean duration of 8.50 ± 0.97 minutes and a frequency (F_o) of 87. After prey ingestion, *C. fuscipes* often spent time in scrupulous cleaning operations (F_o= 58; $\bar{x}$= 4.53 ± 0.34 min). This species is very sensitive to vibrations.

Calathus fuscipes total exploration frequency was F_o= 122 for earthworms, F_o= 113 for pupae and F_o= 65 for fruit flies. By comparing the total frequency of exploration among the three preys, we can put in evidence significant differences (2df; chi-square= 18.78; p<0.01).

Observations followed by food refusal were F_o= 60 for pupae, F_o= 51 for the earthworms and F_o= 57 for fruit flies. Comparing the frequencies of explorations followed by rejection of the prey, there were no differences.

Explorations followed by eating behaviour were F_o= 27 for pupae, F_o= 38 for earthworms and only F_o= 8 for fruit flies. Comparing the frequencies of explorations followed by predation events, we found a highly significant difference (2df; chi-square= 18.96; p<0.01) among the three preys. There was significant differences between fruit flies and earthworms (1df; chi-square$_{earthworms\text{-}fruit\ flies}$= 18.28; p<0.01), and fruit flies and pupae (1df; chi-square$_{fruit\ flies\ -pupae}$= 9.26; p<0.01). In fact, when fruit flies were found by the predator, they were rarely ingested. There was no significant difference between earthworms and pupae.

Total frequencies of explorations on the remains of preys were F_o= 26 for pupae, F_o= 33 for earthworms and F_o= 0 for fruit flies. Comparing the frequencies of exploration on the remains of preys there was a highly significant difference (2df chi-square= 29.86; p<0.01) among preys with fruit flies less preyed than earthworms (1df; chi-square$_{earthworms\text{-}fruit\ flies}$= 31.03; p<0.01) and pupae (1df; chi-square$_{pupae\text{-}fruit\ flies}$= 24.04; p<0.01). There was no difference between earthworms and puparia.

In the following table (Table 4.1) the value of mean first exploration and ingestion latency of tested carabid species are reported. There were no significant differences among latency of tested carabid species.

SPECIES	MEAN FIRST EXPLORATION LATENCY (± S.E.)	MEAN FIRST INGESTION LATENCY (± S.E.)
Pterostichus melas	6.50 ± 1.22	9.27 ± 1.62
Calathus fuscipes	10.32 ± 1.44	6.70 ± 1.63
Carabus coriaceus	4.76 ± 2.16	20.03 ± 11.65
Leamostenus cimmerius	7.89 ± 4.62	16.61 ± 11.69
Nebria kratteri	4.18 ± 2.39	12.30 ± 9.05

Table 4.1- Mean first exploration and ingestion latency of tested carabid species.

4.2.b Food preferences

A table showed carabid weight before and after experiments for all tested species of carabids (Table 4.2).

SPECIES	MEAN WEIGHT (g) BEFORE TEST (± S.E.)	MEAN WEIGHT (g) AFTER TEST (± S.E.)
Pterostichus melas	0.2090 ± 0.0049	0.2216 ± 0.0086
Calathus fuscipes	0.1212 ± 0.0054	0.1300 ± 0.0062
Carabus coriaceus	0.2575 ± 0.0101	0.2647 ± 0.0118
Leamostenus cimmerius	1.3544 ± 0.1195	1.3852 ± 0.1198
Nebria kratteri	0.0995 ± 0.0052	0.1108 ± 0.0035

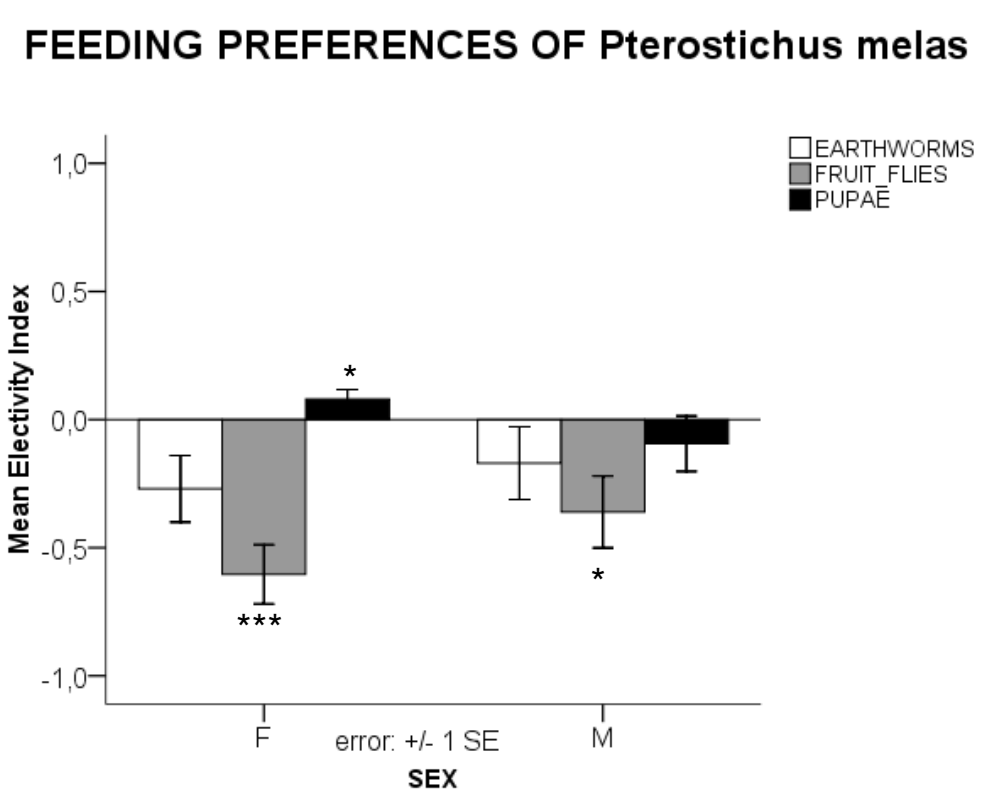
Table 4. 2- Carabid weight before and after feeding choice experiments

Mean olive fly pupa weight is 0.0068 ± 0.0006 (S.E.). Mean fruit fly weight is 0.0008 ± 0.0002 (S.E.). Considering EI mean value, there were differences between males and females of *P. melas* (Table 4.3) and *C. fuscipes* species (Table 4.4). Females of *P. melas* preferred pupae (EI = 0.0799 ± 0.0373 S.E.) (*t* test, t = 2.144; df 14; p=0.050), the males EI for pupae (EI= -0.0940 ± 0.1081 S.E.) was not significantly different from zero (*t* test, t = -1.200; df 14; p=0.250). *P. melas* female Electivity Index for earthworms (EI= -0.2700 ± 0.1300 S.E.) approached the limit, but was not significantly different from zero (t = -2.077; df 14; p=0.057), for males (EI= -0.1700 ± 0.1416 S.E.) was not significantly different from zero (*t* test, t = -1.200; df 14; p=0.250). *P. melas* female Electivity Index for fruit flies (EI= -0.6041 ± 0.1153 S.E.) was highly significant different from zero (*t* test, t = -5.238; df 14; p<0.001), and also for males (EI= -0.3604 ± 0.1403 S.E.) (*t* test, t= -2.568; df 14; p=0.022). EI values are represented in the graphic 4.14.

Pterostichus melas	Pupae EI	Earthworms EI	Fruit flies EI
Males	-0.0940 ± 0.4186	-0.1700 + 0.5483	-0.3604 + 0.5434
Females	0.0799 ± 0.1443	-0.2700 ± 0.5033	-0.6041 ± 0.4466

Table 4.3- *Pterostichus melas* males and females EI mean values for each prey item.

Figure 4.1–*Pterostichus melas* specimens that ingested pupae and earthworms.



Grafic 4.14-*Pterostichus melas* mean EI values for different prey item.

The first prey explored EI of *P. melas* females (first prey explored EI= 0.0405 ± 0.0654 S.E.) was different from zero (*t* test, t = 0,6191; df 14; p=0,5458), so the first encountered prey was the preferred one. For *P. melas* males the first prey explored EI was -0.1023 ± 0.1265 S.E., that was different from zero (*t* test, t = 0.809; df 14; p=0.432) so, generally the first explored prey was discarded.

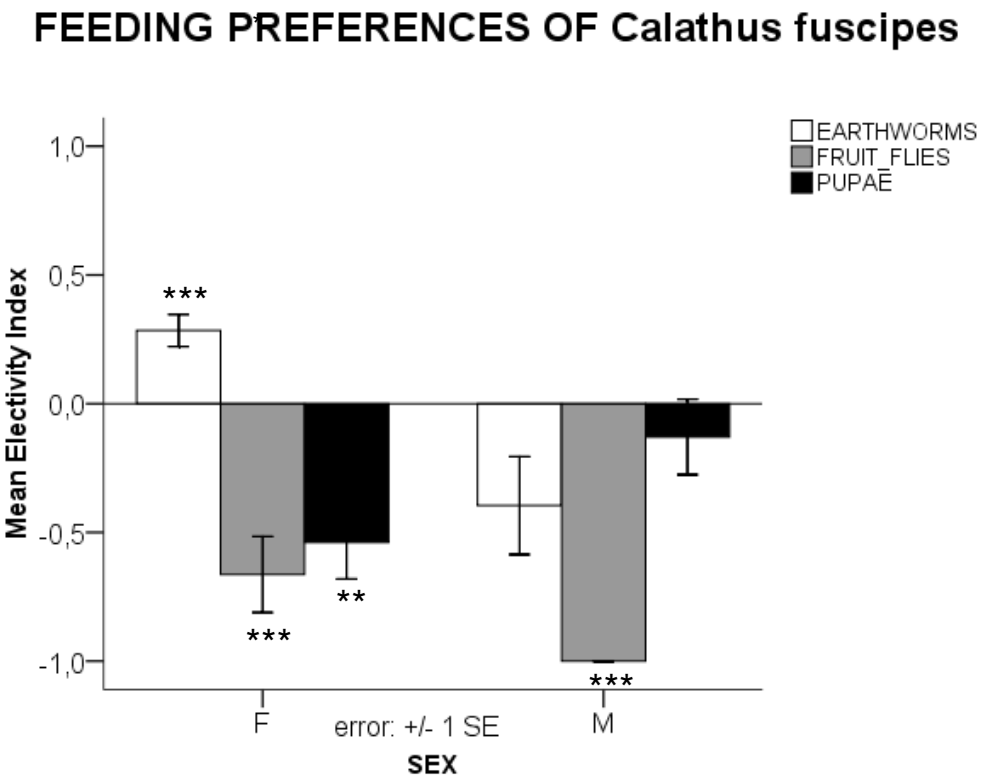
For *P. melas* males and females, the first explored prey was casually found (chi square= 4.044; df 2; p=0.160 Exact Sig.; Fisher Exact Test p=0.160), this means that they did not always encounter at first the same prey, so there were no evidence to sustain presence of chemical cues that could attract carabids to one specific prey. Independently from sex, the number of encounters with each prey type was casual (chi square= 4.200; df 2; Exact Sig.=0.134); furthermore there was concordance between the first explored prey and the first eaten one (Cohen's Kappa= 0.617; Exact Sig. p< 0.001).

C. fuscipes female Electivity Index for pupae (EI= -0.5391 ± 0.14178 S.E.) had a negative value (t = -3.802; df 11; p=0.003), the male Electivity Index (EI= -0.1293 ± 0.1463 S.E.) was not significantly different from zero (t = -0.884; df 12; p=0.394). Females of *C. fuscipes* preferred earthworms (EI= 0.2843 ± 0.0623 S.E.) (t = 4.566; df 11; p=0.001) and the value for males (EI= -0.3954 ± 0.1900 S.E.) approached the limit (t = -2.081; df 12; p=0.059). *C. fuscipes* female Electivity Index for fruit flies (EI= -0.6634 ± 0.1474 S.E.) was significantly different from zero (t =

-4.501; df 11; p=0.001) and for males (EI= -1) it had a negative value. These results are represented in the graphic 4.15.

Calathus fuscipes	Pupae EI	Earthworms EI	Fruit flies EI
Males	-0.1293 ± 0.5276	-0.3954 ± 0.6850	-1.000 ± 0.000
Females	-0.5391 ± 0.4911	0.2843 ± 0.2157	-0.6634 ± 0.5150

Table 4.4- *Calathus fuscipes* males and females EI mean values for each prey item.



Grafic 4.15- *Calathus fuscipes* mean EI values for different prey item.

The first prey explored EI of *P. melas* females (first explored prey EI= 0.0405 ± 0.0654 S.E.) was not different from zero (t = 0,6191; df 14; p=0,5458). Also for *P. melas* males the first explored prey EI was -0.1023 ± 0.1265 S.E., that was not different from zero (t = 0.809; df 14; p=0.432); so the first encountered prey was consumed in a proportional quantity to its availability. For *P. melas*, the first explored prey was casually encountered (chi square= 4.044; df 2; Fisher Exact Test p=0.160), independently from sex, the number of encounters with each prey type was casual (chi square= 4.200; df 2; Exact Sig.=0.134); furthermore there was concordance between the first explored prey and the first eaten one (Cohen's Kappa= 0.617, exact Sig. p<0.001).

C. fuscipes female first explored prey Electivity Index was 0.3029 ± 0.9912 S.E., that was significantly different from zero (t= 3,056; df 5; p=0,028), the first explored prey was preferred. For *C. fuscipes* male first explored prey Electivity Index was 0.8539 ± 0.1769 S.E. with t= 0.483, df 7, p=0.644, the first explored prey was ingested according to its availability.

C. fuscipes did not explore at first the same prey (chi square= 2.413; df 2; Exact Sig.= 0.301) the frequency of the first explorations for each prey type was casual, independently from sex (chi square= 4.200; df 2; Exact Sig.=0.134) and there was concordance between the first explored prey and the first ingested one (Cohen's Kappa= 0.321; Approx. Sig. p=0.037; Exact Sig. p=0.051).

We compared Electivity Index of the first ingested prey, using one-way ANOVA to verify if there were differences among preys. Statistical analysis, considering *P. melas* males and females together, showed that if pupae were the first ingested prey, they were consumed in greater quantities than earthworms (ANOVA, $F_{2,27}$= 5.062; p=0.014; post hoc Tamhane p=0.004). If fruit flies were ingested first, they were consumed in lower quantities than pupae (ANOVA, $F_{2,27}$= 3.997; p=0.030; post hoc Tamhane p=0.001) and earthworms (post hoc Tamhane p=0.017). Earthworms, if first ingested, were consumed in lower quantity than pupae (ANOVA, $F_{2,27}$= 15.726; p<0.001; post hoc Tamhane p=0.001).

Regarding *C. fuscipes* we found high significant value for earthworms compared to pupae (ANOVA $F_{2,27}$= 21,185; df 2; p<0.001; post hoc Tamhane p<0.001).

Figure 4.2- *Calathus fuscipes* specimens that ingested pupae, fruit flies and earthworms.

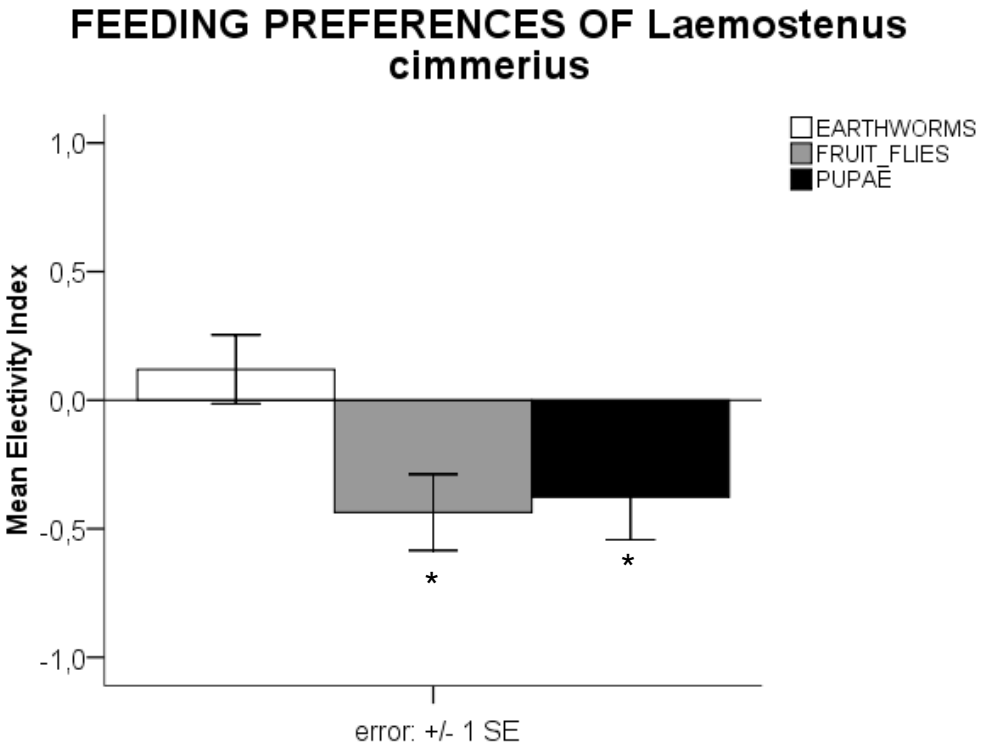
Laemostenus cimmerius, Carabus coriaceus, Nebria kratteri did not show significant differences between males and females in feeding preferences. EI values of alla tested species are summarized in table 4.5.

Laemostenus cimmerius ingested earthworms proportionally to their availability (Electivity Index= 0.120 ± 0.140 S.E.) (t= 0.90; df 10; p=0.39), while they refused pupae (Electivity Index= -0.377 ± 0.174 S.E.) (t= - 2.27; df 10; p= 0.05), and fruit flies (Electivity Index= -0.436 ± 0.491 S.E.) (t= - 2.94; df 10; p= 0.02) (Graphic 4.16).

There was concordance between the first explored prey and the first ingested one for *Laemostenus cimmerius* (Cohen's Kappa=0.86; p<0.001). One-way ANOVA statistical analysis, considering *L. cimmerius,* showed no differences among the first ingested prey quantities.

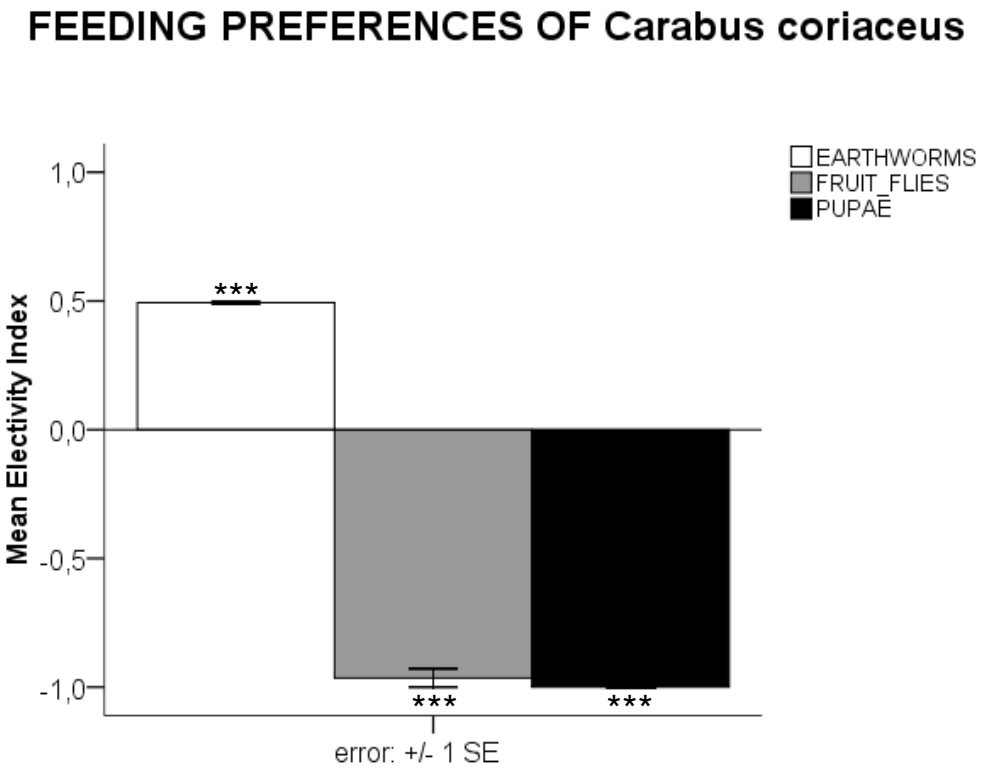
SPECIES	Electivity Index		
	Pupae	Fruit flies	Earthworms
Pterostichus melas	-0.007 ± 0.058	-0.482 ± 0.092	-0.220 ± 0.095
Calathus fuscipes	-0.326 ± 0.108	-0.838 ± 0.077	-0.069 ± 0.123
Carabus coriaceus	-1	-1	0.497 + 0.001
Leamostenus cimmerius	-0.377 ± 0.174	-0.436 ± 0. 491	0.120 ± 0.140
Nebria kratteri	-1	-1	0.495 ± 0.003

Table 4.5- EI values of tested carabid species.



Grafic 4.16- *Laemostenus cimmerius* mean EI values for different prey item.

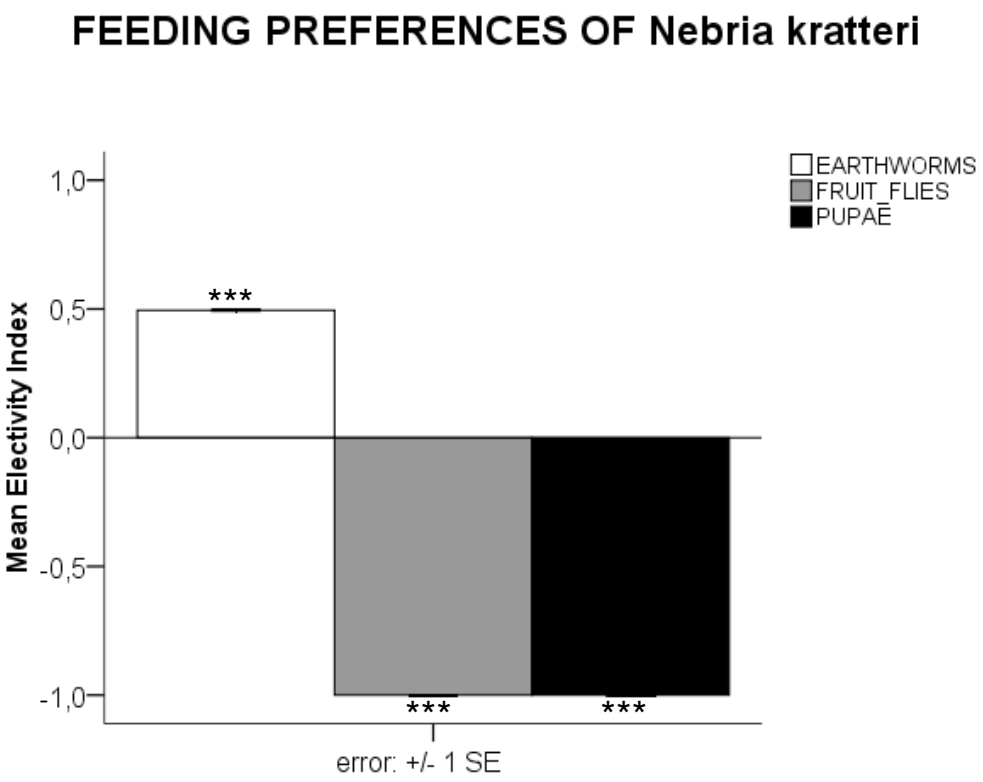
Carabus coriaceus preferred earthworms (Electivity Index=0.497 + 0.001 S.E.) (*t* test, t= 501.9; df 11; p <0.001), while they refused pupae (Electivity Index= -1), and fruit flies (Electivity Index= -1) (Graphic 4.17).



Grafic 4.17- *Carabus coriaceus* mean EI values for different prey item.

Nebria kratteri preferred earthworms (Electivity Index=0.495 ± 0.003 S.E.) (t= 150.3, df 9, p<0.001), while they refused pupae (Electivity Index=- 1) and fruit flies (Electivity Index= -1). *Nebria kratteri* never ingested pupae and fruit flies (Grafic 4.18). *Carabus coriaceus* and *Nebria kratteri* did not prefer pupae and they are probably not good antagonists of olive fly.

Figure 4.3-*Carabus coriaceus* specimen that ingested earthworms.



Grafic 4.18- *Nebria kratteri* mean EI values for different prey item.

4.3 Microscopical gut content analysis

The crop contents of each specimen collected in field in the autumnal periods were examined to identify *B. oleae* fragments. Previously prepared *B. oleae* slides were used as comparison (Photo 5,7,9,11,12,15,16 at the end of this thesis). Diagnostic structures of *B. oleae* might be recognized inside the gut of these predators. *B. oleae* pupae may be easily recognized by the shape of maggot stigmata (Photo 1,2) and mouth parts (Photo 3,4), that persist on the puparium. Larvae and pupae show specie-specific mouth-parts. The cuticle of olive fly puparium is too much tenuous to be clearly identified. The remains belonging to *B. oleae* adults were found in the gut of two *C. fuscipes* (Photo 6,8,10,13,14,17,18). These data put in evidence *C. fuscipes* predation on *B. oleae* in nature. No *B. oleae* pupae remains were found. The crop of *P. melas* and *C. fuscipes* that have ingested *B. oleae* in laboratory, contained only liquid, yellow-coloured food, with no recognizable fragments. These gut liquid contents were unidentifiable. In fact no identifiable fragments belonging to *B. oleae* pupae were found into the analysed gut contents of *P. melas* and *C. fuscipes*.

In carabid guts there were fluids, mush and large recognizable arthropod fragments. The crop contents of carabids belonging to the same species were remarkably uniform. There were similitudes even between the two different species, to underline the uniformity of *pabulum* in

autumnal period and in agreement to polyphagous habits of these species. No significant differences in prey uptake occurred between sexes. According to Evans and Forsythe (1985), the post-ventricular regions contained smaller pieces of cuticle than the crop.

A brightfield illumination digital image gallery was constituted. Spines are present on the internal surface of the proventriculus of both species. Microphotographs show the structure of the inner surface of the proventriculus of *P. melas* and *C. fuscipes* with triangular teeth.

In the crop there were pulverized arthropod remains, visible as hard fragments, fragments of unknown origin and recognizable plant remains, visible as soft fragments. The presence of jelly-like substances, mush or fluid were noted. Some guts contained a deep reddish brown colour material that suggested earthworms, slugs or snails remains. *P. melas* that had been fed on earthworms in captivity had similarly coloured crop contents. However only the presence of chetae may allow earthworm identifications. Snails and slugs may be identified from teeth of the radula. In some cases, crop contains very fine particles or liquid and identification of food was impossible. In accordance to literature data (Davies, 1953) the crop of examined *C. fuscipes* contained liquid food and only a few fragments of arthropodal remains. In numerous cases, it was impossible to identify the belonging order of insect fine fragments.

P. melas and *C. fuscipes* ingested a wide range of invertebrates and a great quantity of insects (Table 4.6, Photo 19-26). These species appear to be strictly carnivorous, although some specimens occasionally consumed food of vegetable origin; however these vegetable materials may be derived from secondary predation too. More than one prey were found into the guts for both species. Gut content analysis, however, showed that these two species regularly ingested earthworms and even adult dipteron, that are the alternative prey used during feeding choice tests.

SPECIES	BEETLES	DIPTERAN	HYMENOPTERS	INSECTS	INVERTEBRATES	EARTHWORMS	SNAILS /SLUGS	SPIDERS	MITES	VEGETABLE	ORGANIC MATERIAL	FUNGAL MATERIAL
Calathus fuscipes	17	9	1	12	3	1	1	2	1	9	26	7
Pterostichus melas	21	6	1	15	3	9	0	6	5	6	19	13

Table 4.6- *P. melas* and *C. fuscipes* ingested food

4.4 Molecular gut content analysis

One pair of oligonucleotide primers was designed from the selected sequences. The sequence of the 5' upstream primer (INTR-F) was 5'- GCAAAGGCAAGGATAGAACG - 3' and the 3' downstream primer (INTR-R) was 5' – TTGCAGCTTTCAGCATTTTG - 3'. When DNA from *B. oleae* pupae was amplified using the primers INTR-F/INTR-R, a single band of 544 bp resulted. The primer pairs amplified fragments of the predicted size.

4.4.a Species specificity

When the primers INTR-F/INTR-R were tested for species specificity against DNA from *B. oleae* and other 16 preys, the 544-bp fragment was only amplified for *B. oleae* DNA, used as control (Figure 4.5a). There was no amplification in other invertebrates preys (Figure 4.5b). This result demonstrated that the INTR-F/INTR-R primers did not cross react with the DNAs of the prey species that the carabid generalist predators may also consume in olive groves.

Figure 5.4a

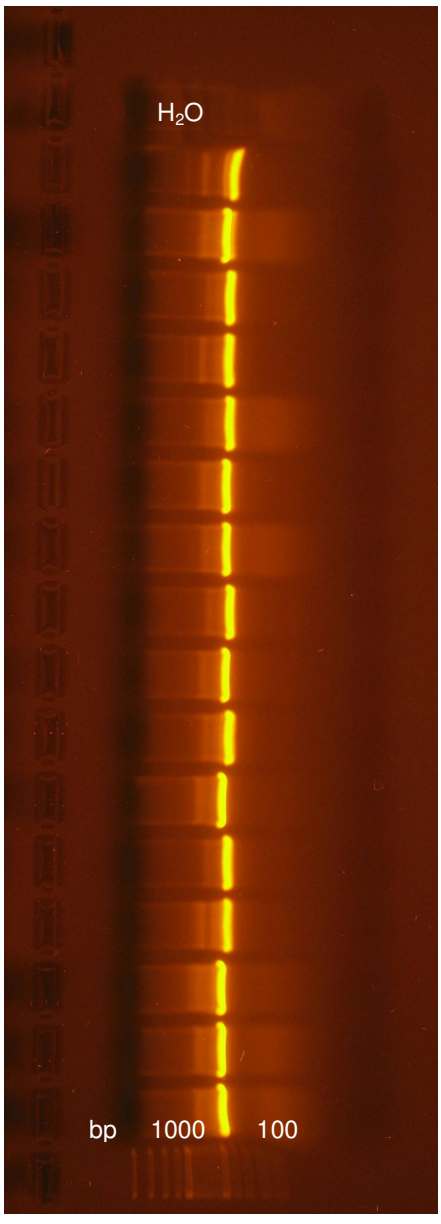


Figure 4.5b

Figure 4.5a–PCR amplification products obtained using INTR-F/INTR-R primers employing as template DNAs extracted from different invertebrate prey samples. The band refers to *B. oleae* DNA sample.

Figure 4.5b-Positive controls; Invertebrate prey DNAs show amplification with generic 18 S ribosomal primers.

4.4.b Detection of *Bactrocera oleae* DNA in predator guts

The INTR-F/INTR-R primers were used to verify the presence of the *B. oleae* DNA in the guts of all carabid beetles that had eaten at least one *B. oleae* pupa and had been frozen at 0 h. It was evident that amplification products were present only using DNA samples extracted from the guts of carabids that had ingested *B. oleae* pupae and using DNA samples extracted from *B. oleae*, used as control. Amplification did not occur using DNA extracted from starved carabids**.** Examples of amplification patterns are shown in Figure 4.6.

Sequencing reactions, carried out by the bmr-genomics (Padova, Italy), confirmed that predator gut DNA sequences were equal to that obtained for *B. oleae* prey.

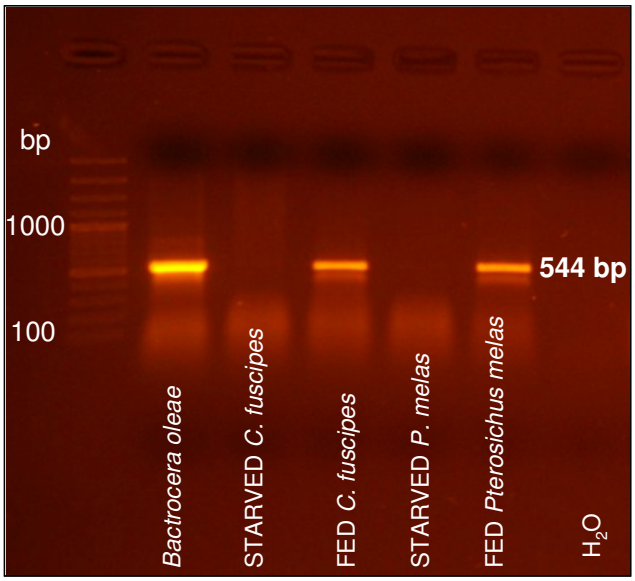


Figure 4.6–PCR amplification products obtained using INTR-F/INTR-R primers on different DNA templates.

4.5 Carabid daily rhythms

This part of the research investigated on carabid daily rhythms, also providing information on the activity pattern of some carabid species, e.g. *P. melas*, for which no data were previously available. In fact, even if a lot of *Pterostichus* species are well documented (Thiele and Weber, 1968), the period of activity is a specific rather than a generic characteristic (Luff, 1978).

Daily rhythm collected data showed that all the tested carabid species (*P. melas, C. fuscipes, C. coriaceus, P. rufipes, P. griseus, N. kratteri, L. cimmerius*) were nocturnal, they were hungry when they began to move and feeding activity was generally manifested during the first hour after the sunset. The number of active individuals of *C. coriaceus* during the dark phase was higher than during the light one (χ^2_1=13,81, p<0,001), the same for *P. rufipes* (χ^2_1=10,81, p<0,005), *P. griseus* (χ^2_1=4,32, p<0,5), *C. fuscipes* (χ^2_1=4,05, p<0,5), *P. melas* (χ^2_1=19,10, p<0,001), *L. cimmerius* (χ^2_1=16,02, p<0,001), *N. kratteri* (χ^2_1=9,03, p<0,001).

Pterostichus melas was hardly active at sunset, but it is possible to notice some diurnal activity in this species, generally strictly associated with feeding behaviour. There are some indications for a second peak of activity at about 9–10 hours after sunset (Fig. 4.7).

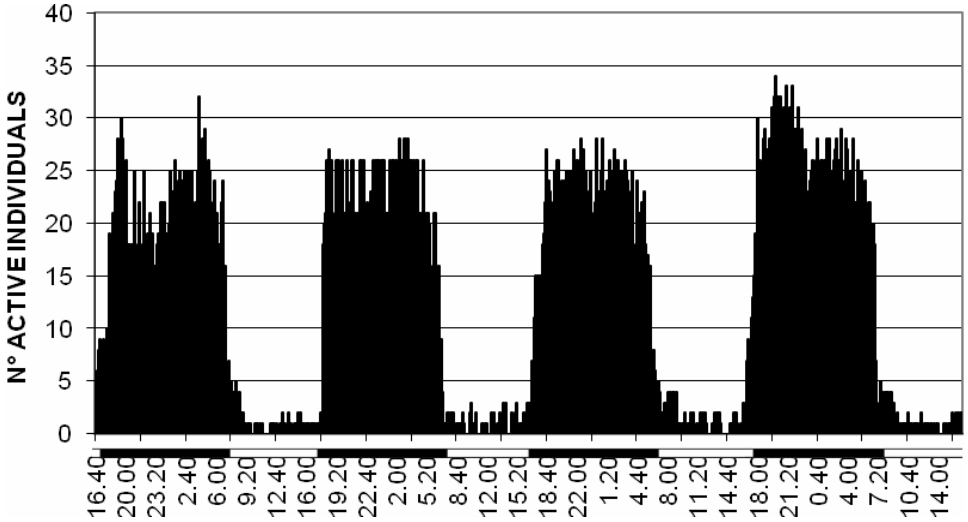


Figure 4.7- Daily rhythm of *Pterostichus melas italicus.*

C. fuscipes was nocturnal and its activity seemed to be constant during the dark phase. There was also some carabids active during the light phase. *C. fuscipes* usually ingested food during the first hour after the sunset, but feeding activity may be seldom observed even during the light phase (Fig. 4.8).

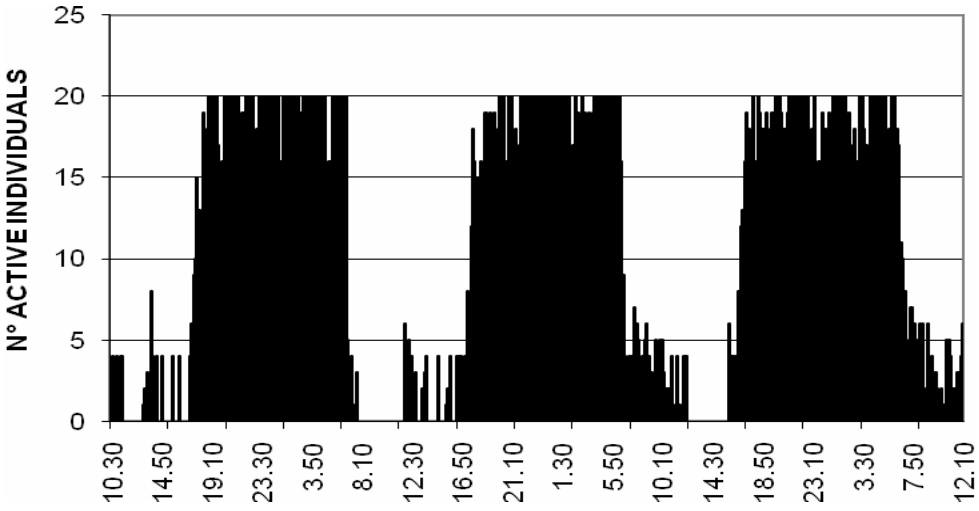


Figure 4.8- Daily rhythm of *Calathus fuscipes graecus.*

P. rufipes was nocturnal and its activity seemed to increase in the second part of the night. Feeding behaviour had been prevalently observed during the dark phase, one and two hours after the light/dark passage (Fig. 4.9).

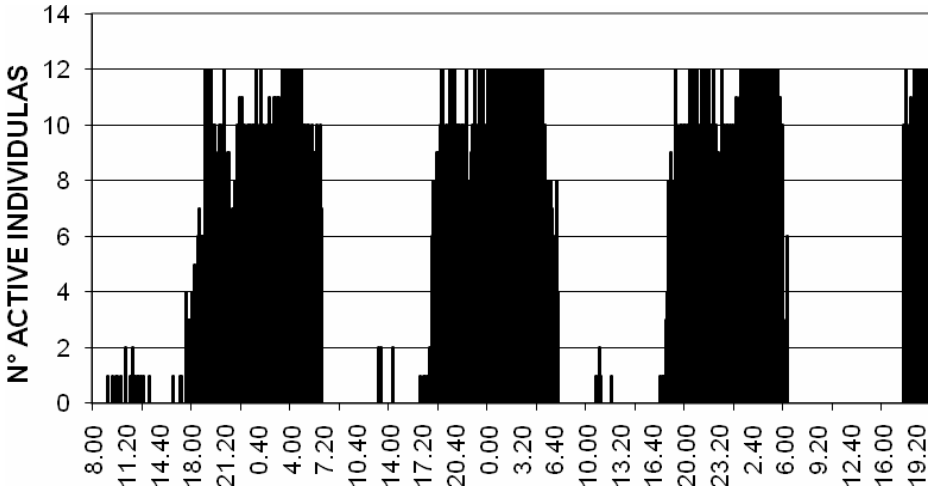


Figure 4.9- Daily rhythm of *Pseudoophonus rufipes.*

P. griseus was nocturnal too, but this species showed some diurnal activity. *P. griseus* and *P. rufipes* digged burrow and carried grain seed inside. Feeding activity was showed during the dark phase, generally from 18:30 to 20:30 (Fig. 4.10).

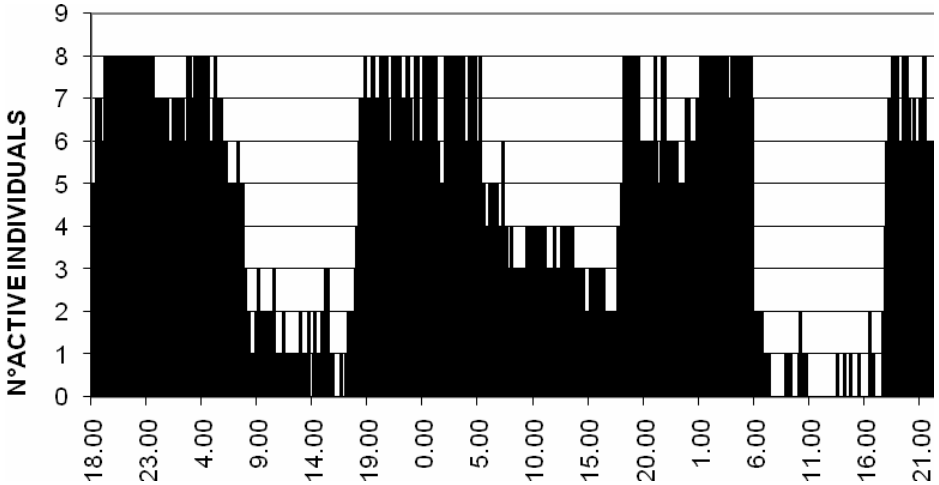


Figure 4.10- Daily rhythm of *Pseudoophonus griseus.*

C. coriaceus was nocturnal and showed two peaks of activity. This species ingested food one hour after the sunset and some time before the dark/light passage too (Fig. 4.11).

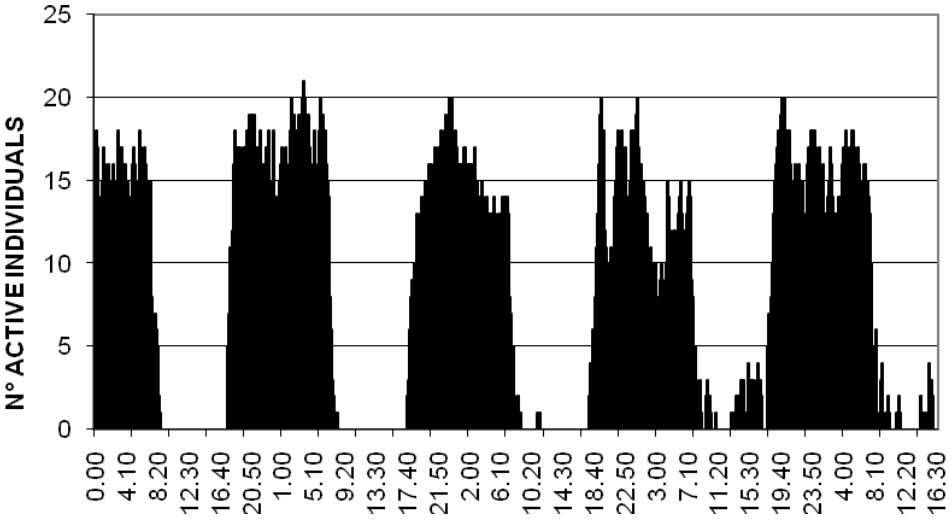


Figure 4.11- Daily rhythm of *Carabus coriaceus mediterraneus.*

L. cimmerius seemed to be active during the central part of the night, and less active at sunset and at sunrise too. This species was strictly nocturnal, carabids rapidly left their refuges when they became active (Fig. 4.12).

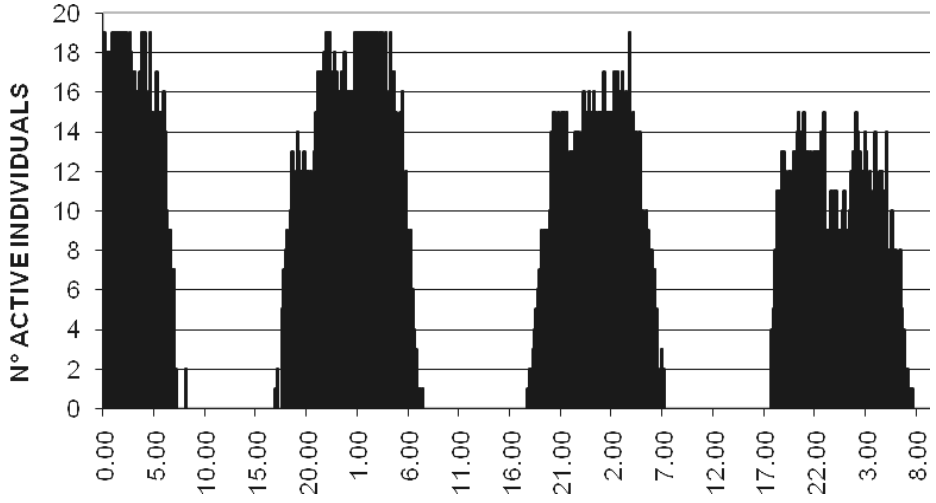


Figure 4.12-Daily rhythm of *Laemostenus cimmerius.*

N. kratteri seemed to be active during the central part of the night, its activity increased slowly and gradually until the peak and then decreased. Feeding activity was recorded during the dark phase (Fig. 4.13).

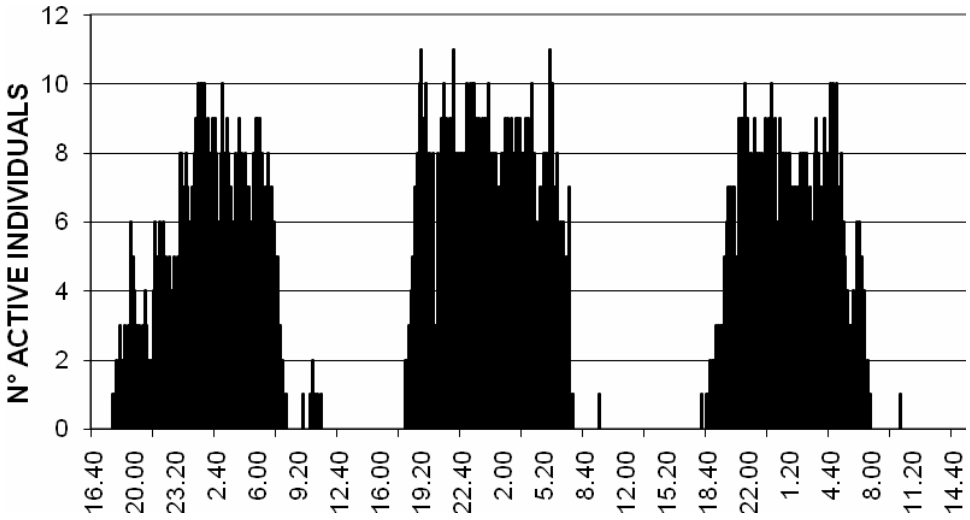


Figure 4.13- Daily rhythm of *Nebria kratteri.*

During this phase of work new data about carabid daily activity were collected, in literature there were no data for *N. kratteri* and *P. melas italicus*.

4.6 Carabid density in olive grove

Within the enclosure 47 individuals belonging to 12 carabid species were found in olive grove. In enclosure A 10 carabids were collected, 12 in enclosure B, 12 in C and 12 in D. Total density of carabid beetles was 5.9 individuals per m^2. *P. melas* mean density was 0.5 individual/m^2. The species with the highest density was *Trechus quadristriatus* (3,6 per m^2). There were a few abundant species and many rare species. All collected data are summarized in the Table 4.6.

Pitfall traps collected 62 carabid belonging to 12 species (Figure 4.14), in the same sampling period (Table 4.7). 9 species are equal to those collected in the enclosures. Pitfall traps data have been accepted as density data in older literature (Thiele, 1977) generating confusion. In our study we have a direct correspondence between these two data. Some litter layer inhabiting carabids, as *T. quadristriatus,* were found in high population densities only in enclosures, that are more effective than pitfall traps for the capture of little species.

SPECIES	ABUNDANCE	DENSITY
Trechus quadristriatus	29	3,6
Pterostichus melas	4	0,5
Nothiophilus geminatus	4	0,5
Harpalus distinguendus	2	0,3
Olisthopus glabricollis	1	0,1
Pseudoophonus rufipes	1	0,1
Asaphidion flavipes	1	0,1
Philorhizus crucifer	1	0,1
Pseudoophonus griseus	1	0,1
Brachinus brevicollis	1	0,1
Leistus fulvibarbis	1	0,1
Calathus cinctus	1	0,1
TOTAL	47	5,9

Table 4.7-Sampled species with their abundance and their density per square meter in the olive grove of Piana della Torre in the autumnal season.

SPECIES COLLECTED IN PITFALL TRAPS	AD
Harpalus distinguendus	0,5
Pterostichus melas	3,0
Calathus cinctus	2,8
Pseudoophonus rufipes	1,2
Brachinus italicus	0,2
B. crepitans	0,5
Pseudophonus griseus	0,2
Leistus fulvibarbis	1,2
Trechus quadristriatus	0,3
Nothiophilus geminatus	0,2
Anchomenus dorsalis	0,2
Nebria brevicollis	0,2
Total n° individuals	62
Total n° species	12

Table 4.8-Carabid species collected by pitfall traps in the olive grove of Piana della Torre

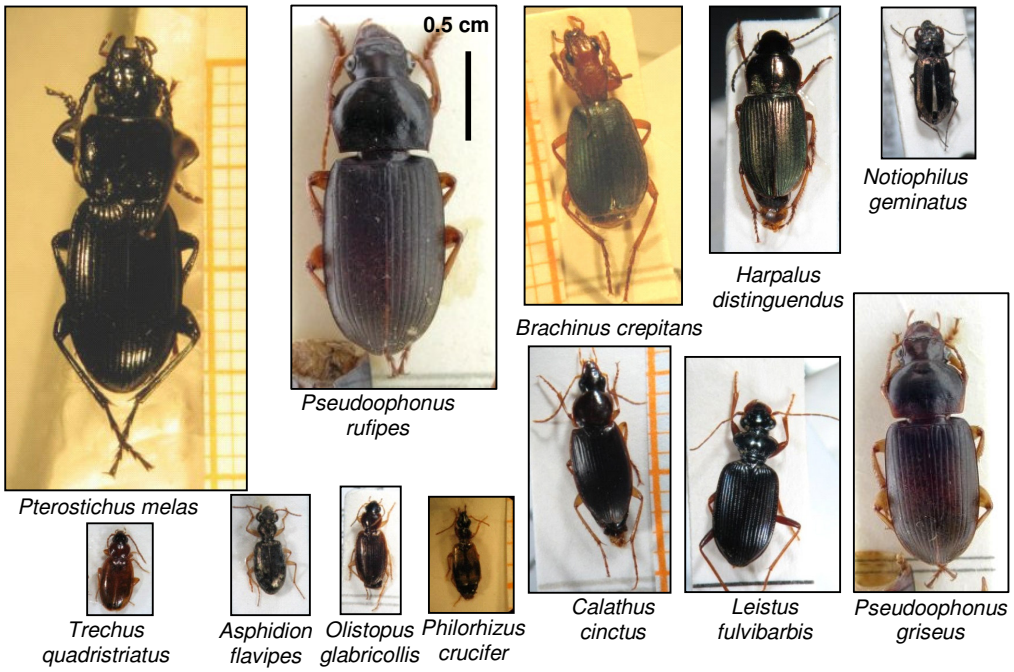


Figure 4.14- Carabid species sampled into enclosures of Piana della Torre

4.7 Simulation of carabid predation on *B. oleae* pupae and larvae

At the end of the experimental period with *D. planus,* only 3 pupae have been found. Soil was carefully sifted. The experiments with *D. planus* and *P. rufipes* have been repeated twice to increase accuracy. *D. planus* caught 17 pupae out of 20 and 14 pupae out of 20. In the terrain of *P. rufipes* no pupae have been found after a week, and after experiment replica 5 pupae out of 20 were found. Soil was carefully sifted. *P. melas* was able to find only 7 pupae. *P. melas* individuals were able to catch fallen larvae, immediately after their fall or at sunset. Larvae generally fell in the morning. During the two weeks of observation 19 larvae fell. Carabids belonging to *P. melas* species captured 10 larvae out of 19 fallen ones.

5. DISCUSSION

5.1 Preliminary test

Adult Diptera are present at high frequencies in carabid gut contents (Sunderland, 1975; Hengeveld, 1980a; b; Pollet and Desender, 1987). For this reason, according to Toft and Bilde (2002), I used *D. melanogaster* as standard comparison food of high quality in my experiments. Fruit flies resulted a very good quality food for generalist insectivores (Bilde and Toft, 1994; 1997a,b; 1999). Drosophilids are common in different habitats, including agro-ecosystems (Toft and Bilde, 2002), as our sampling confirmed. Unfortunately, the value of other Diptera, as well as pupae and larvae, was not so well-known as for fruit flies (Toft and Bilde, 2002), but it is not so improper to assume that *B. oleae* pupae could be high quality food for a generalist predator.

Earthworms have been found in gut contents with high frequency, so this prey is assumed to be high-quality food for some generalist predators (Toft and Bilde, 2002). Larvae of *Abax parallelepipedus* (Symondson, 1994) on a sole diet of earthworms become adults and larvae of *C. coriaceus* in our laboratory have regularly grown to the third stage. Nevertheless earthworms were accepted but not preferred by *Pterostichus melanarius* (Symondson et al., 2000), and are low-quality food for *Pterostichus nigrita* (Thiele, 1977) and *Agonum dorsale* (Bilde and Toft, 1994). This may indicate different ability to digest earthworm components and different degree of adaptation to earthworm predation.

Species that prey olive fly pupae were selected by a preliminary laboratory screening consisting in feeding choice experiments. This preliminary screening may indicate that not all polyphagous carabids species abundant in olive groves have the same preference for *B. oleae* pupae. *B. sclopeta, B. italicus, Anchomenus dorsalis* do not prefer pupae and do not eat them in our experiments. These species have little dimensions and little mandibles too, and may be unable to crush puparia, probably too hard to eat, except for *Distichus planus,* because of its “megacephalic” morphology. Conversely big carabids can grip puparia and ingest them. Prey size seems to be an important factor in prey selection (Pollet and Desender, 1987). Dimensions are important for an antagonist and it is possibly to hypothesise a selection of potential carabid antagonists of *B .oleae* with a dimensional criterion. Nuenschwander et al. (1983) made similar observations about carabid dimension and olive fly pupae predation. Furthermore Jørgensen and Toft (1997) suggested that hard seeds may give more mechanical difficulties for small larvae than for larger ones. The relative sizes of predators/prey are very important in these studies, *B. oleae* is a little prey, easily to be ingested by carabid species with medium size as those tested.

Carabid larvae seem to have the same feeding habit than adults (Grandi, 1951; Lövei and Sunderland, 1996; Toft and Bilde, 2002). According to Thiele (1977) feeding experiments show that *Carabus* larvae preferred earthworms, as the adults.

6.2 Quantitative tests

In *C. fuscipes* and *P. melas* species there were differences between males and females, that ate more quantity and varied food composition than males.

Summing up *P. melas* females preferred pupae, ate earthworms in proportion to availability and refused fruit flies. *P. melas* males ate pupae in proportion to availability, preferred earthworms and they disliked fruit flies. *C. fuscipes* females did not prefer pupae, preferred earthworms and refused fruit flies. *C. fuscipes* males ate pupae and earthworms in proportion to availability, they never ingested fruit flies.

The two tested species behave as generalist predators, they did not always explored at first the same prey, so there were no evidence to sustain the presence of chemical cues that could attract carabids to one specific prey. Weather (1989) underlined that some carabid species found their prey only using tactile cues.

Experimental food choice tests with carabid beetles are common in literature (Weather, 1991; Bilde and Toft, 1994; Kielty et al. 1999, Mundy et al, 2000; Oberholzer and Frank, 2003). Laboratory studies with four types of aphids offered to *Pterostichus cupreus* Linnaeus*, P. melanarius, P. madidus* and *Harpalus rufipes,* reported that the Pterostichini generally eat more aphids over a 15° temperature range (5° to 20°C) than other temperatures (Kielty et al. 1999). The experimental temperature chosen for my feeding and daily activity observations was of 20°C, also used by Toft in a lot of preference feeding tests. Additionally Kruse et al. (2008) found that for the carabid species *C. fuscipes,* the maximum activity was at 10-20°C, both in light and in darkness, and for the species *Pterostichus versicolor* (Sturm, 1824) in light there was a maximum at 20°C, and in darkness the total path travelled was independent from temperature.

Carabids increased their locomotory activity at intermediate temperature, but the relationship between predation rate and temperature differs for different predator-prey combinations (Kruse et al., 2008). Moreover carabids were subjected to a starvation period of only two days before the experiments. Foraging mainly depends on hunger level (Grüm, 1971; den Boer, 1986). In particular, Mols (1987, 1988), studying *Pterostichus coerulescens* L., suggested that initial satiation state was the main motivation for displacement under controlled conditions and that the direction of displacements was not related to hunger. In literature I found that in the carabid *Poecilus cupreus* (Linnaeus, 1758) longer starvation period increased overall consumption, but it did not affect its preference or the order of selection (Langs and Gsödl, 2001). However, a starvation longer than seven days, may possibly influence prey preferences because hungrier predators may be less selective (Bilde and Toft, 1998). Some preliminary tests with two groups of carabids subjected to three or one day of starvation, confirmed that after a too short period of starvation carabids generally did not ingest anything, and after three days of starvation carabids become less selective ingesting all preys during the test. For these reasons I chose a starvation period of two days.

In this laboratory study, finalized to verify predation of some abundant ground beetle species, *P. melas*, *C. fuscipes*, *C. coriaceus, N. kratteri, L. cimmerius* on olive fly pupae,

compared with other types of prey, we found that *P. melas*, *C. fuscipes* and *L. cimmerius* feed on *Bactrocera oleae* pupae, except for *P. melas* females, pupae were ingested but not preferred. These results are not unexpected, in fact tested carabid species are not *B. oleae* specific predators, however it is positive that, even in presence of alternative and habitual preys, they will ingest pupae.

P. melas ingested pupae more frequently than other preys. In fact, exploration followed by eating behaviour is significantly higher for pupae than for other supplied preys. Empty puparia were also explored more often than remains of earthworms and fruit flies. For this species, electivity index for fruit flies and earthworms had a negative value, while for pupae it was similar to zero. Therefore, considering frequency explorations too, *P. melas* seemed to prefer pupae to other preys. Moreover results showed that *S. melas* ingested more quantity of pupae if they were the first consumed prey.

C. fuscipes and *L. cimmerius* consumed pupae, but they preferred earthworms, ingesting the greatest amount of this prey. Regarding the frequency of explorations and ingestions *C. fuscipes* showed no differences between earthworms and pupae.

C. coriaceus ingested some pupae during preliminary tests that lasted one night, but in quantitative tests of two hours, they preferred earthworms and never ingested pupae.

N. kratteri, a forest carabid species almost completely absent in olive grove and used as comparison, never ingested pupae.

The ingestion phase varied in duration probably according to the consistency of preys (Jørgensen and Toft, 1997). Earthworms are soft, while fruit flies wings are indigestible, puparia have an intermediate consistency. Langs and Gsödl (2001) demonstrate that the preference for a certain food type depends on the prey supplied during laboratory trials, but in our experiments all utilised preys were palatable for *P.melas* and *C. fuscipes*.

Both species demonstrated their polyphagous predatory habits, in fact the first explored prey statistically was the first eaten one. *P. melas* ingested the largest amounts of first eaten prey, whatever it was, while *C. fuscipes* showed a similar behaviour only with earthworms. These results agree with literature about carabid feeding habits (Brandmayr et al., 2005, Hengeveld, 1980a; 1980b; 1980c; Toft and Bilde, 2002). A generalist feeding habit may be advantageous by optimizing a balanced, essential amino acid composition in the diet (Greenstone, 1979). The predator is able to make a choice among foods of different nutritional composition and eat greater quantity of the prey rich in nutrients that the predator is deficient in, or to extract specific nutrients from a single prey item (Mayntz et al., 2005). Generalist arthropod predators may forage opportunistically. In other words, the proportion of prey in the predators diet reflects its relative abundance in the environment (Griffiths et al., 1985; Pollet and Desender, 1987). So carabids will be more effective in reducing the population of the olive fly during year of strong infestation, when pupae are very abundant and predator-prey encounters more frequent. In addition, pupae are motionless and easy to catch. Prey vulnerability can determine prey selection (Langs and Gsödl, 2001). Many taxa target dead preys, due to the reductions in energy expenditure and handling times compared to attacks on living prey (Krebs

and Davies, 1993). In most cases prey preferences are probably a mixture of active choice and passive selection, and the predator will optimize using minimal effort in the search and capture of prey.

There are differences in feeding preferences between sexes in *P. melas* and *C. fuscipes*. This experimental datum is supported by Kielty et al., (1999) who found, in their study, that females of Pterostichini generally eat more than males. Moreover experiments were carried out during the breeding season, when females probably needed more alimentary resources. In particular, females must eat a lot to produce eggs and consume high energetic food (Ernsting et al., 1991; Mayntz and Toft, 2001). Maternal diet can have important effects on offspring fitness, as found in different invertebrate groups such as Lepidoptera (Gould, 1988), spiders (Bilde and Toft, 2000), Diptera (Langley et al., 1978), Coleoptera (Kyneb and Toft, 2006; Futuyma et al., 1993; Fox and Mousseau, 1996) and also carabids (Jørgensen and Toft, 1997). Carabid females supplemented with a mixed diet produced more eggs (Bilde and Toft, 1994). Many insects accumulate fat during larval development, larvae and pupae have a high fat content, and are energetic aliments that females need.

Olive fly pupae could be a better energetic source than earthworms and fruit flies due to its fat content probably higher than other preys. This could explain the preference of *P. melas* for olive fly pupae and the consumption of pupae by *C. fuscipes* and *L. cimmerius.* In fact, fats have a higher caloric content (9 kcal/g) than proteins (4 kcal/g) or carbohydrates (4 kcal/g), thus providing a more concentrated energy source (Barker et al., 1994). Allen (1989) reported that mealworm larvae and pupae, and fruit flies larvae contain more fat than adults. Earthworms are easily available preys, but their fat content is generally low. Furthermore, they have a high mineral contents (Edwards, 1985; Allen, 1989; Barker et al., 1998), but insects and earthworms may be a limited dietary source of vitamin A (Barker et al., 1998). Nevertheless, the nutrient composition of annelids varies depending on the composition of the substrate on which they are grown (Barker et al., 1998).

We observed the predation of *B. oleae* pupae in laboratory trials carried out offering only two alternative food sources to tested carabid species. To evaluate the frequency of *P. melas* and *C. fuscipes* predation in the field, we must also consider the intraguild predation (Finch, 1996; Prasad and Snyder, 2004) and other alternative preys that may limit carabid predator efficacy against the olive fly. The motivational state of the beetles and the availability of prey may be greatly different in field situations and has a strong influence on feeding behaviour (Wheater, 1987). These quantitative tests demonstrated distinct differences in prey choice by predatory beetles in laboratory. However, food preferences are always a composite phenomenon depending not only on food value, but also on prey availability, handling, easiness of capture and discovery (Pollet and Desender, 1986; Toft and Bilde, 2002).

6.3 Microscopical gut content analysis

Microscopical gut content analysis guided to the identification of olive fly recognizable fragments in the gut of two *C. fuscipes*. *B. oleae* adult remains in the gut of *C. fuscipes* may derive form scavenging or the predators captured the olive flies inable to fly at low temperature during the night. However, these data are very important and they put in evidence *C. fuscipes* predation on *B. oleae* in nature.

As concerning *B. oleae* larvae and pupae no remains were found into the guts of the predators. This result may be explained considering that during behavioural observation carabids generally eat only the soft internal tissue of pupae, leaving the puparium. Moreover carabids crushed puparium in the middle, where there are no spiracles and mouth parts. Even carabids dissected after pupae ingestion showed no identifiable *B. oleae* fragments in their guts. These evidences allow us to deduce that carabid predation on *B. oleae* pupae may be underestimated using microscopical analysis. There are also other carabids that leave the exoskeleton of their preys, as *Galerita lecontei* (Allen, 1979).

Moreover carabids prey a great range of different invertebrates and pupae might constitute only a portion of their food intake, as our feeding experiments showed.

Concerning other preys found into the gut, it is possible to suggest numerous interesting considerations, even if my goal was not to analyse carabid diet in autumnal season. I agree with the finding of Pollet and Desender (1987), asserting that liquid food may originate from a large range of prey groups, in fact I found different preys associated with it. Prey uptake is positively correlated with prey availability (Pollet and Desender, 1987).

Pterostichus is a mixed feeder that partially uses pre-oral digestion and ingests fragments of cuticles.

Earthworm remains have been also found in *Pterostichus vernalis* (Panz.), *Nebria brevicollis* (F.) and *Clivina fossor* (L.) (Pollet and Desender, 1987).

Collembola and aphids were not found in gut contents. Collembola are fast and little preys, very difficult to catch for not specialized carabid beetles. In general aphids stay on vegetable and not at the soil level. Moreover collembola and aphids are more active during daytime. All these reasons could explain their absence in the gut of *P.melas* and *C.fuscipes,* more active in night time.

Evaluation of the collected data is difficult because of the presence of un-identifiable fragments, the different degrees of pre-oral digestion of the dissected carabid species, prey sizes and availability that certainly can influence the number of different prey groups in carabid guts. Results concerning *P. melas* and *C. fuscipes* diets in olive grove would not be exhaustive. In fact there are seasonal variations in predation and in different seasons different preys are available (Thiele, 1977; Dijk, 1986; Honek et al., 2006), even if seasonal variation in carabid physiology and feeding experience did not influence their preferences (Honek et al., 2006).

6.4 Molecular gut content analysis

PCR analysis may support our research because primers are specific for *B. oleae*.

Studying the gut content of polyphagous predators is necessary for a qualitative identification of indigenous predators of the prey, quantifying the percentage of the predator population attacking the prey throughout the season, as well as for determination of their contribution to pest mortality (Agustí et al., 1999). Data from field experiments are crucial in modeling predation rate and in evaluating different species of predator as biocontrol agents, or in addressing fundamental questions in feeding ecology (Mills, 1997; Harwood et al., 2001). PCR is a useful technique in studying the relationships between predators and preys. We showed that the PCR primer pair INTR-F/INR-R designed as specific *B. oleae* primers could be used to detect its DNA in the gut of *P. melas* and *C. fuscipes.*

Previous research showed that multiple copy genes, both nuclear (Zaidi et al., 1999; Hoogendoorn and Heimpel, 2001) and mitochondrial (Chen et al., 2000; Agustí et al., 2003), should be ideal candidates for detecting predation because they considerably increase the probability and duration of detection (Agustí et al., 2003).

Our method could detect all developmental phases (eggs, larvae, pupae and female and male adults) of *B. oleae*; in fact DNA is invariable during the life cycle of a prey (Agustí et al., 2000), and thus PCR primers and other molecular diagnostic markers can give reliable results. Subsequent research can focus on enhancing the numbers of these predators as part of an overall integrated pest management strategy. The primers developed in this study, used in combination with other primers developed for *B. oleae* and for other pests will allow us to examine multiple feeding events of individual predators in the field. This is the first research that analysed predator gut contents using *B. oleae* specific primers.

B. oleae pupae could represent an important prey source for other ground macro-invertebrate as Geophilidae, Lithobiidae, ants and predatory beetle larvae. Olive fly pupae are highly abundant preys, suggesting that *B. oleae* pupae could represent an important prey source for ground invertebrates during autumnal period within the soil food web. Making opportune laboratory checks, our specific DNA marker might be used also to identify other invertebrate predator of *B. oleae*. In the future, these primers will be tested in the field to detect *B. oleae* predation by several carabid predator species.

6.5 Carabid daily rhythms

The starved carabid beetles were more active than the satiated ones (Fourier and Loreau, 2002). Carabid tested species are nocturnal and they may prey pupae prevalently at sunset. Carabids also may prey migrating larvae at soil level. In fact, even if larvae fall in the morning, they need about seven hours to become pupae and carabids at sunset may encounter some larvae.

The carabid beetle *Calathus fuscipes* (Goeze) is dull black and mainly active at night (Kegel, 1990). Greenslade, (1963) studied *C. fuscipes* daily rhythm in august. This species showed a peak during night, but it began to be active some hours before the sunset, as

confirmed by my experimental data. In spite of this, *C. fuscipes* was included in nocturnal species because it was more active at night. *Nebria brevicollis* is strictly nocturnal (Greenslade, 1963; Thiele and Weber, 1968; Luff, 1987), as collected data showed. *C. coriaceus* behaved as a nocturnal species, as literature suggested (Thiele and Weber, 1968). In field studies, Thiele and Weber (1968) found for almost all night active carabids, except *Abax parallelus* (Duft.), a maximum activity at about 1–2 hours after the beginning of darkness. In laboratory experiments these authors could demonstrate that the beginning of nocturnal activity of *Carabus* species depends on the starting time of "darkness". My results confirmed these observations.

P. griseus made nocturnal migrations in northern China (Feng et al., 2007). *P. rufipes* is a nocturnal species (Greenslade, 1963; Luff, 1987), it showed an activity peak after midnight, and this peak shifted to earlier in the night during September in north-east England (Luff, 1978).

Many *Pterostichus* species are considered nocturnal, some of them plastic, while other diurnal (Greenslade, 1963; Luff, 1987); *Laemostenus* species living in cave, is considered nocturnal as *L. terricola* (Thiele and Weber, 1968), but no data are available for *L. cimmerius* and *P. melas,* so the nocturnal activity of these species was registered for the first time during this study.

Pollet and Desender (1987) sustained that daily activity patterns of prey and predators may be of great importance, in fact a synchronization of activity increases the chance of encounter and is very important in prey selection. Carabids may capture pupae during the critical phase of displacement on the soil surface. So the question of the walking ability of the *B. oleae* larvae is of great importance. Tremblay (1995) sustained that this phase lasted only seven hours and larvae came out from drupes during the morning. The recording of daily rhythm of carabids confirmed that they have prevalently nocturnal habits that do not coincide with the moment of larvae coming out. On the other hand, considering that in autumn there are a few hours of light, a carabid that begins its activity at sunset may still encounter some roaming larvae. Pupae are vulnerable to predators even during the night. An advantage of nocturnal activity is that predators are active when day-active preys (e.g. flies) are less active and less ready to escape, due to the low temperature (Toft and Bilde, 2002). In fact *C. fuscipes* were able to catch more fruit flies at 5°C than at higher temperatures (Kruse et al., 2008). This evidence may explain the presence of flies in gut contents. Moreover, being flies high-quality prey (Toft and Bilde, 2002), high portion of flies in the diet could increase the fitness.

6.6 Carabid density in olive grove

Collected data refer to a limited period and with four replicates, but for a preliminary experiment the methodology used can give interesting results with little investments in cost and time. This study would not be an extensive study of carabid beetles density. Several biases may be found regard pitfall trapping, in fact capture rates depend on vegetation structure, on weather conditions and on insect ability to escape from traps. To reduce biases connected with border effects, circular enclosures without corner was constructed and pitfall traps were placed along the interior border of each enclosure. Carabid body size and temperature are positively

correlated with the probability to catch using pit fall traps (Esch, 2008). Even abundance and activity may play a role. At warmer temperature activity, levels increased and the probability of capture too (Esch, 2008). Despite these limitations, several useful information about carabid density in the field are provided.

The distribution of predator insects in various farmland habitats is of great importance (Sotherton, 1984) also characteristics of vegetation may be important, for example vegetation defends polyphagous predators from cold (Sotherton, 1985). The structure of weed community affected the composition of carabid assemblage (Saska, 2008).

Fourier and Loreau, (2002) used rectangular enclosures made of plastic sheets 50 cm high, vertically maintained with stakes and buried in the soil 10 cm deep, containing pitfall traps to study carabid predation.

Soil invertebrate density has been measured by Pollet and Desender, (1987) using litter quadrat sampling. Carabid density has been measured by Southerton (1984; 1985) using soil sample of 0.04 m^2 in Hampshire in winter. He found that carabids polyphagous predators were higher in field boundaries than in the center of sampled farmland and woodland was a relative poor habitat for overwintering predators. 15 carabid species were found with a density of 11.4 in field boundaries, of 0.6 in grassland and of 0.16 in cereal stubbles. *Harpalus* (= *Pseudoophonus*) *rufipes* had a density per square meter of 0.5 in field boundaries, of 0.03 in wintersown cereals and it was not present in grassland during winter. In our sampling the density was of 0.1.

Trechus quadristriatus had a density per square meter of 1.3 in field boundaries, of 0.5 in woodland and of 0.04 in grassland. In our sampling the density was higher, 3.6, even confronted with other density value (Southerton, 1984). This species can fly and is very little, probably our sampling method, even being efficient for brachipterous and large flying carabids, collected small flying individuals, as happening with sampled dipteral, so this density value was higher. The used method is not suitable for estimating density of flying insect groups, e.g. Diptera, Lepidoptera and Hymenoptera.

Pterostichus melanarius had a density of 0.5 in field boundaries and woodland, and of 0.02 in grassland (Southerton, 1984). Kromp (1999) reports a decline of 1.7 per m^2 versus 0.1 for *P. melanarius*, probably due to insecticide application. In our sampling *P. melas* showed a density of 0.5.

Asaphidion flavipes controlled aphid populations at 15 individuals per 0.25 m^2, roughly natural densities (Hance, 1987). *Agonum dorsalis* Pont and *Pterostichus melanarius* also affected sugarbeet aphid populations, though the former only at low beetle densities, due to *P. melanarius* tendency toward cannibalism (Hance, 1987). Predator complexes which include carabids have been found more important in controlling pest populations than carabid single individuals. Grouped with other polyphagous predators, *Agonum dorsale, Bembidion lampros* Herbst, and *Bembidion quadrimaculatum* Say form inverse relationships with pest aphids (Edwards et al. 1979, Chiverton 1986).

In olive grove there are a mean of 150-300 pupae per square meter (Tremblay, 1995) and our data indicated that there was 0.5 *P.melas* and 0.1 *P. rufipes* potential olive fly predators, per square meter. Considering that a carabid of this species can daily ingest a quantity of food at least equal to its own weight (Thiele, 1977) carabids could have a certain role on the reduction of the number of pupae.

Lövei and Sunderland (1996) reported data about carabid density. The highest density (66.62 for little carabids and 14,32 for large ones) are measured in field boundaries. In arable fields species with size inferior to 5 mm showed a density of 5.96 in annual crops and of 3.61 in biennial ones, while species longer than 5 mm showed a density of 1.83 and 4.82, respectively. These data are comparable with our results of 5.9 carabids per square meter.

Although *P. melas* and *P. rufipes* are not specialist olive fly predators, these species could influence *B. oleae* population and may be effective predators due to their abundance in olive grove.

6.7 Simulation of carabid predation on *B. oleae* pupae into the soil

D. planus, P. rufipes and *P. melas* were able to find pupae at 2.5 cm of depth in a terrain containing 5 cm of moist soil. *D. planus* and *P. rufipes* were able to catch more pupae than *P. melas*. These results were probably related to the burrowing habits of these two tested predator species, however these simple exsperiments demonstrated that some carabids were able to catch pupae even under the soil surface. There are numerous burrowing carabids, *Pseudoophonus rufipes* occurred at depths of about 25 cm during the day, while *Amara* and *Notiophilus* species are found in the upper 5 cm of the soil (Luff, 1978).

Cavallaro and Delrio (1975) confronted mortality after three months among three different pupae groups in field, one group was burrowed in little cages with 1 mm mesh net, the second in cages with 1 cm mesh and the third without cages. Mortality was higher in pupae without protection.

Bateman (1976) studied the effects of abiotic factors and predation on olive fly mortality in Southern France. He buried the pupae at various depths under the soil and found that predation was more prevalent in winter than in the warmer months for a depth of 5 cm. Bateman suggested that predators go deeper than 5 cm during the summer. My experiment refers to a depth of 2.5 cm and was successful, but concerning the experiments of simulation of predation in soil, the different and more complex conditions in the field may be considered.

During my experiment, *P. melas* was able to catch 53 % of fallen larvae. This datum indicates that carabids may reduce not only pupal number, but also the abundance of migrating larvae. The phase of fall and pupation is critical for *B. oleae* and carabids may be important antagonists during this period. Soil moisture influences larvae displacements, as my observation confirmed, and reduces pupation depth (Cavallaro and Delrio, 1975). Larvae caved in the soil in 5 minutes, if there was no grass, they employed some other minutes in the presence of grass, while on gravelly soils they pupated on the surface near stones (Cavallaro and Delrio, 1975). However, olive fly larvae pupate also on the soil surface (Trembaly, 1995)

and it is probable that if the ground is hard and dry, as it is usually in Calabria, the larvae are not able to descent a lot under the surface and may be easily preyed by carabids or other ground predators. Carabids, ants, birds and myriapods were active predators even during the larvae burial phase, representing a non negligible mortality olive fly factor (Cavallaro and Delrio, 1975).

Predatory activity is essential considering that pupae in the soil are the main source of new olive fly generation (Cavallaro and Delrio, 1975).

The number of fallen larvae is not proportional to the number of infested drupe and it differs from tree (Liaropoulos et al., 1978). Pupae density in the soil is variable in space and time. For example considering one tree on 22 November 1975 Liaropoulos et al. (1978) found 147 pupae per square meter under the peripheral foliage of the tree and 85 pupae per m^2 in the soil under the central part of the tree. A peak of 2000 pupae per tree between October and November at Ossi (Sardinia) in 1978-1979 was recorded (Delrio and Prota, 1989). Moreover pupae density in the soil decreased from November to February and the studies on pupae density refer to years of 90 % infestation level (Delrio and Cavallaro, 1975; Liaropoulos et al., 1978).

7. CONCLUSION

This research demonstrated distinct differences in prey choice by predatory carabids in laboratory. This study also increased the biological knowledge of carabid beetle behaviour, ecology, feeding habits and gave new methods for laboratory and field analyses.

Feeding experiments were conducted in presence of two alternative palatable preys (fruit flies and earthworms), consequently results may be considered reliable. Pakarinen (1994) underlined that when alternative prey was available, generalist beetle species were more likely to avoid slugs, unpalatable preys. Dipteral pupae are palatable prey (Toft and Bilde, 2002). The efficacy of carabid predation on *B. oleae* populations may depend on density of alternative prey species or on the prey quality for the predators.

In the absence of natural enemies and other mortality factors, olive fly populations increase as well as damages. Carabids are indigenous predators and could control olive fly with a much smaller economic waste than parasitoid introductions. Furthermore carabids are considered good bioindicators of the environmental states, as they put in evidence anthropic detrimental effects (Brandmayr and Pizzolotto, 1994; Brandmayr et al., 2002; Rainio and Niemelä, 2003). So it is essential to safeguard and promote the presence of indigenous invertebrate predators in olive groves. To increase plant and habitat diversity in agroecosystems will increase abundance and diversity of antagonists (Altieri, 1999). In fact, non-crop habitats are very important as carabid sources, because they utilise adjacent hedges and field margins for shelter, breeding or dispersal (Holland and Luff, 2000). Also soil grassing can enhance biological control by providing refuge areas for these predators (Lee et al., 2001; Tyler, 2008). Biological control could be particularly effective under an organic farming strategy because landscape diversity and a few persistent pesticides would be applied.

On the other hand, Carabid beetles are affected by intensive agricultural cultivation (Kromp, 1999). Mauchline et al. (2004) found that *Pterostichus madidus* Fab., *P. melanarius* Illiger and *Nebria brevicollis* Fab. were unable to distinguish between good and dimethoate contaminated preys, so they can eat contaminated preys and die under conventional farming; the use of pesticides and agronomic practices reduce the number of generalist predators (Thorbek and Bilde, 2004, Iannotta et al., 2007b). Insecticides are toxic to beneficial species (Fleschner, 1959). Entomologic literature contains many examples of these effects of insecticides on biological balance. It is important to develop and use insecticidal treatments which inhibit the least possible the activity of beneficial insect such as selective insecticides. In any case, pesticides have localised and short-term effects, and carabids can rapidly re-invade sprayed crops (Holland and Luff, 2000) if refuge areas are available. The re-colonisation of sprayed olive groves under conventional regime could be quicker than in other conventional crops because pesticide spraying is usually done no more than three times a year. More and more attention must be given to the development of integrated pest-control programs.

O'Neal et al. (2005) suggested that carabid populations can be easily boosted, in fact certain ground covers are better than others for carabids (*Harpalus pensylvanicus* in this case). Carabids were more abundant in clover and re-grass ground cover treatments than in buckwheat or herbicide-treated bare ground in highbush blueberry fields (O'Neal et al., 2003). Habitat island or field boundaries of olive groves could serve as refuges and recolonization sites.

This research has potential implications on the future management of olive grove, including suggestions for insecticide use strategies to promote generalist ground predator populations.

Studying Carabidae as a pest antagonists is difficult. In fact carabid beetles have often been mentioned as predators on several pests, even if consumption of the pest is probably of little importance to predator fitness because it just forms a small fraction of the food intake (Toft and Bilde, 2002). However if beetles are numerous, predation may have a significant impact on pests. Carabid beetles may reduce *B. oleae* population even if they are not specialized olive fly predators and do not show particular preference for pupae, due to their abundance in olive grove. This hypothesis needs other complex field investigations to be proved.

Another difficulty is that the majority of carabid life cycles are not in synchrony with those of their prey (Lövei and Sunderland, 1996), but this is not true for autumn breeding carabid species (Brandmayr et al., 2005) living and abundant in groves that showed a coincident phenology with the olive fly. Morris et al. (1999) found in olive grove a peak of predator coincident with oviposition by adult of *P. oleae*, *S. oleae* and the olive psyllid, *Euphyllura olivine* (Costa). They hypothesize that this may be an adaptative response to the phenology of the olive moth. The autumnal peak of abundance of *P. melas* and *C. fuscipes* coincided with the highest infestation of olive fly in the field (Delrio and Prota, 1989; Mazzei et al., 2005; Iannotta et al, 2007a). In fact, during the autumn many infested olive drupes fall on the ground where olive fly larvae pupate inside the olive or in the soil (Delrio and Prota, 1989; Iannotta et al., 2003) and are exposed to generalist predators. Bateman (1976) found that predation of *B. oleae* pupae was prevalent in winter. In autumn carabid tested species are the most abundant, after Isopoda, in olive groves at soil level in comparison with other insect groups (Iannotta et al., 2007a). In autumnal season carabids look for food after the aestivation period and during autumnal breeding season carabids need more food and the motivation to hunt increases as well.

Night-active, olfactory-tactile, polyphagous carabids are the most probably pupae predators among carabid guild. Olive fly pupae are motionless, and carabid species *Bembidion properans* Stevens and *Agonum muelleri* (Herbst) preferred inactive prey items than prey with high soil surface activity (Pollet and Desender, 1987).

An olfactory-tactile predator must find prey using tactile and chemical cues and this is easier with motionless or slow prey. Pupae are available and palatable food, that may be ingested and in some cases preferred by several carabid species, as my experiments showed.

Results confirm that carabids can play a role in biological control and may reduce olive fly populations at the pupal stage, in agreement with previous studies where carabids are

considered potential predators of olive fly at the pupal stage (O'Neal et al., 2005; Bigler et al., 1986; Bateman, 1976; Delrio and Cavalloro, 1977; Nuenschwander et al., 1983; Orsini, 2006). In fact, because of their predatory polyphagous diet, *P. melas, L. cimmerius* and *C. fuscipes* may be natural predators of olive fly pupae at soil level. Pupae in the soil are the main source of new olive fly generation (Cavallaro and Delrio, 1975).

The role of carabid beetles as control agent of *B. oleae* has not yet been elucidated for many aspects. This research could not be exhaustive, but would only provide an aid to better understand carabids role as pest antagonists in olive grove agro-ecosystem. Even if further laboratory and field experiments will be necessary, this work of thesis gives a first contribution to solve the open question of carabids role in olive fly reduction and, after this study, it is reasonable to expect that carabids in field may reduce olive fly pupae numbers in the soil and may reduce new spring and early summer *B. oleae* generations and, consequently, the autumnal ones.

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