INTRODUCTION

Cork is the cambium bark of the cork oak tree, *Quercus suber*. It is peeled away from the tree truck every 9-10 years, the interval needed for regrowth, after a tree has reached about 45 years in age (Figure 1). This harvesting is often done traditionally with hand tools, although a mechanized method has been developed recently. This low impact harvesting allows for other enterprises such as cattle grazing, game hunting and mushroom harvesting to take place in the understory. A characteristic Iberian landscape, the dehesa, is made of cork forest managed for pork and cattle grazing and cork harvesting, conforming a classical example sustainable forest management. Unlike the oil-based alternatives, the finished product is also biodegradable. Many wildlife and plant species depend on the cork forest, among which are the endangered Bonelli's eagle, Spanish Imperial eagle, Iberian lynx and the Barbary deer along with wolves, wild boar and genets.

Figure 1. Several aspects of cork tree and cork industry. Cork trees are harvested by hand by specialized workers. The most profitable product from cork is the cork stopper.

Cork oak forests cover over 2.4 million hectares in Portugal, Spain, France, Italy, Morocco, Algeria and Tunisia (Figure 2). A third of these areas are in Portugal, which produces 50% of cork products worldwide. Cork is Morocco's most valuable currency-earning commodity. Each year, US$1.7 billion is earned from cork stoppers and US$700 million in construction materials (FAO) with 15% of that amount going to Morocco, Algeria and Tunisia (ISO). However, the industry is seeing declining revenues. Between 2001 and 2004, for example, Portuguese exports to Australia and the U.S. experienced a 24% decline from 142 million Euros to 108 million Euros. This decline is directly associated with the transition from cork stoppers to synthetic stoppers in the wine industry.

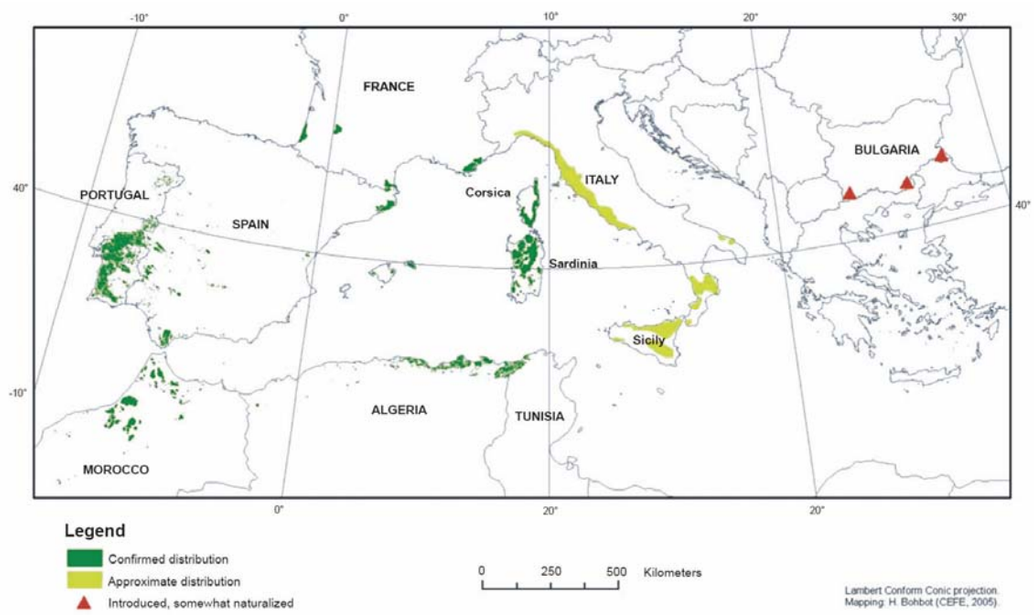


Figure 2. Areas of the World where cork trees are grown.
http://earthtrends.wri.org/updates/node/224

In addition to declining demand, the cork oak industry is being affected by a small but powerful enemy, the larva of the beetle *Coraebus undatus*. This is a medium-sized beetle, (Figure 3) that feeds on young cork oak leaves in the summer causing negligible damage to the tree. The females lay eggs on the bark of the trunk, which upon hatching tunnel the bark until they reach the cortical cambium on which they feed for the next two years. Upon completion of development, the larvae make a chamber in the bark and

transform into a pupae and then into an adult which eventually exists through a hole, mates and completes the cycle. As it feeds the larvae makes galleries underneath the bark which supposedly cause little damage to the tree (Figure 4). For the farmer, however, these galleries suppose a tremendous impact because the value of the cork from an infected tree can be reduced 10 fold. The reason for the decrease in the economic value of infected cork is double. First, the “pana” (piece of bark peeled away of the tree) is normally peeled in one piece (Figure 1). However the larval galleries cause the cork to stick to the inner bark so that the pana brakes down into smaller pieces as it is peeled away. The value of broken pana is smaller than of an entire piece, and more work is needed to extract pana from an infected tree. The second reason is that cork with galleries results in cork stoppers with holes. Cork of good quality is used for making cork stoppers and it is of the highest economic value. However cork of low quality is not used in cork stopper production and its value is several times lower than the high quality cork.

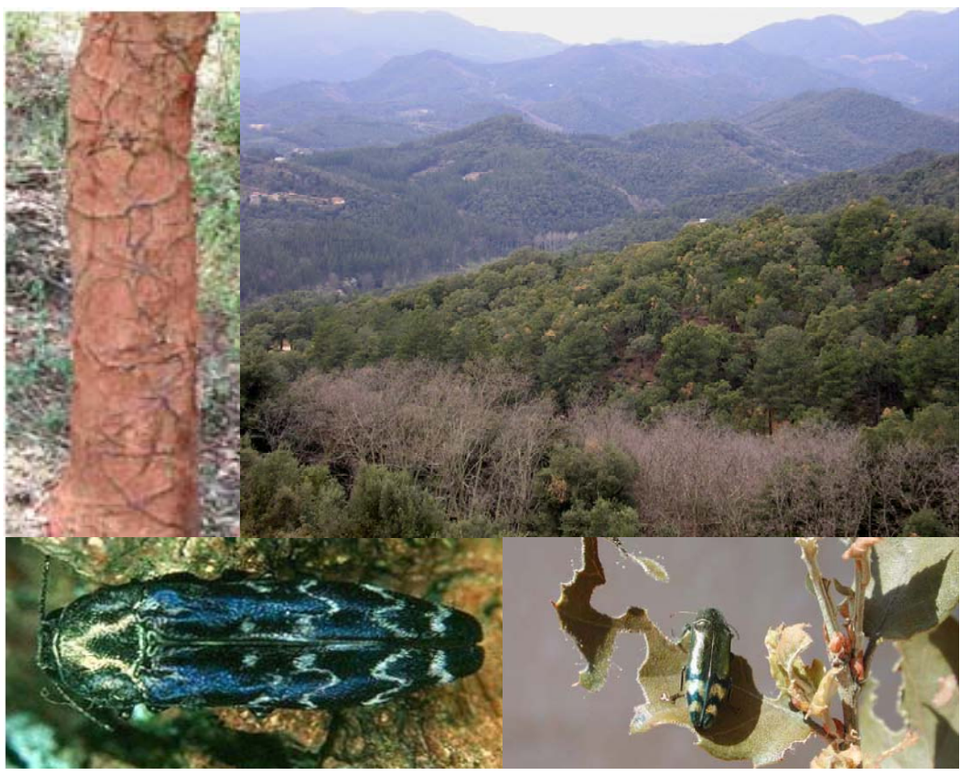


Figure 3. The main cork pest, *Coraebus undatus*, and its sister species, *C. florentinus*. *C. undatus* has narrow white bands on the elitra (lower left) whereas *C. florentinus* has white

dark green bands (lower right). As adults both species feed on oak leaves (lower right), but the larvae have very different ecology. *C. undatus* feeds underneath of the bark of the stem, making long galleries (upper left), whereas *C. florentinus* feeds on the small branches, killing them at the end of its development (upper right).

The farmer that collaborated in this study provided a rough estimate of the damage produced by *C. undatus* in his farm. Normally he produces 20 % cork of poor quality which is used in house insulation, floors, etc., and is of low economic value, and 80% cork of good quality which is used in cork stopper industry and provides the highest benefit. In the last years, due to *C. undatus* damage, he is producing 30 % good quality cork and 70 % poor quality cork. A more recent estimate indicates that in Extremadura, Spain, every year 5 million € are lost due to *C. undatus* damage

(http://www.mma.es/portal/secciones/biodiversidad/montes_politica_forestal/sanidad_forestal/pdf/guadalupe08_Iprocor.pdf).

Although *C. undatus* is not a new insect in cork oak forests, it has always remained below the economic threshold level, and only in the last 10-15 years has become a serious pest problem. This may explain the low scientific production on the biology and control of this insect, at least with respect to articles written in English and published in indexed journals which, as far as we know, are non existent. Since the larvae feed underneath the bark, direct control with insecticides is very difficult. Other means of control are thus necessary.

One alternative means of control is the use of pheromones of sex or aggregation to attract or confuse the insects and so reduce the population numbers either directly by removing them from the population or indirectly by reducing the probability of mate finding and subsequent progeny numbers. In order to study the sex pheromone adult insects are needed, and they need to be virgin. There are two possible methods to obtain adults. One of them is to collect them in the field. This is difficult given that they are not easy to capture. Several methods have been used, light traps, funnel traps with chemical attractants, housing of bark, and screen traps around the tree trunk to collect the emerging adults, but none of these methods has given good results. However purple traps have shown to be effective in attracting buprestid beetles in other parts of the world (Francese *et al*., 2004). In fact, a pilot study has demonstrated that *C. undatus* is captured in purple sticky traps (C. Gemeno, personal communication). However this test was done just with purple traps, with no control traps, so the questions remains open of whether *C. undatus*

was attracted to this colour or was captured by passive intersection with the sticky panels. The **first objective** of this study was to determine if the attraction of *C. undatus* to purple traps in 2007 was active or passive. For this purpose we placed purple and clear traps in the field and monitored captures for the flight season.

A recent publication has shown that males of the buprestid pest *Agrilus planipennis* are attracted to the sight of females, even if these are dead (Lelito *et al*., 2008). We thought that a similar phenomenon may be happening with *C. undatus*, so we did this experience with it, and with a sister species, *C. florentinus*, which is sympatric and shares a similar ecological niche (Figure 3). *C. florentinus* feeds on the small branches of *Quercus* spp., and it causes the death of the branches by eating a ring of vascular tissues around the branch right before molting.

A second way to obtain adults is to rear in captivity larvae collected in the field. Larvae are easy to obtain during the harvest of cork and large numbers can be produced in a short time period. However rearing larvae of this insect is challenging because very few wood boring insects have been reared in diets, and no wood boring buprestid has been reared in diets as far as we know. Our **second objective** was to attempt the rearing in captivity of field collected larvae with the aim of obtaining virgin adults for pheromone research. For this purpose we collected the larval food ingredients from cork trees and prepared diets with them. The larvae restrict themselves to a thin section of the bark consisting of the inner part of the

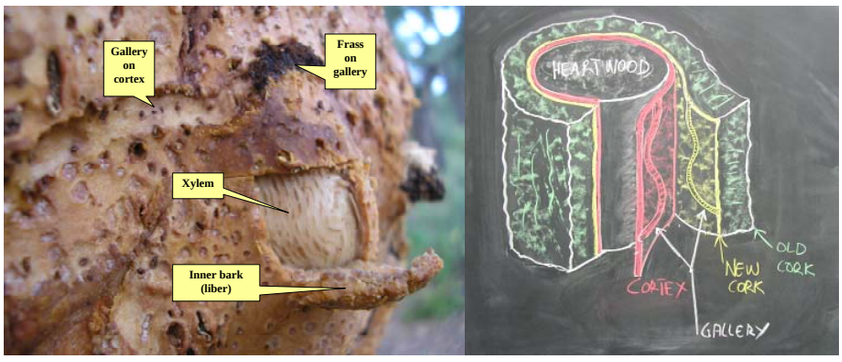


Figure 4. Picture of cork tree alter removing teh bark (left) and schematic representation of the nark and hardwood tisúes (right). The tree shows the internal half of a *C. undatus*

gallery chewed off by a larva on the external part of the liber (called cortex) represented in red colour on the drawing. The other half of the gallery is made on the inner layer of the external bark (the cork itself), painted yellow in the diagram. Between cortex and inner cork there is a layer of cambium cells (the cortical cambium) which produces bark towards the outside.

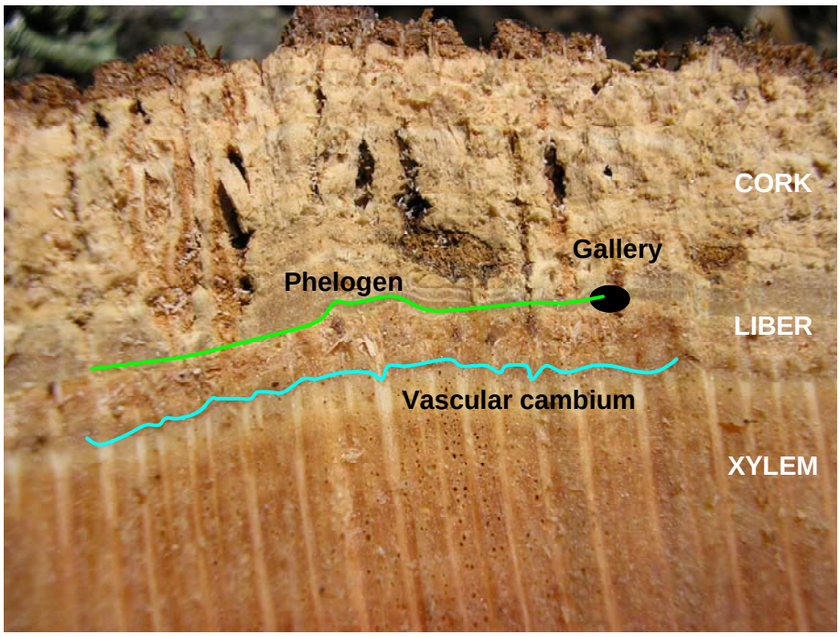


Figure 5. Picture of a transversal section of the branch of a cork oak. The hard wood (xyllem) is followed towards the outside by the vascular cambium (in blue) that produces xyllem towards the inside and phloem towards the outside. Another layer of cambium cells (cortical cambium, or phelogen) produces outer bark (cork) towards the outside. The new cork has a high water content and is of sweet flavor, as opposed to the old cork which is dry and unsweet. The larvae of C. undatus make their gallery on the new cork and the outer layer of the inner bark, or cortex, which has a bitter taste and is of granular and fibrous nature.

MATERIALS AND METHODS

1. Field trapping

A. General

Description of the study area

The study area is situated in a cork forest of Arbucies, Girona, Spain (Figure 1). Total area of the forest is about 273.63 hectares. The forest area is about 450 m above sea level and located in between 41° 50'28.65" North latitude and 2° 29'13.95" East longitude. The main species of the forest are *Quercus suber* and *Q. ilex*. Other important species includes *Pinus radiata*, *P. pinaster*, *Castanea sativa* and woody shrubs such as *Arbutus unedo*. Density of the main tree species is about 390 trees/ha. The average age of *Q. suber* is 100 years and *Q. ilex* is 75 years approximately. The forest is located in a sloppy area crossed by a forest road. There is a small river nearby. The tree cover creates a lot of shadowed areas underneath the trees (Figure 2). The undergrowth vegetation is scarce in some areas and deep in others.

Figure 6. Location of Arbucies in Catalonia, Spain. The black line in the map indicated the distance of the weekly tour to check the traps between Lleida and Arbucies.

The weather of Girona on average is mild. It has a mild climate with plenty of sunny days and not too much rain. There are differences on the coast, which is little bit warmer but has a cold sea breeze. There are also differences in the mountainous area as there is plenty of snowfall all the year round.

	Spain		Girona	
Month	Temperature	Rainfall	Temperature	Rainfall
Jan	7	50	7	141
Feb	7	48	8	94
Mar	11	55	11	112
Apr	13	44	13	109
May	16	47	17	87
Jun	22	13	22	47
Jul	24	8	24	44
Aug	24	18	24	53
Sep	20	39	20	72
Oct	16	78	17	188
Nov	10	60	11	158
Dec	7	55	7	131
Mean	14.75	42.91	15.08	103

Table 1. Monthly average temperatures (°C) and rainfall (mm) in Spain and Girona. Source: http: http://www.kyero.com/weather/17-girona-weather, and the Spanish Institute of Meteorology).

Sampling period

Previous year samplings suggested that the flight period of these species could range between late May and mid September (J. M. Riba, personal information). Traps were placed in the field on May 19, 2008 and glue was applied to them on June 15, 2008. The first trap inspection was done on June 24, 2008 and continued at weekly intervals until there were no captures on 2 consecutive weeks, in September 9, 2008.

Preparation for field trip

Before going to field a fieldtrip plan for every week was prepared. The following material was taken to the field:

- 60 plastic cups (25 ml) coated with glue with paper tag with pencil written date of collection and trap number

- Hard and soft forceps to collect insect from the traps
- Rope and cutter to fix traps
- Rags and mineral oil for cleaning glue
- Latex gloves
- Pen marker
- Plastic bags
- Table with list of trap number for data recording
- Digital camera

The trip from Lleida to Arbucies was made by bus (Figure 6). Two buses were taken, from Arbucies to Girona, and again from Girona to Arbucies. Once in Arbucies, the forest owner (Joan Garolera) or his workers took me and my bicycle to the field, some 7 km up in the forest. The return to Arbucies from the forest was by bicycle, and the return from Arbucies to Lleida was by taking the two buses as before. Due to the bus schedule and the time required for sampling all the traps the field trip required spending a night in Arbucies. There were two possible bus combinations: leaving Lleida at 6:15 AM and arriving Arbucies at 12:15 PM, and leaving Lleida at 2:30 PM and arriving Arbucies at 8:30 PM. It was found that the second combination was better. The return was on the following day at 3:20 PM and arrival Lleida at 10:45 PM.

Sample collection

All traps were inspected visually for the presence of

Coraebus undatus

Coraebus florentinus

Crysobotris spp.

Other buprestids

Other beetles of a minimum size (larger than 2-4 mm)

Other insects those were abundant

Coraebus beetles were always collected from all the traps. *Chrysobothris* spp. was always recorded in the data sheet. For the rest of the insects a set of 10 traps was chosen at random in which every beetle larger than 2-4 mm was collected and placed in the glue-

coated plastic cups. This set of traps varied from the first to the second and from the second to the third week, but it was maintained constant from 8th July. From week 3, all types of insects were collected from traps A8, A9, A11, A13, A16, A18, A22, A23, A24, and A25 (Table 2). Later in the sampling period *Zygaena* sp. (butterflies) captured were also recorded in the data sheet.

Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
24-Jun								x	x	x	x		x			x	x					x	x		x					
1-Jul								x	x		x		x			x	x						x	x	x					
8-Jul								x	x		x		x			x		x				x	x	x	x					

Table 2. Traps from which all insects were collected. The same 10 traps were used after week 3 (July 8).

Cleaning the traps

Each and every week while observing the traps for any capture dirty materials such as leaves, pollen, branches of trees and other undesirable insects and butterflies glued on the traps were cleaned. For this purpose gloves, forceps and towels were used so that the glue used in the traps was not in contact with hands.

Removing the glue from the insects

For proper identification process the glue had to be removed from the insects. Once in the laboratory the glue is removed from the insects using the following procedure:

1. Using a pencil write down the treatment number and date in a small piece of paper
2. Put clean xylene in a vial, introduce the label, and close up the vial
3. Put a small quantity of xylene in the plastic cup where the samples are
4. No more than 2 minutes later, transfer the insects using hard forceps to a small beaker containing a small quantity of xylene
5. Using soft forceps transfer the insects to the xylene vial with the appropriate label
6. Insects remained in the xylene vials for some time (hours, days). Afterwards they were poured into a small beaker with the help of a needle
7. From the beaker, the insects were then transferred to Petri dishes covered with filter paper with the help of fine forceps. Special attention was taken while transferring the insects so that they were undamaged
8. Petri dishes were tagged using a pencil writing down the treatment number and date in a small piece of paper.

9. All the Petri dishes were left in the fume hood for couple of days for better drying.

All these steps were done inside the fume hood not to be contaminated with xylene vapour.

Species and sex identification

After proper drying, primary identification of the insects was done in the laboratory by observing them in the microscope and taking help from books. Types, size, shape, colour, length were observed carefully. *Coreabus undatus* and *Chrysobothris* sp. were easily identified by talking help from book and from other literature available in the laboratory. For other insects and Buprestidae, samples were sent to expert for identification. After taxonomic study, all insect samples were preserved in Eppendorf tubes with date and treatment number and kept in a paper box for further reference and use.

The sex of *C. undatus* was determined by extracting the genitalia before they dried out completely. Males have a very large aedeagus which is very difficult to miss. The absence of an aedeagus indicated that the specimen was a female. The sex of *Chrysobothris* sp. was determined by visual inspection of the last abdominal tergite which is sexually dimorphic. Males have indentation that females lack.

B. Chromatic trap experiment

Traps

Two types of plastic traps were used for the purple trap experiment. One is a piece of hollowed opaque purple plastic, and the other a piece of completely transparent PMMA (poly methyl methacrilate) plastic (control trap). Both were cut in 30 cm x 30 cm pieces of a 3 mm thick material. A 5 mm diameter hole was cut in each corner of the trap with the help of an electric drill or a cork borer for the clear plastic and purple traps, respectively. The holes of the purple trap were reinforced with metal eyelets. The purple trap material was purchased in the USA (because they do not sell this color in our area), and the PMM was bought and cut in a hardware store in Lleida, Spain.

Setting traps in the field

30 pairs of a purple and a clear plastic traps were randomly placed in the filed at 50 m or more between each pair. After finding a suitable location, a nylon rope was tied to one tree and passed through two holes of a purple and a control trap and tied at the same height on a second tree. A second rope threaded the other holes of the traps and was tied to

the two trees so that the traps ended in a vertical orientation one beside the other (Figure 7). The height and distance between the two traps of each pair is shown in Table 3.

Figure 7. Placement of clear plastic (left) and purple (right) trap pairs in the field.

Pair	Distance between purple and control traps	Distance of lower rope from ground	Pair	Distance between purple and control traps	Distance of lower rope from ground
A1	110	180	A16	41	173
A2	77	190	A17	52	180
A3	77	185	A18	84	165
A4	97	195	A19	76	195
A5	93	200	A20	61	150
A6	65	197	A21	98	175
A7	97	195	A22	90	170
A8	90	75	A23	97	185
A9	119	165	A24	56	200
A10	124	190	A25	60	145
A11	89	210	A26	112	180
A12	88	175	A27	122	190
A13	68	180	A28	62	172
A14	76	145	A29	58	173
A15	55	180	A30	45	178
			Mean	84.23	129.93

Table 3. The height and distance between the traps (cm)

Application of Glue on the traps

After setting the traps in the field, purple and control traps were coated on both sides with an even layer of tangle trap glue, which is manufactured by Tanglefoot Co. (Michigan, www.tanglefoot.com) and distributed in Spain by Biagro S. L. (Valencia). The glue was applied with the help of a metal spreader. Figure 8 below shows the application of glue on control and purple traps.

Figure 8. Application process of glue on control (right) and purple (left) traps.

Most of the purple and control traps were re-glued for better functioning and capturing of insects (Figure 9). Tags were attached to the stem of the tree in such a manner that they were easily visible from a far distance (Figure 7).

Figure 9. Appearance of the purple traps after first glue (left) and after re-gluing (right).

For convenience traps were placed along the forest road or following a small creek. Traps of different experiments were placed on separated areas (Figure 10).

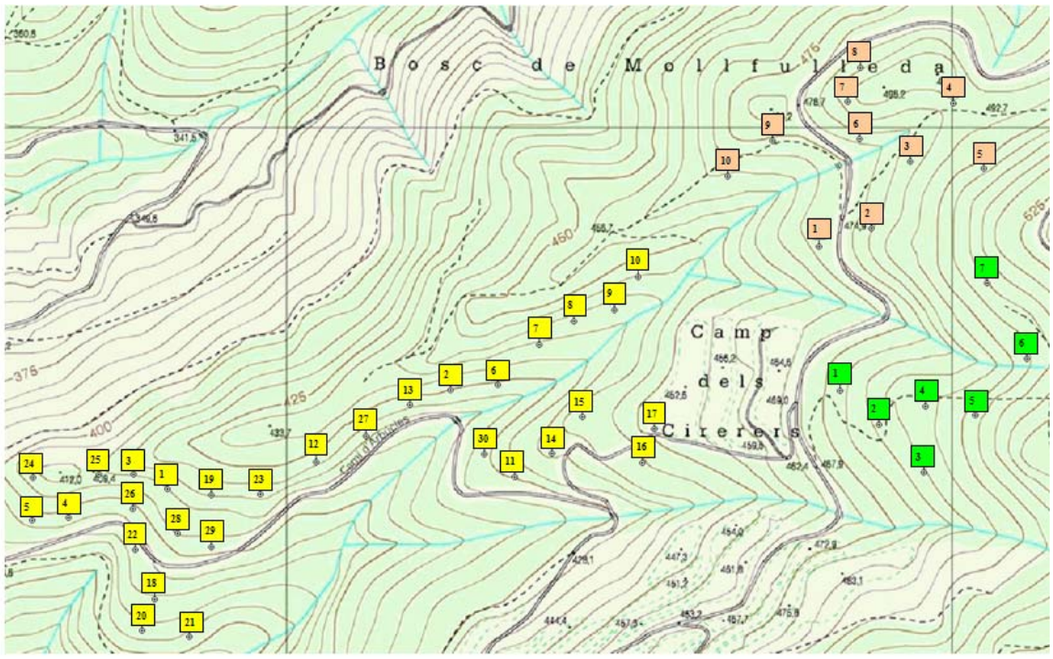


Figure 10. Location of the traps position of both chromatic (yellow) and dummy insect (green: *C. undatus*, pink: *C. florentinus*) experiments.

C. Dummy insect experiment

Adult attraction could be mediated by visual signals coming from other individuals. Two type insect species such as *Coreabus undatus* and *C. florentinus* were used as dummy insect for capturing other live *Coreabus* sp. in the forest area. The experiment done with *C. undatus* was termed as experiment B and in the same way for *C. florentinus* was treated as experiment C. Traps were loaded with a male, a female or nothing (control traps)

Setting of the traps

The traps were PMMA sheets, 20 cm x 20 cm and 3 mm thick with two holes on opposite sides (Figure 11). There were 7 replications for the experiment B and 10 for experiment C. After preparing the trap materials and sampling design, all 51 traps were put in the field site area. Several different suitable locations were selected for putting the traps, but they were put in a randomize manner. Three different treatments, male, female and control, were put at about 5 m from each other in each replication.

Figure 11. Setting process of the traps for dummy insect experiment (left) and field orientation after setting (right).

All the traps were placed on the top of shrubs or small trees of *Q. suber* or *A. unedo*. The distance between each treatment was about 5 meters. On the other hand, the distance between each replication was about 50 meters. The height of the position where traps were set horizontally was about 1.5 meters from the ground. After putting the traps on the top of the shrub, they were tied strongly by using plastic fasteners and placed horizontal to the land surface as much as possible. The tags were attached to the stem of the tree in such a manner that they were easily visible from a far distance (Figure 11). Later the position of the traps was recorded in the GPS system also (Figure 10).

Gluing and setting *Coreabus* spp. on the traps

After putting the traps on the top of the shrub, glue was applied only on the upper surface area. Special precaution was taken while applying glue to avoid contact with clothes, hands and body. Gloves were used all the times while putting glue and dummy insect samples on the traps. Glue was applied on the traps in such a manner that it produced a flat thin layer and even distribution of glue on the surface. Insects were put at the centre point of each trap in a gentle manner by using fine forceps. For experiment B 14 *C. undatus* (7 of each sex) were put on the centre of the traps, and for experiment C 20 *C. florentinus* (10 of each sex) were set on the traps. In all cases, control traps were left blank. At the same time, tags marked with M, F and C were put close to the each trap in order to indicate the sex of the put insects. The insects used in this experiment have different

procedences but all of them were rinsed for at least 24 h in methylene chloride to remove any volatile stimuli.

After two weeks it was found that there was no capture of any *Coreabus* spp. in the traps and that the colour of the *Coreabus undatus* and *C. florentinus* had become darker.

Figure 12. New *Coraebus undatus* (a) and *C. florentinus* (b) put first time on the traps and altered colour of the newly put *C.undatus* (c) and *C.florentinus* (d) on the traps.

We suspected that contact with the glue was the cause of this change in coloration, so we devised a new strategy to put the insect on the traps. Insects glued to a plastic platform, of green colour to resemble the colour of the environment, and the platform was placed on the glue of the traps (Figure 13).

Figure 13. Setting of green plastic base by using super glue (left) and new *Coraebus* spp. placed (right).

2. Larvae rearing

A. Larvae collection

The larvae were collected at three different locations in Spain: Cadiz, Castellón and Girona (Figure 14) from cork oak forests during the commercial cork extraction period.

Figure 14. Three different locations in Spain from where the larvae of *C. undatus* were collected.

Larvae were put in Petridishes filled with humidified vermiculite and placed in a cooler (Figure 15). They were shipped by currier and kept in the refrigerator until put in diet.

Figure 15. Supplied insect larvae in petridishes filled with vermiculite.

B. Larva measurement and handling

Once in the laboratory the width of the head capsule and the body length of each larva were measured with a digital calliper (Figure 21). Each larva was assigned a different code number. Larvae were handled with soft forceps and with the hands wearing latex gloves (Figure 16). To put larvae in the cups, the diet was removed from it and the larva was placed at the bottom, and then it was covered with the diet. The cup was labelled and holes were made on the lid using a 1 mm diameter pin (Figure 21d).

Figure 16. Measurement of larvae head width (left) and body length (right).

At the beginning, 5-10 holes were made but latter one hole was made per cup. The cups were maintained inside cardboard boxes at 25 ± 2°C in obscurity to mimic the natural conditions.

C. Diet

Eight different diets were made using several ingredients (Table 4). As nutritional ingredients the diet had cortex and immature cork. Nutritional additives were fructose and vitamins. As food preservatives we used methyl-paraben, and in one occasion sorbic acid. Water was provided in the agar.

Preparation of tree tissues

The larva makes its gallery on the internal side of the external bark, the **immature cork**, a yellowish, rubbery tissue adhered to the cork when this is taken out of the tree, and the external side of the internal bark, the **cortex**, a woody, hard and fibrous tissue that forms the outer layer of the tree when the bark is removed. The immature cork is strongly adhered to the mature cork and to remove it from cork a sharpened chisel or some other tool needs to be used (Figure 17 and 18). One efficient way of using the chisel is to orient it at 45-90° from the cork and motion it towards the worker while at the same time pressing down on the cork. A very useful tool was found in a kitchen tool used to make butter curls (Figure 17a). To remove the cortex the chisel can be used in the manner that it is intended to for carpentry (Figure 17b). To maintain the sharpness of the chisel, it was rubbed with a sharpener as needed.

Figure 17. Removal of the immature cork layer with chisels (b+c) and butter kitchen tool (a).

Figure 18. Removal of cortex with chisel.

The tissues are grinded separately (Figure 19) and put in an oven at 60°C until completely dry out (1-2 days) and stored in the refrigerator or in a dry place. Some diets included cork tree acorns to supplement or even replace the natural nutrients. The acorns were collected in Extremadura. They were peeled, and the meat was grinded and kept in the freezer until used.

Figure 19. Cortex (a) and young cork (c+b) before and after grinding (d).

Insect diet preparation

Initially we boiled the tree tissues and then mixed them with the agar and the rest of the diet ingredients. Later we realized that the water in these tissues did not mix properly with the agar and run away from the diet flooding the bottom the cups. We changed the

procedure and added the dry diet ingredients directly to the boiling agar, and then added the rest of the ingredients like vitamins and methyl-paraben except the sucrose, which was put directly in the agar water (Figure 20).

Figure 20. Insect diet preparation process

[a: Boiled agar media in the pot; b: pouring of methyl-paraben mixed with alcohol in the agar media; c: Pouring of agar media in the tree tissues; d: stirring of diet materials with a stir; e: Transferring of cold diet in the taper; f: Closing of taper lid with the aim to preserve them in the refrigerator].

Methyl-paraben and vitamins were added at the end when the diet was about 60°C. Methyl-paraben was diluted in 20% ethanol. In the first diets we used a ratio of 1:1 for dry cortex and cork, respectively. However, assuming that the insect eats the same amount of cortex and immature cork from the tree, given that cork is about 70% water, and cortex is about 30% water (unpublished results), we should put a ratio of about 2:1 of dry cortex and cork, respectively, in the diet in order to provide the same amount of natural ingredients in the diet as they eat in nature. This procedure was maintained for final diet. In the FINAL diet (diet #9) we doubled the concentration of methyl-paraben and more than doubled the quantity of water (Table 4). Special care was taken to stir the diet materials so that other materials in the diet like vitamins, sucrose, methyl-paraben mix properly with tree tissue to avoid fungal infection and other unexpected incident. In this manner the diet was protected from fungi and had a sticky consistency which helped to maintain it at the bottom of cups.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	FINAL
Cortex	18	18	-	-	-	-	100	100	100
Cork	36	36	50	50	-	-	100	100	100
Acorn	145	145	150	150	200	200	-	-	-
Fructose	10	10	10	10	10	10	10	10	10
Vitamins	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben	-	3	-	3	-	3	-	3	6
Agar	27	20	20	20	20	20	20	20	20
Water	400	400	400	400	400	400	400	400	714

Table 4. Ingredients of diet used in the study (gms). Final is the diet chosen and used at the end of the study.

Maintenance of insects

Cups were filled with diet to the middle. The diet was pressed down repeatedly with the fingers so that it was compacted. Then the diet was taken out from the cup and the insect was placed at the bottom of the cup. After that the diet was placed back in the cup on

the same position as it was. The diet was pressed **gently** over the insect. Special care was taken with insects that were molting. At the beginning the insects in the no-fungicide diets (1, 3, 5, and 7) were changed to new diet cups every other day because of fungal problems. With the FINAL diet insects had to be changed only once per week.

Figure 21. Handling process of larvae in the diet cup

[a. Putting larva in the diet cup with a soft forceps; b. Larva placed at the bottom; c. Placing insect diet softly on the top of the larva with a forceps; d. Making of a hole by using a pin].

RESULTS

1. Field traps

A. Chromatic traps

Species

Both purple and clear sticky traps collected several insect taxa (Figure 22). A total of 1,606 insects were collected from the traps and taken to the laboratory for processing and identification. Of these, 282 (18%) belonged to the family buprestidae, of which there were at least 4 species. The most abundant was *Agrilus* sp. (125, 44%), followed by *Chrysobotris affinis* (83, 29%), *Coraebus undatus* (37, 13%) and *Anthaxia* sp. (37, 13%). Specially abundant were the Elateridae (637, 40%) composed mainly of 1 unidentified species, and the flower beetles (544, 34%, Prionoceridae + Oedemeridae + Mordellidae), that together made almost 75% of the captures. Some of the other beetle species captured in the traps were the cerambycids *Chlorophorus pilosus* and *Steneurela* sp., several Coccinellidae species, and many small bark beetles such as *Platypus cylindrus* (Platypodidae) which, for being small, were not counted.

Taxa	Order	Family	Total captures contro trap	Total captures purple trap	df	F	P
Coraebus undatus	Coleoptera	Buprestidae	2	35	1	21.9	**< 0.01**
Chrysobotris afinis	Coleoptera	Buprestidae	42	41	1	0.01	0.93
Agrilus sp.	Coleoptera	Buprestidae	104	21	1	16.85	**< 0.01**
Anthaxia sp.	Coleoptera	Buprestidae	21	16	1	0.31	0.58
Lobonyx aeneus	Coleoptera	Prionoceridae	95	144	1	0.84	0.36
Oedemera sp.	Coleoptera	Oedemeridae	163	52	1	5.62	**0.02**
??	Coleoptera	Mordelidae	67	23	1	10.64	**< 0.01**
??	Coleoptera	Elateridae	248	391	1	1.04	0.31
several	Coleoptera	Curculionidae, cerambycidae, coccinellidae..	61	43			
Zygaena sp.	Lepidoptera	Zygaenidae	8	29	1	10.47	**< 0.01**

Table 5. Total number of individuals of the different insect groups captured in purple and control traps and ANOVA results for the comparison among the two trap types. The green coloured cells indicate when a treatment captured more individuals within an insect group.

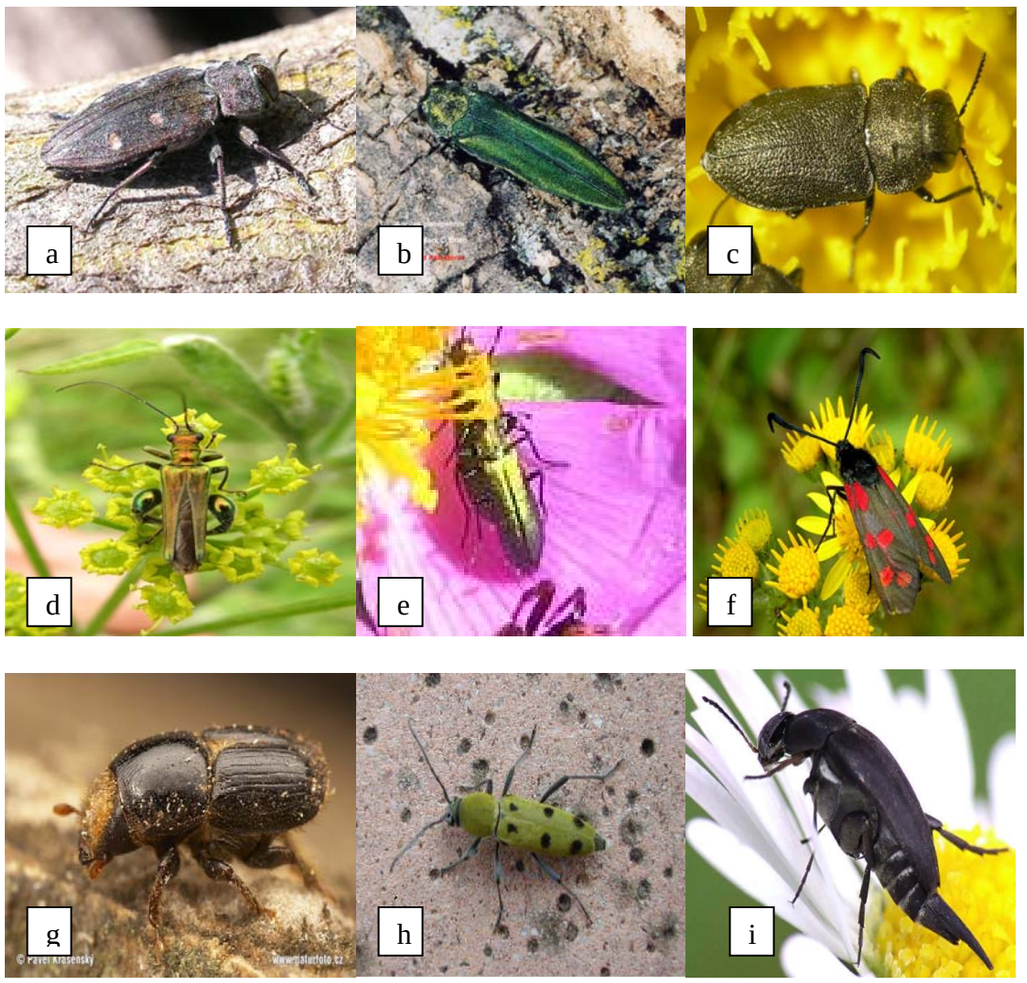
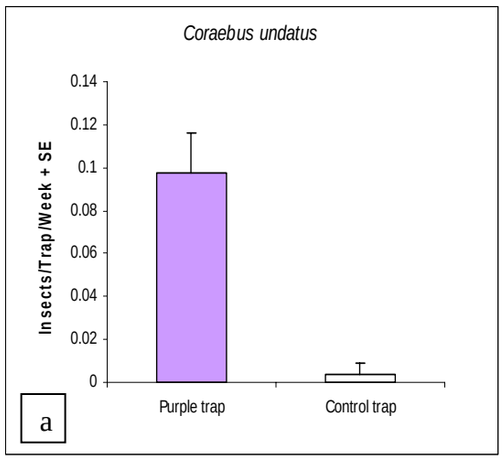
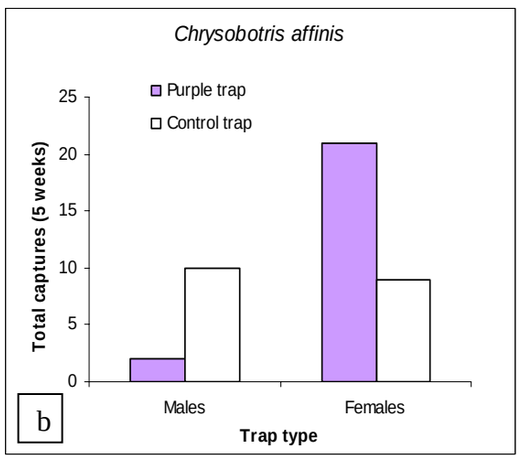


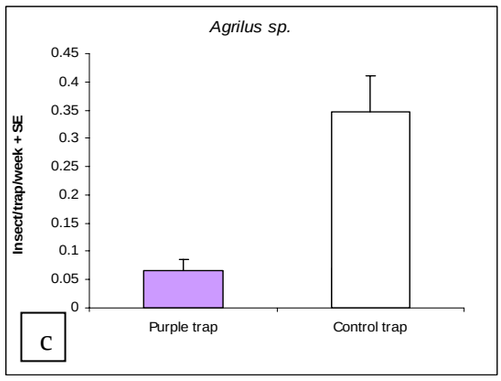
Figure 22. Insect samples collected during the study period. a. *Chrysobothris affinis*; b. *Agrilus* sp.; c. *Anthaxia* sp.; d. *Oedemera* sp.; e. *Lobonyx aeneus*; f. *Zygaena* sp.; g. *Platypus cylindrus*; h. *Chlorophorus pilosus*; i. Mondelidae.

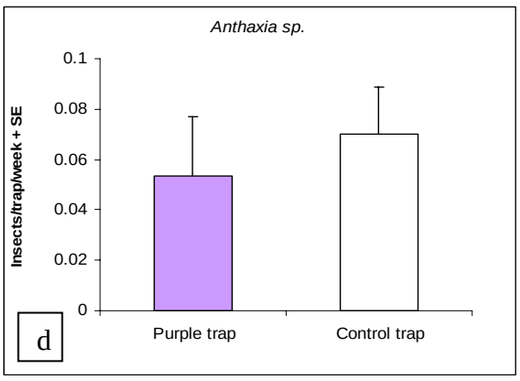
Traps

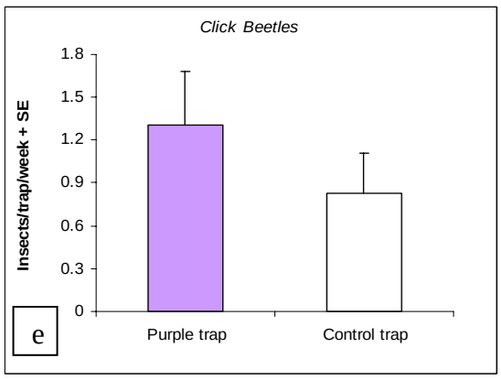
Some taxa were more abundant in the purple traps (*Coraebus undatus*, *Zygaena* sp.) (Figure 23a and Figure 23j), other were more abundant in the control traps (*Agrilus* sp., *Oedemera* spp., and Mondelidae) (Figure 23c, Figure 23h and Figure 23i), while others were attracted in equally to both traps (*Chrysobothris affinis*, *Anthaxia* sp., *Lobonyx aeneus*, Elateridae) (Figure 23b, Figure 23d and Figure 23f and Figure 23e). All the *C. undatus* collected were females. *Chrysobothris affinis* females preferred purple over control, whereas males did not have a preference (Figure 23b).

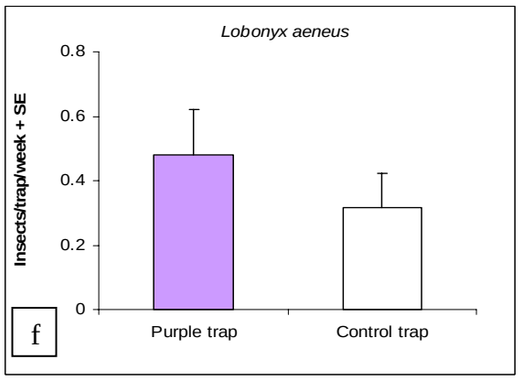

Coraebus undatus
Insects/Trap/Week + SE
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Purple trap
Control trap
a


Chrysobotris affinis
Purple trap
Control trap
Total captures (5 weeks)
25
20
15
10
5
0
Males
Females
Trap type
b


Agrilus sp.
Insect/trap/week + SE
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Purple trap
Control trap
c


Anthaxia sp.
Insects/trap/week + SE
0.1
0.08
0.06
0.04
0.02
0
Purple trap
Control trap
d


Click Beetles
Insects/trap/week + SE
1.8
1.5
1.2
0.9
0.6
0.3
0
Purple trap
Control trap
e


Lobonyx aeneus
Insects/trap/week + SE
0.8
0.6
0.4
0.2
0
Purple trap
Control trap
f

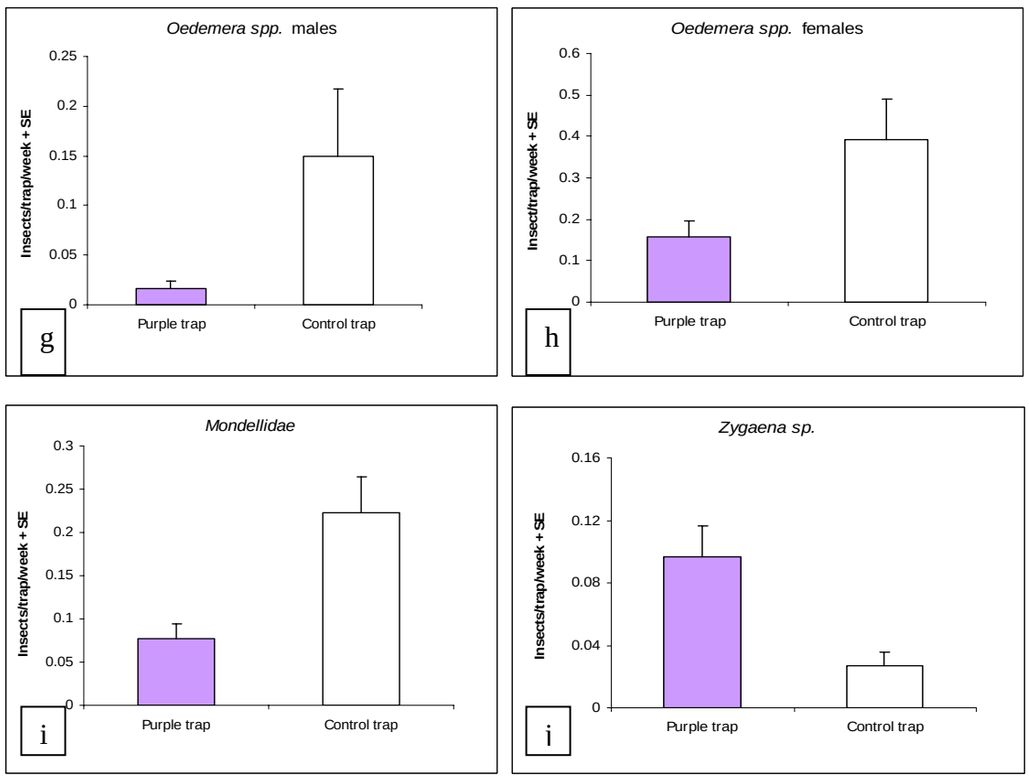


Figure 23. Capture of insect taxa in purple and control traps

[a. *Coraebus undatus*; b. *Chrysobothris affinis*; c. *Agrilus* sp.; d. *Anthaxia* sp.; e. Click beetles; f. *Lobonix aeneus*; g. male *Oedemera* spp.; h. female *Oedemera* spp.; i. Mondelidae; j. *Zygaena* sp].

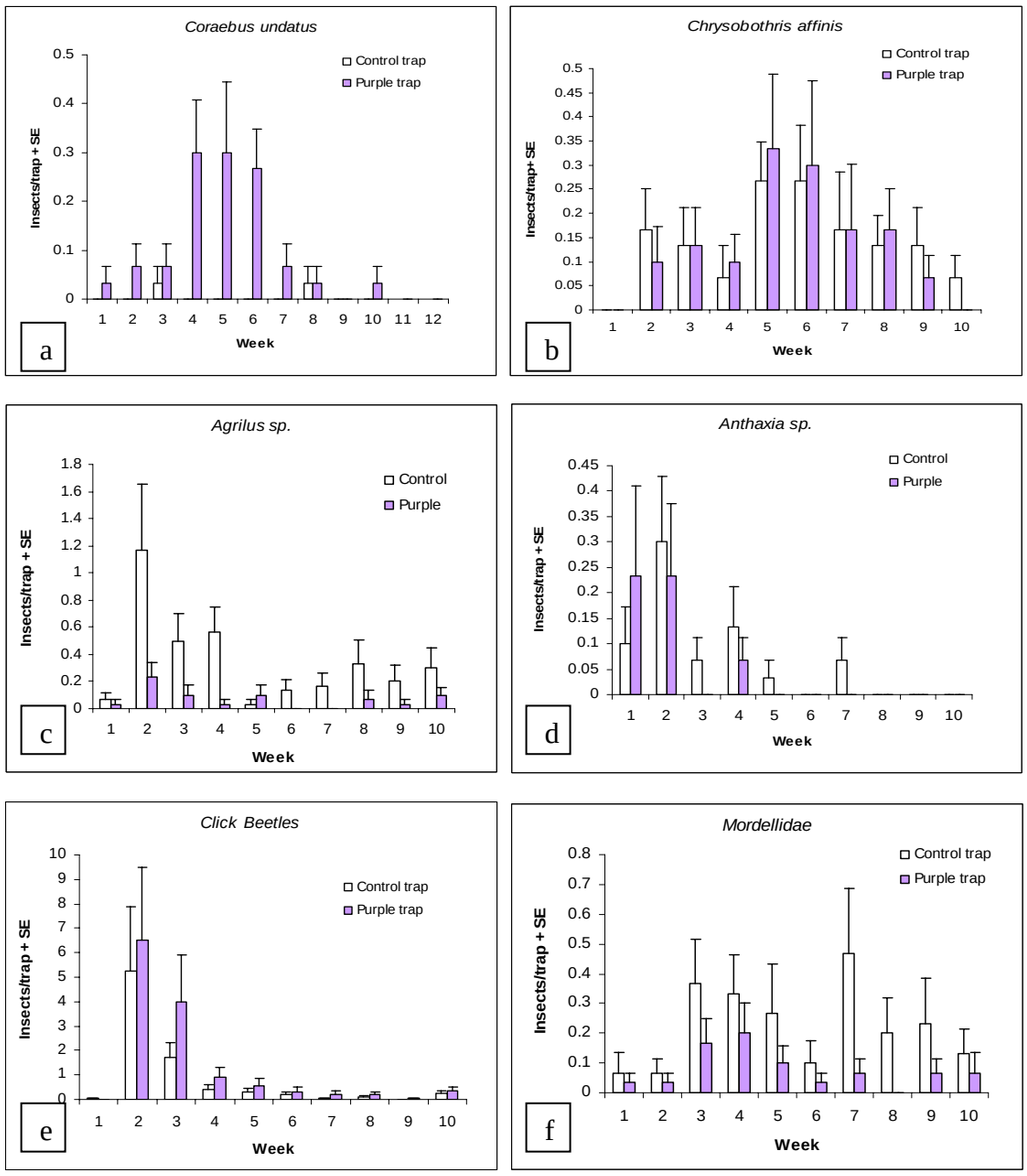
Dynamics

Week 1	2	3	4	5	6	7	8	9	10	11	12
June 24	July 2	July 9	July 16	July 23	July 30	Aug 5	Aug 12	Aug 19	Aug 26	Sept 2	Sept 9

Table 6. Weekly travels to field keep up a correspondence to respective date.

All the species presented population peaks during the sampling period. *Coraebus undatus* was collected from the first sampling week (1 individual, June 15-24) and every week thereafter until week 10 (August 27). A peak was observed between weeks 4 to 6 (July 10 - July31) (Figure 24a). The captures of *C. undatus* varied depending on the location of the traps in the field (Figure 25). *Chrysobothris affinis* also presented a peak around weeks 5 and 6 (Figure 24b), and the other two buprestids, *Agrilus* sp. (Figure 24c) and *Anthaxia* sp. (Figure 24d) peaked at the beginning of the sampling season. Click

beetles were very abundant in weeks 2 and 3, but afterwards their numbers declined considerably (Figure 24e). *Oedemera* spp. and *Lobonix aeneus* were also abundant at the beginning of the sampling period (Figure 24g, Figure 24h and Figure 24i), whereas the Mondelidae were relatively stable throughout (Figure 24f). The *Zygaena* sp. butterflies appeared in week 6 and peaked afterwards (24j).



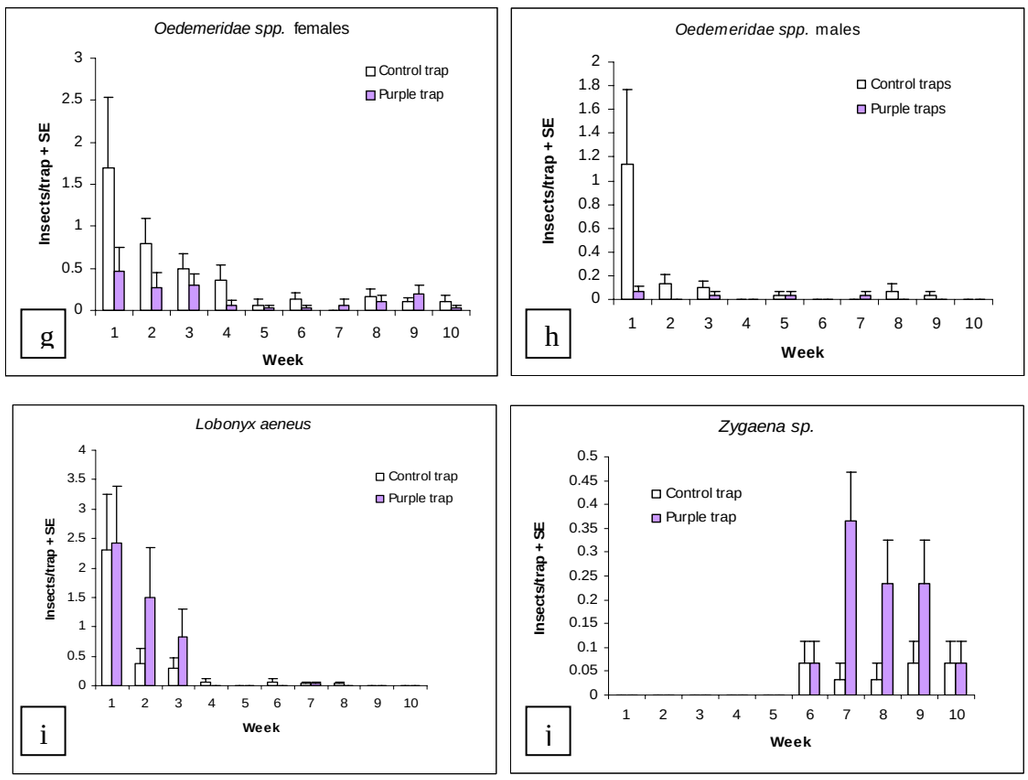


Figure 24. Number of capture/trap of different insect taxa from week1 to week 10 [a. *Coraebus undatus*; b. *Chrysobothris affinis*; c. *Agrilus* sp.; d. *Anthaxia* sp.; e. Click beetles; f. Mondelidae; g. female *Oedemera* spp.; h. male *Oedemera* spp.; i. *Lobonix aeneus*; j. *Zygaena* sp.].

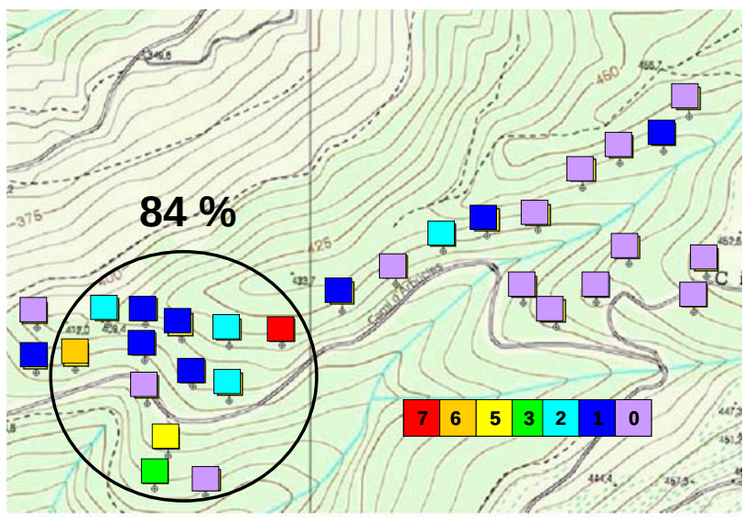


Figure 25. Distribution of captures of *C. undatus* in the field. About 50% of the traps in a particular area captured 84% of the insects

B. Dummy insect traps

Problem in the field

The main aim of the study related with dummy insect traps was to attract other *Coraebus* spp. by visual stimulation. On the basis of the purpose we put *Coraebus undatus* and *C. florentinus* as the dummy insect samples on the traps. There were both male and female on traps besides the control ones. After putting the insect samples in the trap with glue, we found that the colour of the both species converted into black within two weeks (Figure 12c and 12d, in materials and methods). This might be because of the corrosive action of glue. No capture of *Coraebus* spp. was found in the traps as well. So finally we decided to replace the old insects with the new ones.

Problem in the eppendrof

As the colour of the dummy insect samples converted to black colour on glue in the field, we planned to put new insect samples on a green plastic base to avoid direct contact with glue. Accordingly, 51 green plastic bases were prepared in the laboratory together with 51 eppendrof. All insect samples were attached to the plastic base by using super glue. So we put the insects on plastic base and kept them in the eppendrof for half an hour. After half an hour we found the colour of the insect also changed to a greyish. In order to identify the causes of conversion of natural colour of insects, we did another test in the laboratory. This test was done with *C. florentinus* only. To find out the reason of colour conversion, we took three fresh looking insects and treated as control, glued one, and last one is glued plus put inside the eppendrof. After half an hour, we did not find any change to control set, a bit change with glued one and completely black which was put inside the eppendrof with glued position (Figure 26).

Figure 26. Colour alternation of the *C. undatus* into black because of the effect of glue and eppendrof.

Because of this incident we did not use the insect samples and had to use other new set of insect samples instead of the black ones. However for *C. undatus* no more males were available and we could use only females and control treatments. We keep on constant observation every week for any capture of *Coraebus* spp. starting from June 24th 2008 to August 26th 2008, but we could not have any capture during our whole study. First we assumed that this might happened because of change of colour of the insect samples, but we got the same result with a right-colour insect batch. Finally we decided not to keep on weekly observation of the traps and left experiment in the field.

2. Larva rearing

Larva samples

The majority of the larvae arrived from Cádiz, and in lower numbers from Girona and Castellón (Table 7 and 8). Between 63% and 72% of the larvae arrived alive. The others died during collection and shipment.

Location	Dates	Number of larvae	Number alive	% alive
Cádiz	June 12-August 6	204	148	72.55
Castellón	July 24-August 8	14	9	64.25
Girona	July 21-July28	33	21	63.63

Table 7. Percentage of living larvae and total number of larvae collected from Cadiz, Castellon and Girona.

Location	Collected	Num. Larvae
Cádiz	12-Jun	3
Cádiz	16-Jun	6
Cádiz	17-Jun	9
Cádiz	23-Jun	4
Cádiz	24-Jun	1
Cádiz	25-Jun	3
Cádiz	30-Jun	15
Cádiz	1-Jul	7
Cádiz	3-Jul	7
Cádiz	7-Jul	14
Cádiz	8-Jul	3
Cádiz	9-Jul	4
Cádiz	10-Jul	7
Cádiz	15-Jul	4
Cádiz	16-Jul	7
Cádiz	17-Jul	1
Cádiz	18-Jul	13
Cádiz	21-Jul	12
Cádiz	22-Jul	8
Cádiz	23-Jul	15
Cádiz	31-Jul	21
Cádiz	4-Aug	15
Cádiz	5-Aug	19
Cádiz	6-Aug	6
Girona	21-Jul	5
Girona	23-Jul	14
Girona	24-Jul	4
Girona	28-Jul	10
Castellón	24-Jul	5
Castellón	8-Aug	9

Table 8. Number of larvae collected on the basis of dates from three different locations.

Larval size distribution

Larvae arriving from the field were in different developmental stages. In the sample of larvae from Cádiz it can be observed that the smallest larvae (head width < 2 mm) appeared mostly on days 10-35. Also an increase in head width can be observed from day 10 to day 40.

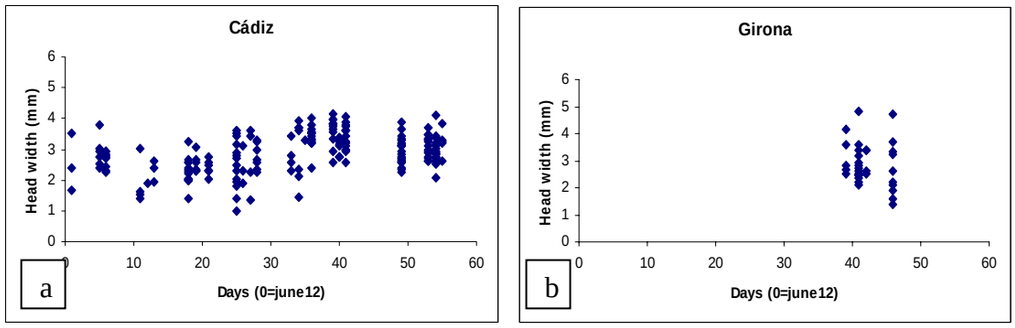


Figure 27. Distribution of head width of larvae collected from Cadiz (a) and Girona (b)

There was a good correspondence between head width and body length, so this other morphological character, although more variable, can be used to estimate the less variable head width (Figure 27e and Figure 27f).

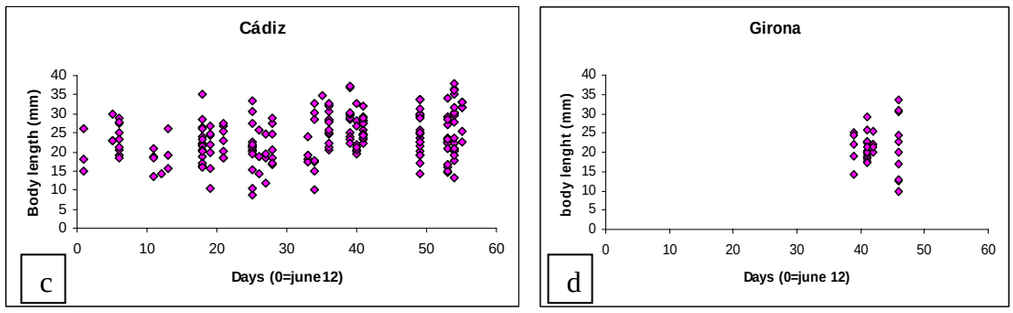


Figure 27. Distribution of body length of larvae collected from Cadiz (c) and Girona (d)

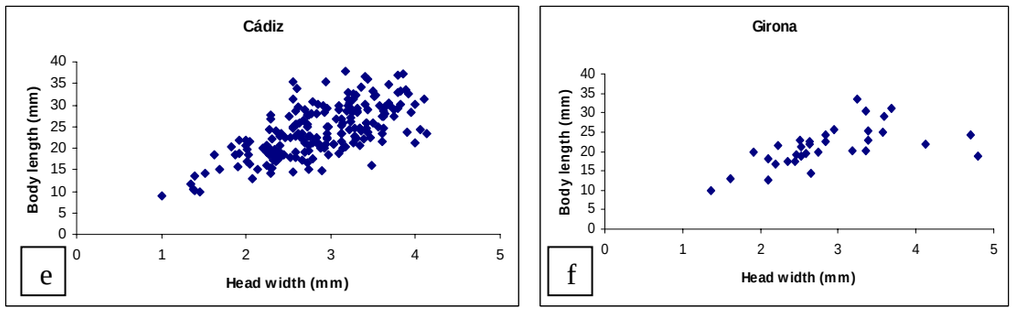


Figure 27. Correlation between head width and body length of larvae collected from Cadiz (e) and Girona (f).

Larva survival in diets

A total of 178 larvae were put in diets. Of these 120 were used to compare diets 1 to 8 and the rest were put in diet #9. About 70% of the larvae have died at the time of writing this thesis. Roughly 45 % have died to fungal growth in the diet, 45 % to natural causes and 10% to sorbic acid, tested as an additional diet preservative.

All the diets without fungicide (#1, 3, 5, and 7) developed fungus very quickly (Figure 28) so the insects in these diets had to be changed every 2 days. At the beginning the fungus stayed in a small area of the diet, but after successive transfers of insects with fungal infection the cups became completely covered with fungus in a short time and the insects had to be thrown away.

Figure 28. Diet cup showing the formation of fungal spores.

We lost a significant number of insects and diet to the fungus in the diets without fungicide.

		In the original diet			After transfer to diet #9*	
Diet	N	Dead to fungus	Natural death	N	Dead to fungus	Natural death
1	15	9	2	4	1	2
2	15	0	3	12	6	5
3	15	4	2	9	2	4
4	15	0	7	8	2	1
5	15	5	7	3	1	0
6	15	0	4	11	3	3
7	15	1	4	10	0	3
8	15	2	6	7	5	2
9		-	-	58	11	3
* Excluding those dying to sorbic acid						

Table 9. Death and survival number of larvae in original diets and after transferring to diet 9.

Overall, there were not appreciable differences in larval performance among the 8 diet types. If anything, the diets with acorn (1 - 6) seemed to perform worst than the diets without it (2 - 8), but with the low number of replications and the fungal infections no statistics were performed. After 5 weeks (August 1) the experiment comparing diets was ended (N = 15) and the surviving insects were transferred to the new final diet #9, which was like diet 8 but slightly modified. In its final version this diet contains twice the amount of methyl-paraben and more than double the quantity of water than diet 8, but otherwise it is identical to it.

One of the batches of diet 9 used in august 14 resulted in a lot of fungal growth and mortality on the check of August 25. Because of the scarcity of natural ingredient to make new diet at this time, we decided to rescue this batch of diet. To do this we added extra methyl paraben, so that the initial dosage was doubled, and jut to one half of the diet, another common insect diet preservative, sorbic acid. The larvae were changed to these new diets and the fungal problem did not reappear. We believe that the problem came from having used an outdated surplus of methyl-paraben when this batch of diet was made. However the experience produced an unexpected result: the insects in the sorbic acid diet

got sick and many of them died. The insects in the sorbic acid diet were rescued by changing them back to the diet without sorbic acid on September 9, but the effects of sorbic acid remained and the insects continued dying afterwards.

On September 19, 3 and a half weeks later, of the 23 insects that had been put in the diet without sorbic acid only 4 had died (83% alive), whereas of the 25 insects put in the diet with sorbic acid 11 had died (56% alive). The insects in sorbic acid diet developed some or all of the following symptoms: accumulation of fat body (whitening of body), immobility, and deformities of head capsule during molting (Figure 29)

Figure 29. A healthy insect larva (left) and mal insect larva due to effect of sorbic acid (right).

Interestingly, these same symptoms have been observed in the past when this insect has been reared in previous diets also containing sorbic acid (Cesar Gemeno, personal communication (Figure 30).

Figure 30. Sick larvae from rearing tests in 2007. They resemble the symptoms caused by sorbic acid in this study.

A descriptive analysis of the data shows that overall mortality due to fungus on the no-fungicide diets was higher than in the diets with fungicide. When the surviving insects were transferred to diet #9 the percentage dying to fungus or to natural causes was more similar between these two groups of insects than before (Table 9), however a somewhat larger percentage of insects that had been in the fungicide diets died to fungal causes than those that came from a non-fungicide diet. A similar trend was observed for the larvae that had been put straight in diet #9 from the field. We suspected that this higher mortality to fungal growth could be an artefact of the diet #9 containing a defective batch of methyl-paraben. When the insects dead due to fungal growth in this defective diet were removed, the percentage of larvae reared in diet #9 that died to fungal growth or to natural causes became more similar among the different groups (those coming from non fungal diets, from fungal diets and those put directly on diet #9 from the field) (Table 10). On average, insects reared in diet #9

Before trasnferring to diet # 9		
Diet	% dead to fungus	% dead to natural causes
No fungicide	53	47
Fungicide	6	94
After transferring to diet # 9		
Diet	% dead to fungus	% dead to natural causes
No fungicide	31	69
Fungicide	59	41
Diet # 9 from field	74	26
Correcting for sorbic acid mortality		
Diet	% dead to fungus	% dead to natural causes
No fungicide	31	69
Fungicide	27	73
Diet # 9 from field	33	67

Table 10. Comparison of larval death before and after transfer to diet 9 and mortality rate related with sorbic acid.

Molting

When ready to molt the larvae stopped moving and eating and their physical aspect changed. The body length shortened, segments became closer to each other, the colour of the body became duller and the head capsule elongated (Figure 31).

Figure 31. Molting larva: whitish coloration shortening of the abdomen, approximation of body segments and elongation of head capsule.

Some times it was difficult to determine when an insect was molting and when it was dying. One difference between dying and molting insects is that the first ones are flaccid and bend by gravity when picked up with the forceps. 29 individuals molted once, and 3 of these molted twice. All the insects that molted twice are alive at the time of writing this thesis. 10 of the larvae that molted just once are dead, 6 due to fungal growth and 9 to natural causes. Of the 29 insects that have molted, 25 were reared in diets 1 to 8 from the start, and were later transferred to diet #9, and 4 were put directly from the field in diet #9. The time between the insect was put in the diet and when it molted range from 35 to 70 days (mean = 45 days), with a slight trend for insects that were collected later in the season to molt earlier.

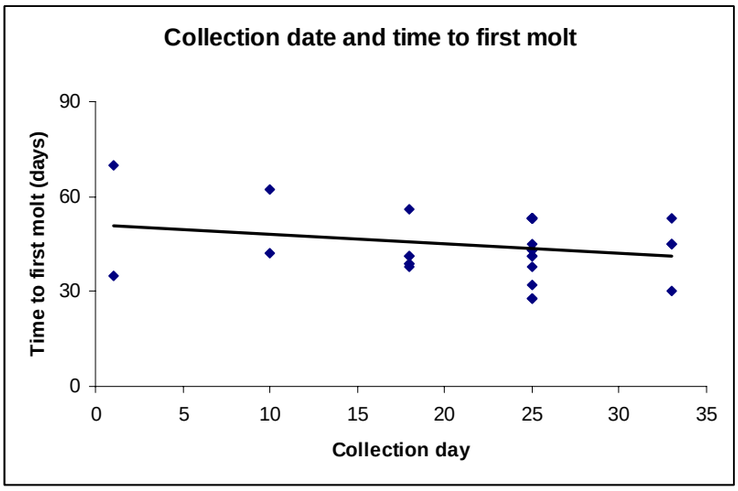


Figure 32. Relationship between collection day and number of days to molt

The number of days elapsed between an insect was detected as starting a molt and the end of the molt ranged from 0 to10, with an average of 5.35 d for the first molt and 7.66 for the second molt. Right after molting the old skin was next to the insect and the insect head was white and soft (Figure 33). Some hours (days) after molting the insect resumed its feeding and tunnelling activity.

Figure 33. A newly molted larva.

DISCUSSION

Coreabus undatus is considered the most important insect pest for cork oak in Spain. Considerable amount of loss has been done by this pest in Spain. From a study it has been found that the yearly neat loss due to the damage caused by this insect is about $5x10^6$ (http://www.mma.es/portal/secciones/biodiversidad/montes_politica_forestal/sanidad_forestal/pdf/guadalupe08_Iprocor.pdf). So it is important to control the pest causing a great economic damage. But not much work has been done on the control or sampling of *C. undatus* by using traps. This is the first attempt from Spain or most probably from the world to trap *C. undatus* by using chromatic traps. Few people took some initiative to trap *Coraebus* spp. in this way in Spain. Besides a few other researchers from different parts of the world have reported on capturing other insects like *Agrilus planipennis* on black, yellow, white, purple, red, green, navy blue traps (Francese *et al*., 2004). On the basis of the information our present study also consists with the study made by other researchers.

Our trapping experiment has demonstrated that the attraction of *C. undatus* to purple traps observed in 2007 is not the result of a random encounter of the beetles with obstacles, but a positive attraction to the purple colour, given that the majority of the insects (35/37) were captured in the purple traps and only 2 out of 37 were captured in the clear plastic traps. The number of insects captured is not very large, but for the history of this species this number is very large. Not only adults have been collected in significant numbers, but we provide the first direct estimation ever of the adult flight curve for this insect. The application of the purple traps is questionable due to the low number of insects collected. However a close inspection of the data shows that 84% of the captures were in 50% of the traps. These traps were located in sunny, well exposed areas, whereas a group of traps in a different area did not capture a single insect in the whole season. Trap position is a determinant factor in the collection of this insect.

Dummy insect experiment to trap insect samples also done for the first time in Spain. There are only a few reports on capturing insect taxa by using dummy insect traps creating visual stimulation. Lelito *et al*., (2007) studied on the mating behaviour of *A. planipensis* which is a pest on North American ash tree. They used *A. planipensis* as a dummy insect to trap male and female on the leaves. But basically they studied only on the mating behaviour of the insect species. The lack of captures in these traps could be

explained by a lack of visual responses of these species to other members of the species. We suspect that other factors may have caused this lack of response. One is related to the fact that not a single *C. florentinus* was collected in the purple traps, neither in 2008 nor in 2007. Since these insects live on the branches it is possible that they never fly in the lower areas of the forest where the traps were located. The second argument is that only females of *C. undatus* were collected in the purple traps. This could be explained like in *C. florentinus* if males of *C. undatus* did not fly in the lower parts of the trees. If this is so, the lack of captures of the dead-insect traps could be the result of the lack of males flying in the area of the traps. Future studies should make observations about the daily activity patterns of *C. undatus* and *C. florentinus*.

Larva of *C. undatus* has reared for the first time in Spain. There is no report on rearing larva of *C. undatus* in the laboratory under the artificial condition. A total of 178 larvae were put in 9 different diets. Of these 120 were put in diets 1 to 8 and the rest were put in diet #9. About 45 % have died to fungal growth in the diet, 45 % to natural causes and 10% to sorbic acid. A significant number of insects lost due to the fungal infection in the diets.

A descriptive analysis of the data shows that overall mortality due to fungus on the no-fungicide diets was higher than in the diets with fungicide. When the surviving insects were transferred to diet #9, the percentage dying to fungus or to natural causes was more similar between these two groups of insects than before (Table 9). A similar trend was observed for the larvae that had been put straight in diet #9 from the field. When the insects dead due to fungal growth in this defective diet were removed, the percentage of larvae reared in diet #9 that died to fungal growth or to natural causes became more similar among the different groups (Table 10).

The developmental time of *C. undatus* from egg to adult is about two years under natural conditions. In the laboratory it can probably be shortened to 1 year. The time limitation of this master thesis did not allow determining if the development of *C. undatus* can be completed in the laboratory with this diet. Although modest, our results are a great step forwards towards rearing this insect in captivity. Given the few examples of buprestids, and wood-boring insects in general, reared under natural conditions our success with *C. undatus* provide a solid ground to develop this methodology further.

CONCLUSSIONS

1. Purple traps attract more *C. undatus* than control, non-colored, traps. This demonstrates that purple is attractive to *C. undatus*.
2. Purple is not attractive to some Buprestid species. In fact control traps were more attractive to *Agrilus* sp. than purple traps.
3. All the *C. undatus* captures in the purple trap experiment were females. *Chrysobothris affinis* was also attracted, but only the females. Attraction to purple is therefore sex-related.
4. With the help of the purple trap we have obtained the first direct estimation of a flight curve of this insect. In the future purple traps could be used to measure *C. undatus* abundance in cork forests
5. The location of the trap is a determinant factor in the success of the captures. Sunny, well exposed places captured more *C. undatus* than shaded areas.
6. A diet containing natural ingredients and a few additives has a great potential for rearing *C. undatus* field collected larvae in the laboratory, however complete development to adulthood is still lacking.
7. Small quantities of sorbic acid have long lasting, deadly effects on *C. undatus* larvae.

REFERENCES

Francese, J. A., Mastro, V. C., Oliver J. B., Lance, D. R., Nadeer, Y. and Lavallee, S. G. 2005. Evaluation of colors for trapping *Agrilus planipensis* (Coleoptera: Buprestidae). Journal of Entomological Sciences, 40 (1): 93-95.

Lelito, J. P., fraser, I., Mastro, V. C., Tumlinson, J. H., Boroczky, K. and Baker, T. C. 2007. Visually Mediated 'Paratrooper Copulations' in the mating behavoiur of *Agrilus planipennis* (Coleoptera: Buprestidae), a highly destructive invasive pest of North American Ash trees. Journal of insect Behavior, 20: 537-552.