# Biology, control and luring of the cockchafer, *Melolontha melolontha*

Literature report on biology, life cycle and pest incidence, current control possibilities and pheromones.

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# Monitoring of the cockchafer (*Melolontha melolontha* L.) and control of the grubs in outside crops

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## 1.1 Introduction

The Common cockchafer (or May-bug or May-beetle), *Melolontha melolontha*, is a common large beetle which often crashes into lighted windows, at night during May. The larvae (so-called grubs) are fat and white and have a curved body shape and live in the soil. They can grow up to 46 mm in length. The adults are feeding with leaves and flowers of a range of deciduous trees, but in general they are not a very serious pest on trees. The larvae however, can be very noxious pests of grasses, cereals and other agricultural crops such as potatoes and strawberries, as they live in the soil feeding on the roots. They can be also serious pests in gardens, orchards and tree nurseries. The larvae feed below ground for 3-4 years, before changing into adult beetles. Control of the larvae is very difficult because they live in the soil.

## 1.2 Biology of the Common cockchafer, *Melolontha melolontha*

### 1.2.1 Morphology, life cycle and behaviour

#### 1.2.1.1 Adult

The Common cockchafer, *Melolontha melolontha*, is a beetle that belongs to the family of Scarabaeidae. The adult beetle is 25 to 30 mm long; dark head, black pronotum covered with short hairs, reddish brown wing cases or elytra each with 4 longitudinal ribs. The abdomen is black, with elongated and flattened pygidium. The last fan-like segments are more developed in the male.

Figure 1. The male of *Melolontha melolontha*.

The adults appear in April-May, fly singly, particularly at dusk, and then migrate towards a feeding site: forest or isolated tree (feeding flight). After 10 to 15 days of feeding, the females have acquired their sexual maturity and make the egg-laying flight, towards fields and meadows in the opposite direction to that of the pre-feeding flight. Each female deposits a batch of about 24 eggs in soft soil, at a depth of 15 to 25 cm. Many egg-laying females die, but about a third return to feed and lay for a second time; some lay eggs for a third time.
The adult beetle is a night-flyer and often comes crashing into lighted windows on warm evenings in early

summer. Its large size and buzzing flight make it eye-catching. They eat the leaves and flowers of many deciduous trees, shrubs and other plants, but rarely cause serious damage. They have a preference for certain tree species (see § 1.2.2).

### 1.2.1.2 Egg

After feeding on tree leaves, the females fly to open fields. They have a preference for soft soils where they quickly dig in to a depth of 15-25 cm. The soft soil closes after the female has dug in, and this place is hardly to find. The dimensions of the oval eggs are 2 x 3 mm, but they enlarge by water absorption. They are laid in batches of about 24 eggs. After 4-6 weeks the larvae hatch. Development of the eggs does not take place in very dry soils (less 10-20% water content) or in very wet soils. The optimal temperature for egg development is 18° C.

Figure 2. The eggs of *Melolontha* are laid in the soil at a depth of 15-25 cm

### 1.2.1.3 Larva

The larva is a so-called 'grub'. It has a whitish curved body, large head, bearing strong mandibles, long, hairy, well developed yellow legs. It takes 3-4 years for the larvae to become fully developed, and they burrow deeper into the soil each winter to hibernate. Directly after hatching, end of June-July, the young larva starts to gnaw the small roots. It moves about horizontally distances of up to 30 cm per day. When the first cold weather appears, it buries itself in the ground and hibernates.
The grub measures 10 to 20 mm in the first autumn, 30 to 35 mm by the following autumn, and reaches its maximum size, 40 to 46 mm, in the spring of its third year – in certain regions in eastern Europe the larval development lasts four year. The larvae can be serious pests of grasses and many crops as they live in the soil feeding on roots, especially those of grasses, cereals and other crops.
There are three ($L_1$, $L_2$, $L_3$) larval stages. At 15° C the $L_1$ egg-larva hatches after 49 days, at 20° C after 32 days and at 25° C after 19 days; in the field mostly after 42 days. Too wet or too hot days in summer can cause high egg mortality. The speed of development of the larva depends on the temperature. Therefore the body length is not a good characteristic for the larval stage. For determine the larval stage of *Melolontha melolontha*, the width of the head capsule is more secure; $L_1$ = 2.7 mm, $L_2$ = 4.5 mm and $L_3$ = 6.9 mm. (for *M. hippocastani* respectively 2.6, 4.2 and 6.5 mm). For orientation in the soil on medium distance, the $L_1$ larvae are attracted to the plants by the $CO_2$ release of the roots. Olfactory and taste stimuli are used for orientation on short distance. Unsuitable food plants can be avoided – within its life span from egg-larva to pupa, a larva can reach a horizontal movement in the soil of 1.5-5.5 m.

Figure 3. Some larvae of *Melolontha melolontha* (photo Alterra)

Damage done by $L_1$ larvae is hardly to notice, only at extreme densities of >1000 larvae per $m^2$, the damage is visible After several feeding stops, the $L_2$ larvae appear at the end of August-September with a subsequent feeding period during 4-6 weeks. After that, the first hibernation takes place. In the next spring, when soil temperature (on a depth of 30 cm) in April is exceeding 7°C, the $L_2$ larvae ascend to the root region and feed until the end of May-June. At that time the larval weight has increased from 0.15 to 0.8 g. During this second year the larva is extremely voracious. In autumn, the $L_3$ larva appears, the weight has increased to 3.8 g and the damage to feeding has increased subsequently. In this stage, the second hibernation takes place. In the next spring, larval feeding starts again until pupation at the end of June.

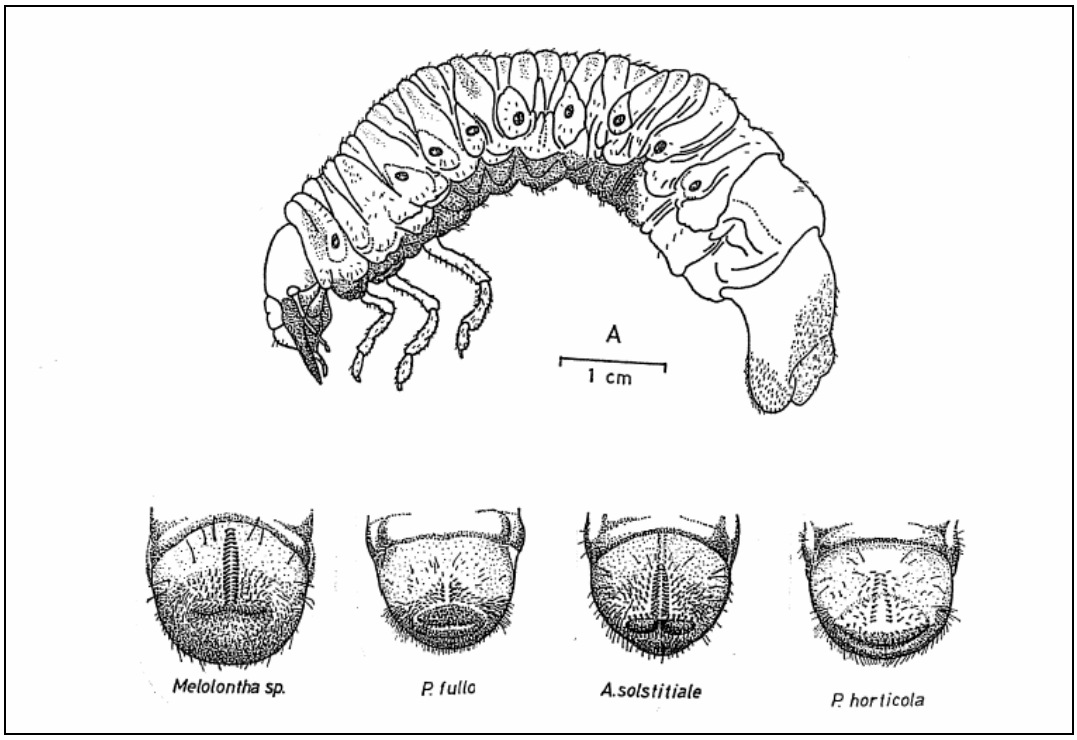



Figure 4. The larvae of different soil pest insects can be identified with characteristics at the end of the abdomen (Schwenke, 1974).

### 1.2.1.4 Pupa

Pupation takes place in June in a small cell at a depth of 15-100 cm – depending of soil and climate type, mostly at a depth of 30-40 cm. Duration of the pupa varies from 25 days at 25° C to 100 days at 12° C. The young adult beetles appear from August-September but they and stay in the soil for a third hibernation. The adults are formed in August but remain inactive until the next spring.

Figure 5. The beetles leave the soil by characteristic exit-holes

### 1.2.2 Host plants of the adults

The adults are leaf consumers, of trees and shrubs and rarely cause serious damage, but only occasionally they are harmful in cherry or plum orchards because they feed also on the blossoms. The adult cockchafers feed on leaves of trees in road-side plantings, in forest edges and in orchards. The adults appear in April-May, fly particularly at dusk, then migrate towards the feeding trees. After 10 to 15 days of feeding, the females have acquired their sexual maturity and make the egg-laying flight, towards fields and meadows. The adults have specific preferences.

Figure 6. Oak is the most preferred feeding tree for adult beetles.

Table 1. Host tree preferences of the adult.

| Highly preferred trees | Rare feeding on | No feeding on |
|---|---|---|
| *Quercus* - oak | *Castanea* - chestnut | *Tilia* - lime |
| *Acer* – sycamore, maple | *Aesculus* – horse chestnut | *Robinia* - acacia |
| *Carpinus* - hornbeam | *Salix* - willow | *Fraxinus* - ash |
| *Fagus* – beech | *Populus* - poplar | *Ulmus* – elm |
| *Prunus* – plum | *Betula* - birch | Conifers, except *Larix* |
| | *Corylus* - hazel | |

This list is relative, depending on the situation – whether the adult beetles have a choice or not.

### 1.2.3 Flight pattern of the adults

The first beetles who are leaving the soil are the males. When temperature is favourable, the beetles stay on the low vegetation. De beetle flight starts at dusk and ends at darkness. It is curious that the first flight occurs in swarm lanes and on silhouettes of forest edges or groups of trees which form silhouettes against the sky at dusk. This kind of orientation does not exceed more than 3 km – the maximum sight of the beetle. Single trees are not preferred. They also fly onto contrasting objects such as groups of buildings or coniferous trees.

One larva per $m^2$ means 10.000 beetles per ha which fly onto suitable feeding trees – thus forming large concentrations of beetles in a small area. From here, the beetles search for suitable feeding trees during short flights. Mating occurs on the trees and last for several hours. After 10-20 days, the flight back to the field starts. Also the peak of this flight is around sun down. The deposition of eggs takes place from 200-900 m from the feeding trees up to a distance of about 1500 m. The females land abrupt on the soil, avoiding the vegetation and search for a sandy place to dig in. Depending of soil structure and soil humidity they deposit their eggs 15-25 cm deep in a batch of about 24 ± 14 eggs. They stay for 2-4 days in the soil. Most females die after this first egg deposition – about ⅓ survives. These surviving females fly again to the trees. Maturation of the eggs depends on temperature: 5-8 days at 27° C or 23-32 days at 15° C. The amount of the second flight is about 16 ± 8 eggs.

### 1.2.4 Host plants of the larvae

The larvae are very polyphagous, they attack the roots of weeds and various crops cereals, red beet, potato, lettuce, raspberry, strawberry, meadow grasses, fruit or forest trees. In meadows, *Taraxacum* and *Plantago* are highly preferred. Leguminosae are preferred above Graminaceae.

Figure 7. The weed *Taraxacum* is an important food plant for the larvae.

The larvae are occasional pests in pastures, nurseries, gardens, and in grassy amenity areas like golf-courses. The injury to grassland and lawns results in poorly growing patches that quickly turn brown in dry weather; the grubs can be found immediately below the surface, usually lying in a characteristic comma-like position. Injury to the roots and rootstock causes small saplings and tender tap-rooted plants like lettuce, which can wilt suddenly or show stunted growth or premature shedding of leaves. Plants growing in rows are usually attacked in succession as the grubs move along from one plant to the next. The injured tissue can favour the development of bacterial or fungal diseases. The roots of fruit or forest trees are peeled. The strongest feeding damage occurs in the year before pupation.

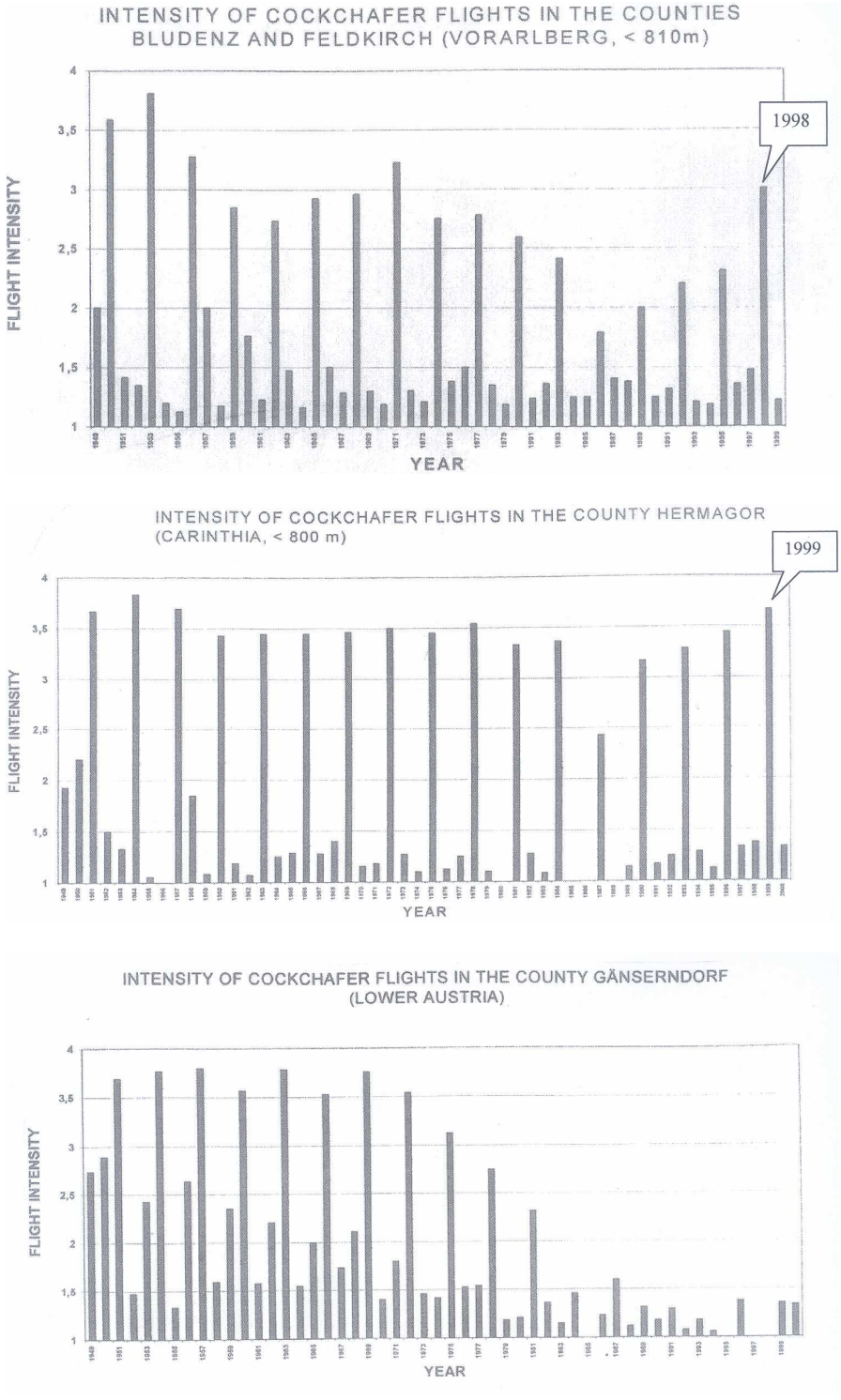
Figure 8. The larvae gnaw on the roots of many crops such as strawberry

Figure 9. The larvae are able to destroy the roots of young fruit- and forest trees.

# 1.3 Pest incidence

## 1.3.1 Pest cycles

In west European regions, the developmental cycle lasts 3 years. So the "major flights" take place every 3 (sometimes 4) years but the year differs from one region to another.




Figures 10 a,b,c. Cockchafer flight intensity in three regions in Austria in the years 1949-2000 (Cate, 2002).

In the figures 10 a,b,c it can be seen that the last peak in the Austrian region Vorarlberg happened in 1998, while the last peak in Carinthia happened in 1999. In Lower Austria the pest incidence diminished during the last decades.

### 1.3.2 Phases of a 3-year life cycle

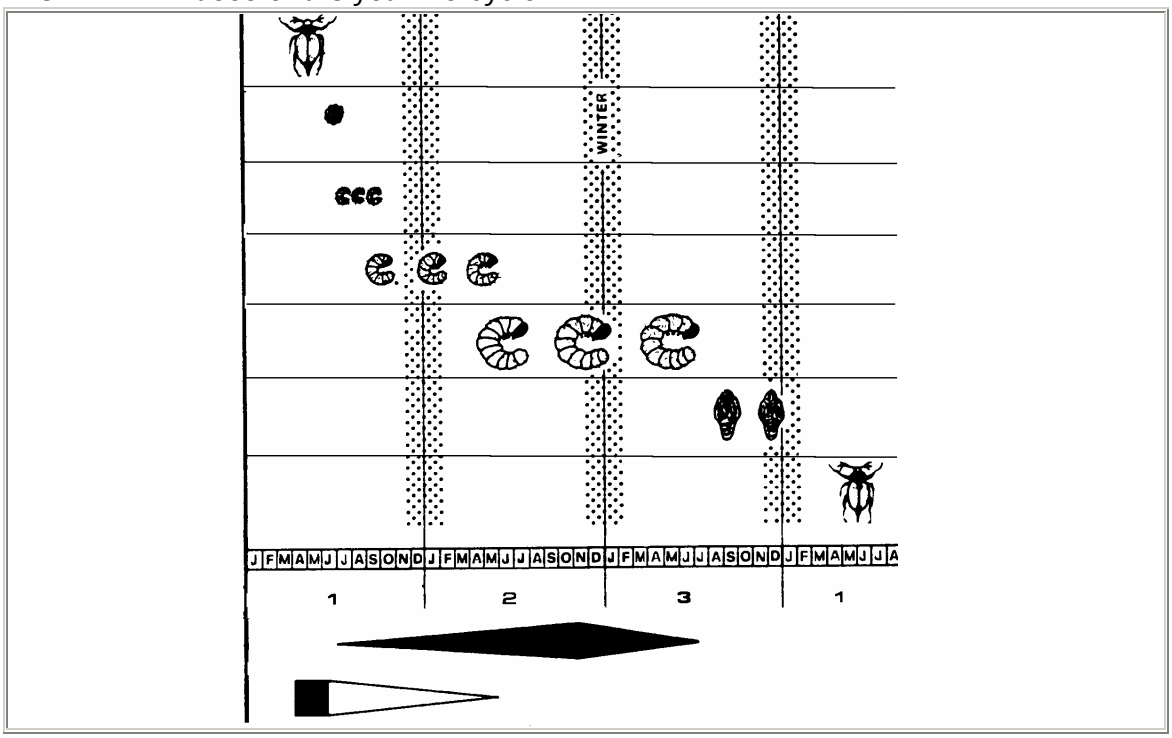



Figure 11. Scheme of the 3-year life cycle of *Melolontha melolontha.* (Source: "Krankheiten und Schädlinge im Obst- und Weinbau", H. Oberhofer, Südtiroler Beratungsring; http://www.rebschutzdienst.at/Krankh_Schaedlinge/Kr_Sch_Beschr_Bilder/24_Maikaefer/Maikaefer.htm)

**Year 1** – flight of adults in April-May, mating, feeding on trees during 2-3 weeks. Flight to the open fields for egg-deposition (females in the soil for 3-4 days); second flight to the trees and feeding during 2-3 weeks. Second flight to the fields for depositing eggs. Sometimes there is a third flight. Hatching of the eggs after 6 weeks; Larval $L_1$ instar during 2 months; $L_2$ instar end August-September; hibernation as $L_2$.

**Year 2** – Larva $L_2$ starts feeding from mid-April; $L_3$ appears in June which causes serious damage to the crops; hibernates as $L_3$.

**Year 3** – Larva $L_3$ starts feeding from mid April – damage less severe compared to previous year. Pupation end of June and subsequent rest during 2 months. The adult beetle appears in August - hibernates as an adult which stays in the pupal chamber just below the frosted soil 5-40 cm deep but up to 100 cm deep in the soil.

**Year 4** – The beetles leave the soil when the soil temperature has reached 10-11° C on a depth of 10-20 cm, during two subsequent days. Flight of the adults takes place in April-May. A drop of temperature stops the beetle's activity – during a late sudden frost, the beetles go even deeper again in the soil.

### 1.3.3 Phases of a 4-year life-cycle

During a 4-year life-cycle, the larval $L_2$ stage appears one year later, in June of the second year. The $L_3$ appears in September of the third year. Pupation occurs in July and the adults appear in September of the fourth year. For *Melolontha hippocastani* it is known that this species can have a 3-6 year cycle.

### 1.3.4 Pest eruptions

Except the annual differences in pest occurrence (3-year cycles) there are also long term cycles. As an example: in Germany in the years around 1900, populations of beetles were six times higher compared with the years around 1920.

### 1.3.5 Impact of climate

Climate conditions can act as negative abiotic factors: rain and cold periods during the flight cause shorter life span of the beetle and a pronged maturation of the eggs in the body. The young L1 larvae are affected by extreme high temperatures and drought. The older larvae are able to avoid this by moving to deeper soil. Winter frost is not a serious factor because the larvae can dig deep in the soil.

## 1.4 Monitoring for pest prognosis

### 1.4.1 Monitoring larvae

In threatened areas, the number of larvae can be monitored by digging 25 soil samples of 50x50 cm per ha. In summer 40 cm (two spades) is deep enough. But in spring and autumn, the larva are deep up to 1 m.

Table 2. Indication of damage in relation to numbers of larvae per $m^2$ depends on the type and age of crop.

| | **gardens** | **meadows /cereals** | **peas and beans** | **susceptible crops** |
|---|---|---|---|---|
| number $L_1$ | 5-15 | 30-40 | 5-10 | 2-3 |
| number $L_2$ | 3-15 | 20-30 | unknown | unknown |
| number $L_3$ | 1-2 | unknown | 3-4 | 2 |

### 1.4.2 Monitoring adults

The begin of the flight can be expected when the sum of the mean daily temperatures from April 1 st, amounts over 355 degrees Celsius.

*Shaking trees*

Monitoring of adult beetles can be done by shaking small feeding trees or certain branches of taller trees in the early morning when the beetles are cold. Estimating the number of fallen beetles gives an idea of the pest incidence.

*Lamp lights*

The beetles are attracted to lamp lights. Counting or estimating the number of crashed beetles give also a rough idea.

Figure 12. Adult beetles are attracted to lamp light. The number of crashed beetles can give a rough idea of the beginning of the flight

and the population densities.

*Pheromone traps*
The suitability and effectiveness of traps baited with pheromones will be discussed in another chapter within this report.

Figure 13. The so-called Unitrap.

## 1.5 Natural enemies

Several biotic factors can have a negative effect on the larvae such as pathogenic micro-organisms: bacteria, viruses, fungi (*Beauveria tenella*) and nematodes. Most important is the bacteria-like *Rickettsiella melolonthae* which can cause high larval mortality.

### 1.5.1 Parasitoids

The long-legged *Dexia rustica* (Diptera; Tachinidae) is a typical parasitic fly of *Melolontha* larva, it attacks also *Phyllopertha* and *Amphimallon* species. The eggs are deposited on the ground; the larvae are hatching directly and begin to search for grubs. Even 30-35 cm deep in the soil 10% of the grubs can be parasitized by 1-6 fly-larvae per host. The fly-larva hibernates in the host which is killed in spring. Pupation occurs in May and the adult flies appear in July/August. In Europe usually found in meadows, fields and woodland margins. There is one generation per year ( Belshaw, 1993; http://www.tachinidae.org.uk/site/get-species.php?brcno=1601; http://www.faunistik.net/ponline/diptera/tachinidae/dexiinae.html

### 1.5.2 Predators

Predatory insects such as Carabidae, and Formicidae are able to decimate the adults of *Melolontha* above ground; Elateridae decimate larvae of *Melolontha* subsoil. However, birds such as Starlings, Crows and Gulls can be much more effective – especially after ploughing.

## 1.6 Other important soil pest insects

There are other relevant beetles from which the larvae are very similar to the Cockchafer, *Melolontha melolontha* and to each-other, such as: *Amphimallon solstitialis*, *Phyllopertha horticola, Melolontha hippocastani* and *Polyphylla fullo.*

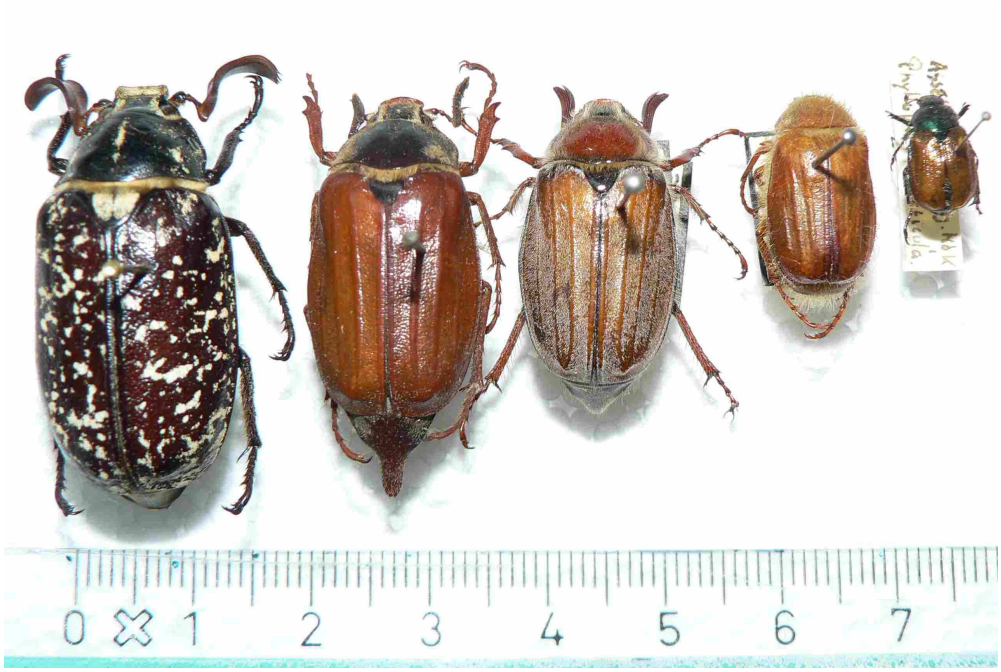



Figure 14. The most important soil pests, From left to right: *Polyphylla fullo* - July beetle or Walker; *Melolontha melolontha* – Cockchafer; *Melolontha hippocastani* – Chestnut cockchafer; *Amphimallon solstitialis* - June beetle; *Phyllopertha horticola* - Rose chafer.

***Amphimallon solstitialis* – June beetle.**
Adults are yellowish-brown (14-18 mm long), have their flight activity during warm evenings in June/July.

Figure 15. *Amphimallon solstitialis* (photo: www.koleopterologie.de)

The larvae are white and elongate (up to 30 mm long). The larvae overwinter one time – so usually they complete their development within two years. The larvae feed on the roots of various herbaceous plants including ornamentals and roots of nursery trees. Although the larvae can cause considerable damage, especially in their second summer, the larvae are usually present in only small numbers (Alford, 1991).

Figure 16. The June beetle (source: www.koleopterologie.de)

***Phyllopertha horticola*** – **Rose chafer.**
Adults are colourful, metallic green and reddish-brown, 7-11 mm long, are especially common in light-soiled grassland areas. They occur mainly in May and June, often flying during daytime in warm sunny weather. The larvae are relatively small - up to 15 mm long. They hibernate one time – so usually they complete their development within two years. The adults feed on leaves, flowers and fruits but their damage is not really important. The larvae feed on plant roots, especially grasses, extensive damage can be seen in lawns - but not in crops.

Figure 17. Larvae of *Phyllopertha horticola*.

Figure 18. The adult of *Phyllopertha horticola*.

***Melolontha hippocastani* – Chestnut cockchafer.**
This species is closely related with the Common cockchafer, *Melolontha melolontha* - their morphology and biology is very similar.

Figure 19. *Melolontha hippocastani* (photo: www.koleopterologie.de)

*M. hippocastani* is more adapted to drier climate conditions and dry sandy soils. *M. melolontha* deposits its eggs preferably in open fields and *M. hippocastani* more in forests and open spots in forests. The adults of *M. hippocastani* are smaller and have a brown instead of a black pronotum.

***Polyphylla fullo* – July beetle or Walker.**
Adults are dark brown with irregular white spots; length 32 to 40 mm. Antennae of the male are well-developed, lamellated.

Figure 20. *Polyphylla fullo* (photo: www.koleopterologie.de)

The larva is 60 to 80 mm long, similar to *Melolontha melolontha*. The larvae live in very sandy soils such as dunes and dry sandy river valleys where they feed on roots of several tree species and common wild plants (e.g. *Eryngium maritimum*, *Psamma arenaria*), as well as the plants cultivated on these soils, particularly vine and pine seedlings. The adults fly after sunset. The female deposits her eggs in the sandy soil neighbouring the trees. The larvae overwinter three times in the soil and the adult appears from mid-June to mid-July. The eggs are deposited near edges of pine forests or vineyards. The adults are harmless to the crops – they prefer the needles of pine trees sometimes leaves of deciduous trees; the larvae attack the roots of vine and young pines (Schwenke, 1974).

## 1.7 Pest control

### 1.7.1 Chemicals

In the past in Europe, the adult beetles on the feeding trees were treated with insecticide applications. Masses of dead beetles are not a guarantee for good results, without additional control of larvae in the soil, this method is insecure. Furthermore, by spraying trees, the impact on the environment is very high. The suitability and effectiveness of chemical control of larvae with insecticides will be discussed elsewhere in this report (Schwenke, 1974).

### 1.7.2 Nematodes

The suitability and effectiveness of control of larvae with entomopathogenic nematodes will be discussed elsewhere in this report.

### 1.7.3 Fungi

The suitability and effectiveness of control of larvae with entmopathogenic fungi will be discussed elsewhere in this report.

### 1.7.4 Nets

The use of nets by covering the soil is very effective in prevention of egg-laying by the adult beetles (Meinert et al., 1997). However, this method is unpractical and much too expensive for the use on a large scale.

### 1.7.5 Soil and crop management

Damage by cockchafer grubs can be reduced by cultural techniques, and by the use of chemical and/or biological control methods if available. Thorough cultivation and good weed control will generally have the

result that plant losses are minimal.
Infested plants may benefit from adequate watering and fertilizing to stimulate good growth of the crops. By ploughing the soil, especially by rotating machines, many larvae are killed or released for birds. Depending on the larval stages and the number of plough activities, 40-80% of the larvae can be killed. Ploughing is only effective when applied during summer, because in winter time, the larvae are too deep in the soil. In some countries such as France, *Melolontha* have become rare nowadays and cause almost no damage. This is most probably due to the widespread use of mechanical cultivation which kills the very fragile larvae, as well as to the change in production systems (Schwenke, 1974).

Figure 21. An ideal habitat for *Melolontha*: the bare soil between the rows of strawberries facilitates the adult beetle to dig in easily for egg-laying on a depth of 15-25 cm

### 1.7.6 Landscape management

The larvae are very polyphagous, in meadows they attack the roots of several wild grasses and weeds. Host plants are: *Rumex, Chenopodium, Stellaria, Achillea, Festuca* and *Cirsium*. But *Taraxacum* and *Plantago* are highly preferred. In experiments it was shown that the roots of *Taraxacum officinale* are the best source of nutrition and that the beetles are capable in the field to select these weeds for oviposition. In stony soils even with *Taraxacum*, less larvae are present compared with sandy soils (Haus & Schütte, 1978). Leguminosae are preferred above Graminaceae (Schwenke, 1974). The occurrence of *Taraxacum officinale* and *Melolontha melolontha* in Europe over the past 30 years, showed that the conditions for propagation of the 2 organisms have been changing. Over the past decade, in some regions the abundance of *Taraxacum* has increased in relation to decreasing herbicide usage. In an experiment, this weed was reduced to 12% of its abundance by spraying herbicides; by that, the abundance of the larvae was reduced to 55%. Nowadays, many meadows and pastures are partially covered by *Taraxacum* - these conditions are favourable for mass occurrence of *Melolontha* (Schutte, 1996).

Figure 22. In Ukraine, large areas of unused land covered with grasses and suitable weeds provides the ideal place for propagation and increase of *Melolontha melolontha*.

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# 2 Current possibilities to control white grubs

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## 2.1 Introduction

White grubs are larvae of Scarabaeidae. As a group, they are a major pest throughout the world. In Europe, the major species attacking agricultural and horticultural crops, are the cockchafer (*Melolontha melolontha*), June beetle (*Amphimallon solstitialis*) and garden chafer (*Phyllopertha horticola*). Cockchafer has a three-year life-cycle in most countries, June beetle has a two-year life-cycle and garden chafer has a one-year life-cycle. Despite varying life cycles, grubs of all three species can cause serious attack to crops.
In Ukraine, damage caused by the cockchafer grubs is an increasing soil pest in outside crops. Particular in strawberries damage can be great, but also vegetable crops, meadows and ornamental plant production like rose bushes can be seriously attacked. Depending on the year, losses of 20-25% can occur resulting in substantial economic damage. The high infestation of fields with grubs is caused by the yearlong neglect of the pest: large areas of fellow, unused land provided the ideal place for propagation and increase of the pests. In addition the grubs can only be fought effectively in the period of May-June when they surface to the root level to cause the damage. So far fighting the grubs (larvae) proved to be very difficult. No effective crop protection product is available on the market in the Ukraine. Effective fighting will partly require specific equipment to apply insecticides, which isn't available currently in Ukraine. No experiments have been done to find biological ways to control the pest in Ukraine.
This report aims at providing an update on the current state of scientific research on the topic. This focuses on possibilities to control both adults and larvae of *Melolontha melolontha*, *Amphimallon solstitialis* and *Phyllopertha horticola*. Biological and chemical control measures are discussed, as well as cultural control measures. The search was limited to the last ten years (from 1996 onwards).

## 2.2 Biological control

### 2.2.1 Entomopathogenic nematodes

Entomopathogenic nematodes are potentially capable of killing varying species of grubs and actively disperse through the soil, but efficacy of most species against grubs is quite low (Gerritsen et al., 1998). Particularly *P. horticola* can be controlled successfully with *Heterorhabditis bacteriophora*. However, Peters (2000) stated that field control of *M. melolontha* with nematodes is not economically feasible. The nematode strain that shows the best results against larvae of *M. melolontha* is *Steinernema glaseri* (Berner et al., 2001). Berner et al. (2002) found that treatment with *H. bacteriophora* could reduce the numbers of grubs with up to 65%, at an application rate of 1 million nematodes per sqm.
Peters (2004) reports on a new string of *Steinernema*, *S. scarabaei*, for which *M. melolontha* was very susceptible in a laboratory trial. Mass rearing of this strain was not successful so far.

### 2.2.2 Entomopathogenic fungi

The fungus *Beauveria brongniartii* is considered the main natural enemy of *M. melolontha* (Keller, 2000). It can attack all of its development stages. In developing a way to control M. melolontha larvae with B. brongniartii, two methods have been developed: one is spraying adults with blastospores, which they carry with them to the breeding sites, the other is soil application of barley kernels colonised with fungus. The lather has been developed into a commercially available product (Melocont®-Pilzgerste), mainly because, of the two possible solutions, this one has a direct effect on the larvae. This product is mainly used to control *M. melolontha* in meadows and orchards. Both methods of application showed long-term effects.
For survival and spreading out of B. brongniartii humid conditions are required, which are not always present (Meinert et al., 2001). It also is important to apply the fungus deep enough into the soil (5 cm), to

make sure it is not inactivated through UV light.
Strasser (2004) stated that an immediate effect of treatment with *B. brongniartii* is not to be expected. In contrast to the mode of action of chemicals, of which the effect fades out, the effect of treatment with *B. brongniartii* builds up. Higher *M. melolontha* populations quicken the efficacy of *B. brongniartii* and the fungus is claimed to act on all development stages. The effect should also last for three to four generations of the cockchafer.
Against *P. horticola* the fungus *Metarhizium anisopliae* is favoured, according to Strasser, based on its specificity and high pathogenicity.

### 2.2.3 Others

Meinert et al. (2001) applied 3 l/ha NeemAzal-T/S (azadirachtin) as a spray application by helicopter. The product does not have a direct killing effect, but should diminish or stop feeding. In laboratory tests, Hummel et al. (2004) found 100% mortality only after 9 days. He also found, that NeemAzal-T/S lowered the numbers of eggs per female from 14.9 to 3.9 and lowered the percentage of eggs to hatch from 53 to 15%.
Bacteria may have an affect on chafer larvae, but have not been tested extensively. Both *Bacillus thuringiensis* and *B. popiliae* could have an effect on larvae, but no successful consistent efficacy has been reported (Mann, 2004).

## 2.3 Chemical control

Grubs are difficult to control chemically, as they complete their complete larva state subterranean. On top of this, they move up and down the soil, which complicates a successful control further. Nevertheless, several reports on experiment with chemical control are available.
The efficacy of chlorpyrifos to control white grubs is doubtful. Strasser (2004) mentioned the compound as intended to use for control of *P. horticola*. On the other hand, Mann (2004) called it ineffective to control chafer grubs.
Trials have been carried out to control *M. melolontha* adults with spray applications by helicopter. Meinert et al. (2001) reported to have used phosalone (2.5 kg/ha Rubitox®) on woodlands, based on positive results in the past. In this research, phosalone was able to show 80 to over 95% control of adults and larvae. However, questions can be asked concerning environmental impact of such a treatment.
Chemical control has also been used in a combination with cultural control measures or biological control measures. After treatment with thiamethoxam or acetamiprid and subsequent soil cultivation before planting strawberries, in 2001, a control level of over 80% was achieved (Łabanowska et al., 2003). The test population was considered high, at 14 larvae/sqm.
Sublethal doses of the neonicotenoid imidacloprid were reported to enhance the susceptibility of *Cyclocephala hirta* and *C. pasadenae* to *H. bacteriophora*. American research has shown 94% control of "chafer larvae" with imidacloprid but was less effective in the 3rd instar (Mann, 2004). Also contrary results on imidacloprid have been reported.

## 2.4 Cultural control

Grubs are sensitive to soil cultivation, if they are located in the topsoil. However, for the growing of a lot of agricultural and horticultural crops, this cannot be applied successfully, since the grubs are not yet in the topsoil when the soil is cultivated (Strasser, 2004). Postponing planting or sowing moment may be helpful in achieving a higher level of control of cultivation.
Several cultural control measures, such as lime application, using heavy rollers, urea application or aerification were found not to be successful, but not irrigating during egg-laying could reduce the numbers of eggs laid in a certain area (Mann, 2004).
Trials have been carried out applying nettings in orchards and vineyards, preventing the adults of the May beetle to fly to the trees where they forage, as well as the flight back, to lay eggs (Meinert et al., 2001). After treatment with an insecticide to prevent emerging adults to copulate, growers reported covered areas

to be as good as free of larvae.

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# 3 Pheromones and Melolontha melolontha

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## 3.1 Introduction

The word pheromone is a contraction of the Greek words 'pherein', which means to transfer and 'hormon', which means to excite. Pheromones are defined as substances, which are secreted to the outside by an individual and when perceived by a second individual of the same species, they trigger a specific response. Several types like alarm, trail and aggregation pheromones are known to exist for insects. When a pheromone is released with the intention of attracting members of the opposite sex for mating, it is called a sex pheromone. In moths, most sex pheromones are released by females to attract conspecific males, however in many other species of insects the pheromone is released by the male and attracts females. Also in some primitive moth species, males, or both the males and females release a sex pheromone.
If the substance that initiates a behavioral response is actually released by the insect, they are called pheromones. At the other end there have been described thousands of attractants for many insect species. An overview can be found at internet site: www.pherobase.com.
The first insect sex pheromone was isolated and identified in 1959 by Butenandt. He and his co-workers extracted and purified about 12 milligrams of a, to the males, highly attractive compound from 500,000 females of the oriental silk moth *(Bombyx mori)*. They identified this compound as (E,Z)-10,12-hexadecadienol (Bombykol). In these early pioneering years it was never considered that a sex pheromone might consist of more than one compound. Later it became obvious that multiple component pheromones were more a rule than an exception. In 1978 it was discovered that sex pheromone gland extracts of *Bombyx mori* contained, in addition, the corresponding aldehyde of Bombykol, namely Bombykal, which was part of the sex pheromone.
Male moths are extremely sensitive to their sex pheromones. For example, amounts of less than 10 pg (10-11 gram) of the sex pheromone of *Bombyx mori* when offered on a piece of filter paper to the males elicit a behavioral response. Other research shows that male moths are able to detect and to respond to sex pheromone concentrations as low as picograms per liter of air. Experiments have been carried out with *Adoxophyes orana*, marked with radioactive 32P, to determine the distance over which these moths were able to locate a source with virgin females. It turned out that the males were able to locate the females over a distance of 75 meter in just one night. Measured over several nights, males were even capable of reaching sources that were several hundreds of meters away. Insects like beetles and weevils sometimes are less sensitive towards their pheromone and as a consequence produce larger quantities of it.
Insects are very sensitive towards scents that they can use for finding mates and food sources. On the other hand they can be quite insensitive towards smells (volatiles) that are less essential for surviving. With help of their antenna, which in fact is the nose of the insect (see figure 23), the insect is able to recognize potential food sources, friends and/or threats. Qualitative as well as quantitative information is gathered and used for orientation.

Figure 23. Antenna of male *Melolontha melolontha*.

## 3.2 Sex pheromones and integrated pest management

In contrast to pesticides, sex pheromones are substances that are produced and used by insects themselves. Therefore, it is unlikely that resistance against them will develop. When sex pheromones are chemically identified and available, they can be used in pest control in four different ways: (1) monitoring, (2) mass trapping, (3) mating disruption and (4) the attraction and subsequent killing of the insects without trapping them, known as attract-and-kill.
Monitoring is the most common use of pheromones. As a monitoring tool, sex pheromones are used to attract exclusively the species of interest and, therefore, provides data about the presence and abundance of the insect pest. The appropriate time for pesticide application can be calculated, so that pesticides will only be used at the moment when they are most effective and needed.
The second way in which sex pheromones can be used is mass trapping. This method is not used very often, especially not in first-world countries. One reason for this is that mass trapping is less thorough than the application of pesticides. Another reason is that the application of sex pheromones for mass trapping is a rather time consuming way of controlling a pest because one needs a lot of traps which have to be installed and maintained. In first-world countries where labour is expensive, the use of sex pheromones in mass trapping is commercially conceivable only in few cases.
The third approach is mating disruption. Here, the sex pheromone is applied in such high concentrations onto the crop or in storehouses that the male pest insects are no longer able to locate the female insects. In this way, no copulation will occur and, as a consequence, no new offspring will develop. This method has advantages over mass trapping because it is relatively easy-to-use. In practice however, there are still few cases where mating disruption has shown to be of practical value in pest control. Not all insects are sensitive to this method and insect sex pheromones are often too expensive for the application as mating disruptant. Another important cause is the commitment to register the sex pheromones in many countries before they may be applied for mating disruption, which is an expensive and time consuming procedure.
The fourth method which involves insect sex pheromones in the control of insect pests was developed as "Attract and Kill". The sex pheromone is formulated into a glue-like liquid UV-absorber (for light protection) with a small amount of a very potent insecticide. It is applied in droplets onto the plants that have to be protected. The male insect is attracted to the sex pheromone, touches the source and picks up some of the glue together with a (sub)lethal dose of the insecticide. If such a male copulates with a female later on, there is a good chance that she is poisoned as well. This method is used with success, for example, against *Pectinophora gossypiella* in cotton fields in Egypt and against *Ephestia kuehniella* in flour mills in Italy.
In developing countries, the newer, expensive pesticides are not always available. Because the threshold for damage is much higher than in first-world countries, and the costs of labour are much lower, the application of sex pheromones in pest control programs could be a solution. Sex pheromones are already used in the control of some insect pest species in third-world countries. One established example is the use of the sex pheromone of Phthorimaea operculella, which was identified in 1976 by TNO and IPO. ***PHERO*BANK** (Wageningen) synthesises this sex pheromone on a commercial scale. This sex pheromone has been applied in Peru, Venezuela and Tunisia for years with great success in mass trapping of *Phthorimaea operculella* It appears to be cheaper and more effective than the formerly used pesticides.

## 3.3 Scarabaeidae pheromones and attractants

*Melolontha melolontha* belongs to the family of the *Scarabaeidae*. Of this family several pheromones and/or attractants have been identified. Annex 1 shows a summary of the pheromones and/or attractants that have been described within this family (from: www.pherobase.com)
The pheromones and/or attractants that have been identified in the subfamily of *Melolonthinae*, to with *Melolontha melolontha*, belongs are summarized in annex 2.
As can be seen from this annex there is no clear relation between species. Some species use similar compounds or similar kind of compounds but also related species sometimes apply compounds which are chemically not related.

## 3.4 Introduction to cock chafer semiochemicals

European cockchafers, *Melolontha melolontha* L. (Coleoptera: Scarabaeidae: *Melolonthinae*), are a severe pest in agriculture and horticulture when calamitous mass breeding occurs. The polyphagous larvae feed upon plant roots, while the adults may heavily damage above-ground foliage. As a pest, the species has extensively been studied in many biological aspects. One option to control a herbivore pest insect is the use of those naturally occurring chemicals that mediate its sexual communication or its location of the host plant.

Cockchafers perform a spectacular swarming flight around host trees at dusk. Counting of flying and resting beetles revealed that males exclusively perform the swarming flight while females remain on the host tree leaves they feed or have fed upon. In a landing cage bioassay conducted in the field, swarming cockchafer males preferred cages baited with females to cages baited with males. Gas chromatographic analysis of beetle extracts with electroantennographic detection revealed the presence of electrophysiologically active compounds, among them toluquinone, phenol, and 1,4-benzoquinone, the sex pheromone of the closely related forest cockchafer, *M. hippocastani*. In funnel trap bioassays none of the quinones is attractive to males per se. Volatiles from mechanically damaged leaves and a mixture of green leaf volatiles (GLV) mimicking the bouquet of mechanically damaged leaves are highly attractive to *M. melolontha* males. The attractiveness of the same GLV mixture is synergistically enhanced when toluquinone is added to the lure. In the same setup, 1,4-benzoquinone is behaviourally not active. Thus, based on a sexual dimorphism in flight behaviour, GLV act as sexual kairomones and attract males to sites of female feeding damage. Toluquinone as a sex pheromone indicates that conspecific females are actually present, and synergistically enhances the attractiveness of the GLV. This constitutes the first report about an insect sex pheromone not being attractive on its own, but needing the concomitant presence of host plant volatiles to attract males to potential mates.

Phenol has been identified in female full body extracts from both, *M. hippocastani* and *M. melolontha*. In the field, phenol attracts males of both species and enhances the attraction of cockchafer males to the green leaf alcohol (Z)-3-hexenol. If equal ratio mixtures are compared, a mixture of phenol plus (Z)-3-hexenol is less attractive for M. hippocastani males than a mixture of (Z)-3-hexen-l-ol plus 1,4-benzoquinone, whereas phenol plus (Z)-3-hexen-l-ol attracts as many *M. melolontha* males as a mixture of (Z)-3-hexen-l-ol plus toluquinone. In both species three component mixtures containing phenol, (Z)-3-hexen-l-ol, and the respective benzoquinone in equal proportions do not capture more males than two component mixtures consisting of only (Z)-3-hexen-l-ol and the benzoquinone. These results show that phenol is another male attractant common to M. hippocastani and *M. melolontha*. However, when optimized ratios of (Z)-3-hexen-l-ol and the respective benzoquinone are used, addition of phenol reduces numbers of attracted males in both species. The exact function of phenol remains to be elucidated, and the term male attractant is used instead of sex pheromone.

Since toluquinone, 1,4-benzoquinone, and phenol are present in female full body extracts from *M. melolontha* and *M. hippocastani*, field experiments were performed addressing the question, whether swarming males discriminate between conspecific and heterospecific females. Males of both species prefer females when given the choice between females and males of the other species. However, they prefer conspecific females when females from both species are offered simultaneously. The results suggest that species-specific pheromone blends contribute to precopulatory reproductive isolation in sympatric populations of *M. melolontha* and *M. hippocastani*. But in contrast to findings in other sympatric scarab beetles, a blend emitted by forest cockchafer females is not a behavioural antagonist to European cockchafer males and vice versa. Furthermore, the respective blends are not indispensable prerequisites to find and select a mate, as it is the case in other insects.

Responses of *M. melolontha* cockchafers to host plant volatiles were investigated both in the field and using electrophysiological techniques. Male cockchafers are attracted by volatiles from mechanically damaged leaves of *Fagus sylvatica* L., *Quercus robur* L., and *Carpinus betulus* L. Odours from intact *F. sylvatica* leaves are not attractive to *M. melolontha* males. In total, 16 typical plant volatiles are shown to elicit electrophysiological responses on cockchafer antennae, among them many green leaf volatiles typically emitted by damaged leaves. In the field the green leaf alcohols (Z)-3-hexenol, (E)-2-hexenol, and 1-hexanol attract males, whereas the corresponding aldehydes and acetates are behaviourally inactive. Thus, the function of sexual kairomones in the mate finding process of *M. melolontha*, can only be attributed to green

leaf alcohols. Interestingly, the close relative, M. hippocastani responds only to (Z)-3-hexenol, not to the other leaf alcohols. Females are not attracted by any of the tested volatile sources.
To elucidate the structure-activity relationships of aliphatic alcohols, i.e., green leaf alcohols and non-natural analogues, both behavioural and physiological responses were studied in male and female M. melolontha. The compounds tested were saturated aliphatic alcohols with chain lengths between five and eight carbon atoms. Further-more, the cockchafer's responses to six-carbon alcohols with (E)-2-, (E)-3-, (Z)-2-, (Z)-3-, and (Z)-4-configurated double bonds were tested. All compounds elicit dose-dependent responses on the antennae of both sexes. In general, males show a stronger normalized EAG response to the stimuli than females. In the field, only the naturally occurring six-carbon alcohols, i.e., 1-hexanol, (E)-2-, (Z)-3, and (E)-3-hexenol are attractive to *M. melolontha* males. The attractiveness depends on the molecules' structure. Females are not attracted by any of the tested compounds.
The results of the field and physiological experiments with natural and synthetic host plant volatiles highlight the function of the green leaf alcohols as sexual kairomones. No evidence was found that males or females use plant volatiles for host location.
To optimize cockchafer lures, specific binary or ternary blends of (Z)-3-hexen-l-ol with phenol, and toluquinone or 1,4-benzoquinone, respectively have been tested in funnel trap experiments. In both species, *M. melolontha* and *M. hippocastani*, binary lures containing (Z)-3-hexen-l-ol combined with toluquinone or 1,4-benzoquinone, respectively, at a ratio of 10:1 are the most potent male attractants.
In 2004 and 2005 several experiments in The Netherlands have been conducted with pheromones and with light to attract the *Melolontha* adults. It was concluded that the beetles could be well attracted with traps with pheromones plus kairomones. Traps based upon a strong light (>300 W) appeared also attractive. The traps that have been used in these trials, where constructed from small swimming pools (diameter 1-2 meters) plus a strong lamp and placed at a height of 2.5 meters. With this system it appeared that 50% of the flying insects could be trapped away. These tests were conducted in an isolated field. Later observations in this isolated field showed that the number of grubs (figure 24) had been reduced.

Figure 24. Grub larva of *Melolontha melolontha*

Figure 25. Yellow UNITRAP with barrier crosses

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# Annex 1. Insect genera of the Scarabaeidae family of which pheromones and/or attractants have been described

| **Genus:** | **subfamily** | **: tribe** |
|---|---|---|
| Amphimallon | Melolonthinae | : Chasmatopterini |
| Anomala | Rutelinae | : Anomalini |
| Antitrogus | Melolonthinae | : Melolonthini |
| Aphodius | Aphodiinae | : Aphodiini |
| Blitopertha | Rutelinae | |
| Cetonia | Cetoniinae | |
| Costelytra | Melolonthinae | |
| Cotinis | Cetoniinae | : Gymnetini |
| Cyclocephala | Dynastinae | : Cyclocephalini |
| Dermolepida | Melolonthinae | : Melolonthini |
| Dyspilophora | | |
| Epicometis | Cetoniinae | |
| Euphoria | Cetoniinae | : Cetoniini |
| Exomala | Rutelinae | : Anomalini |
| Heptophylla | Melolonthinae | |
| Holotrichia | Melolonthinae | |
| Hoplia | Melolonthinae | : Hopliini |
| Kheper | Scarabaeinae | : Scarabaeini |
| Lepidiota | Melolonthinae | : Melolonthini |
| Liparetrus | | |
| Macrodactylus | Melolonthinae | : Macrodactylini |
| Maladera | Melolonthinae | |
| Melolontha | Melolonthinae | |
| Onthophagus | Scarabaeinae | : Onthophagini |
| Oryctes | Dynastinae | : Oryctini |
| Osmoderma | Trichiinae | |
| Oxycetonia | Cetoninae | |
| Oxythyrea | Cetoniinae | |
| Pachnoda | Cetoniinae | |
| Phyllopertha | Rutelinae | : Anomalini |
| Phyllophaga | Melolonthinae | : Melolonthini |
| Popillia | Rutelinae | : Anomalini |
| Potosia | Cetoniinae | |
| Protaetia | Cetoniinae | : Cetoniini |
| Rhizotrogus | Melolonthinae | : Melolonthini |
| Scapanes | Dynastinae | |
| Strategus | Dynastinae | : Oryctini |
| Valgus | Cetoniinae | |

# Annex 2. Pheromones and or attractants described within the subfamily Melolonthinae

| | | |
|---|---|---|
| *Amphimallon solstitiale* | Tolasch. 2003 | |
| r-acetoin | P F | |
| *Antitrogus consanguineus* | McGrath, 2003 | |
| delta-9,10-24:Hy | P | |
| delta-9,10-25:Hy | | |
| delta-9,10-26:Hy | | |
| delta-9,10-27:Hy | | |
| delta-9-25:Hy | | |
| 25:Hy | | |
| 3me-25:Hy | | |
| *Antitrogus parvulus* | Chow et al, 2005 | |
| 4me6me8me10me16me-22:Hy | P F | |
| 4me6me8me10me16me18me-22:Hy | | |
| *Costelytra zealandica* | Harrison, 1970; Henzell, 1970 | |
| CO2 | A | |
| | Galbreath, 1988 | |
| phenol | P F; P | |
| *Dermolepida albohirtum* | McGrath, 2003 | |
| delta9,10-23:Hy | SP | |
| *Heptophylla picea* | Kakizaki, 1998, 2000; Leal, 1996a | |
| R,Z7,15-hexadecadien-4-olide | P; P F; P | |
| *Holotrichia consanguinea* | Ward, 1996; Leal, 1996b | |
| anisole | P M; PA M | |
| *Holotrichia loochooana* | Yasui, 2003; Arakaki, 2003 | |
| anthranilic acid | P F; P F | |
| *Holotrichia parallela* | Leal, 1993c | |
| R-linalool | P F | |
| l-isoleucine methyl ester | | |
| | Leal, 1992b | |
| me-2S-amino-3Sme-pentanoate | P F | |
| *Holotrichia reynaudi* | Ward, 2002 | |
| anisole | P M | |
| *Hoplia communis* | Imai, 1998 | |
| 2-phenylethanol | K | |
| *Hoplia equine* | Zhang, 2003b | |
| 14-2Kt | P F | |
| *Lepidiota crinita* | McGrath, 2003 | |
| delta9,10-29Hy | P | |
| delta9,10-31Hy | | |
| *Lepidiota negatoria* | McGrath, 2003 | |
| delta9,10-25Hy | P | |
| *Lepidiota picticollis* | McGrath, 2003 | |
| delta9,10-23Hy | P | |
| *Macrodactylus murinus* | Arredondo-Bernal, 1995 | |
| caproic acid | A L | |
| valeric acid | | |
| octyl butyrate | | |
| *Macrodactylus nigripes* | Arredondo-Bernal, 1995 | |
| caproic acid | A L | |
| valeric acid | | |
| octyl butyrate | | |
| *Macrodactylus subspinosus* | Lingenfelter, 2003 | |
| caproic acid | A L | |
| valeric acid | | |
| octyl butyrate | | |
| E2-9OH | | |
| alpha-ionone | | |
| | Williams, 2000 | |
| caproic acid | A | |
| valeric acid | | |
| octyl butyrate | | |
| E2-9OH | | |
| alpha-ionone | | |
| | Williams, 1990 | |
| caproic acid | A L | |
| valeric acid | | |
| octyl butyrate | | |
| *Maladera matrida* | Yarden, 1996 | |
| Z,E-alpha-farnesene | K | |
| | Ben-Yakir, 1995 | |
| eugenol | A | |
| *Melolontha hippocastani* | Ruther, 2002b | |
| Z3-6OH | K | |
| | Ruther, 2002a | |
| phenol | P | |
| | Ruther, 2001b | |
| 1,4-benzoquinone | P | |
| toluquinone | | |
| | Ruther, 2001a | |
| 1,4-benzoquinone | Al F DS | |
| | toluquinone | |
| | toluquinone | |

| | |
|---|---|
| *Melolontha melolontha* | Imrei, 2003b |
| Z3-6:Ac | K H |
| Z3-6:OH | |
| benzaldehyde | |
| E2-6:OH | |
| 6:OH | |
| | Reinecke, 2002 |
| toluquinone | P F |
| 1,4-benzoquinone | |
| 9:Ald | |
| | Ruther, 2002 |
| phenol | P |
| 1,4-benzoquinone | |
| 9:Ald | |
| *Phyllophaga anxia* | Alm, 2004 |
| l-valine methyl ester | P L |
| l-isoleucine methyl ester | |
| | Zhang, 1997 |
| l-valine methyl ester | P F |
| l-isoleucine methyl ester | |
| | Poprawski, 1992 |
| caproic acid | A |
| *Phyllophaga congrua* | Crocker, 1999 |
| phenyl propionate | A |
| eugenol | |
| geraniol | |
| *Phyllophaga crassissima* | Crocker, 1999 |
| anethole | A |
| | Zhang, 1997 |
| l-valine methyl ester | A |
| l-isoleucine methyl ester | |
| *Phyllophaga crinita* | Robbins, 2003 |
| me-2-me-thio-benzoate | P F |
| | Crocker, 1999 |
| phenyl propionate | A |
| eugenol | |
| geraniol | |
| *Phyllophaga elenans* | Leal, 2003 |
| l-isoleucine methyl ester | P F |
| formyl-isoleucine methyl ester | |
| acetyl-l-isoleucine methyl ester | |
| *Phyllophaga forsteri* | Alm, 2004 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| | Zhang, 1997 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| *Phyllophaga fraterna* | Zhang, 1997 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| *Phyllophaga fusca* | Alm, 2004 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| | Zhang, 1997 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| *Phyllophaga futilis* | Zhang, 1997 |
| l-valine methyl ester | A |
| l-isoleucine methyl ester | |
| *Phyllophaga hirsuta* | Alm, 2004 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| *Phyllophaga hirticula* | Zhang, 1997 |
| l-valine methyl ester | A |
| l-isoleucine methyl ester | |
| *Phyllophaga lanceolata* | Nojima, 2003c |
| l- leucine methyl ester | P F |
| l-isoleucine | |
| l-valine methyl ester | |
| *Phyllophaga marginalis* | Alm, 2004 |
| l-valine methyl ester | A L |
| l-isoleucine methyl ester | |
| *Phyllophaga sp.* | Camino-Lavin, 1996 |
| methyl acetate | A L |
| ethyl acetate | |
| *Phyllophaga squamipilosa* | Nojima, 2003c |
| l- leucine methyl ester | A L |
| *Rhizotrogus majalis* | Nojima, 2003d |
| 2R3R-butanediol | P M&F |
| meso-2,3-butanediol | |
| *Rhizotrogus majalis* | McGovern, 1970 |
| propyl 1,4-benzodioxan-2-carboxylate | A |
| | Tashiro, 1964 |
| butyl sorbate | A |
| *Rhizotrogus vernus* | Imrei, 2003a |
| 1,4-benzoquinone | P |
| phenol | |