IOBC / WPRS

## Working Group "Integrated Control of Soil Pests"

### Subgroup "*Melolontha*"

OILB / SROP

## Groupe de Travail "Lutte Intégrée contre les Organismes du Sol"

### Sous-Groupe "*Melolontha*"

# Proceedings of the meeting
# Compte-rendu de la réunion

at

**Freiburg, Germany**
**23-25 October 1995**

Edited by Siegfried Keller

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# Introduction

The *Melolontha* group held its third international meeting, but the first one as subgroup of the IOBC/WPRS working group "Integrated Control of Soil Pests". The meeting was held from 23-25 October 1995 in Freiburg, Germany. The local arrangements and a half-day excursion to *Melolontha* sites of the Kaiserstuhl region were successfully organised by Günter Schruft and Manfred Fröschle.

Over 40 participants from 6 countries attended the meeting and presented 22 oral contributions and 5 posters.

Main themes were:

- Population development, distribution and damages.
- Pathogens and microbial control.
- Integrated control.

This Bulletin contains the proceedings of this meeting.

On behalf of the *Melolontha* group I would like to thank Günter Schruft and his staff for the excellent manner in which they organised the meeting at the Staatliches Weinbau Institut in Freiburg. The next meeting will be in Switzerland in autumn 1998.

Siegfried Keller
Convenor of the Subgroup

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**Integrated control**

# Population development, distribution and damages

# Occurrence of the Common Cockchafer (*Melolonta melolontha* L.) in the State of Baden-Württemberg/Germany

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Summary:

The State Institute for Plant Protection at Stuttgart has initiated various investigations to elaborate the status quo of the occurance of the Common Cockchafer in the State and the damage caused by the grubs. Although single specimens of the beetle were found to be scattered around the state, severe damages on crops were recorded in rather restricted areas. These include:

1. Schwäbische Alb:
   The region is characterised by permanent grassland alternating with arable land. Damage was recorded only on grassland.
2. Western Kraichgau:
   The region comprises mostly arable crops and some apple orchards. Damage by the grubs were recorded only on the roots of pome trees.
3. Kaiserstuhl:
   There is a wide range of crops, which are grown on rather small fields. All of them are known to be susceptible to attack by Common Cockchafer.

The highest damages were found to be in the Kaiserstuhl, followed the by Kraichgau and the Schwäbisch Alb.

Between 1930 and 1970 frequent infestations by the Common Cockchafer were recorded over the whole State with serious damage on grown crops. The occurrence at that time was scaled and documented in an infestation map (Fig.1) which one can still rely on even today. In the late seventies the Common Cockchafer has not been recorded. In 1982 a farmer from the northern Kaiserstuhl area (Fig.2) reported a new occurrence of the pest. However, this was underestimated for many years. In the region of Ortenau the beetle turned to be a part of the local fauna, due to the rather extensive pattern of agriculture in that region. In the region of Bodensee in 1981 a late frost had apparently caused an extremely high beetle mortality. This has retarded the start of a new cycle for about 12 years. Accordingly, a weak flight was observed 1993 in that region. At the present time the Common Cockchafer can be found again all around the state, but without crop damage, except in the three mentioned regions.

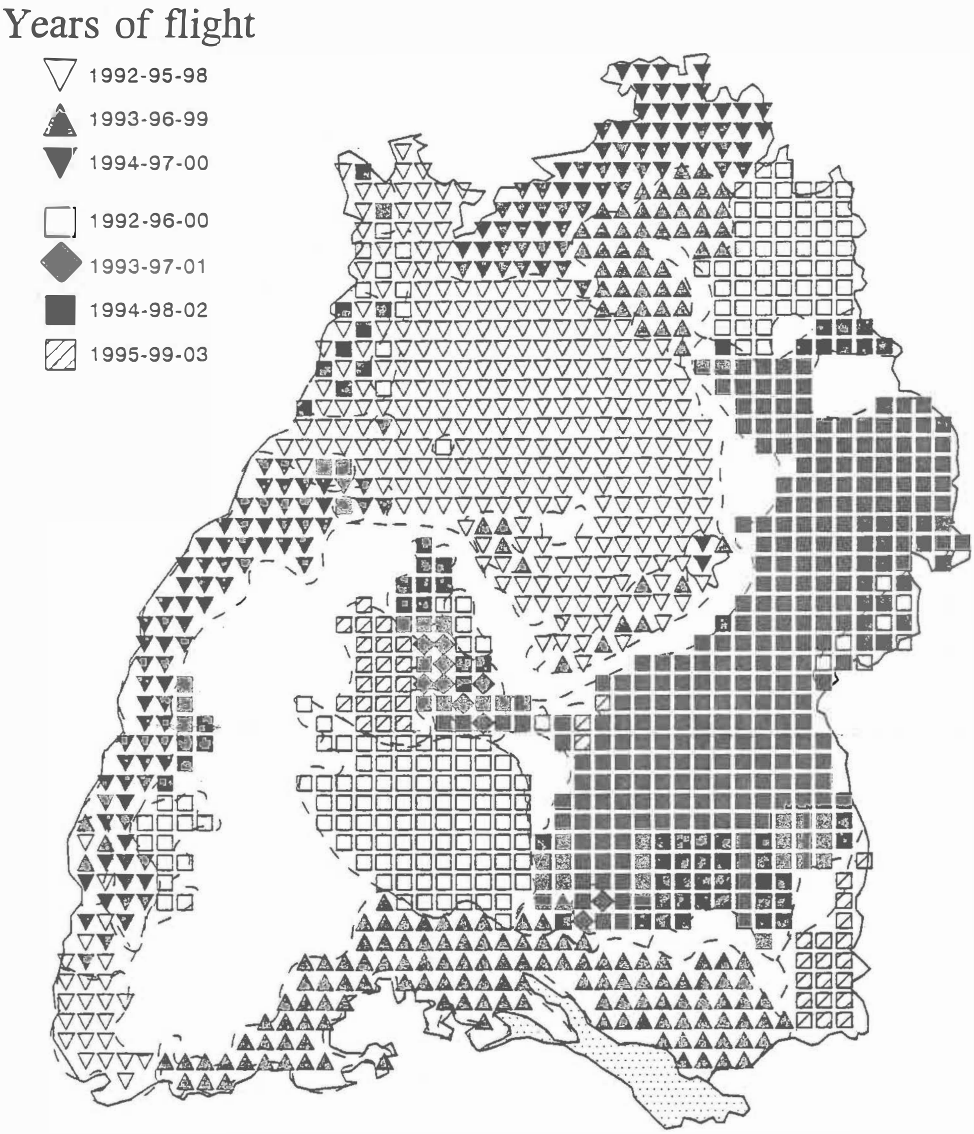
1. Schwäbische Alb (four-years-generation):
A section of some few kilometres parallel to the western borders of this mountain chain is recorded as infested. In 1992, two meadows located at 900 and 750 NN were completely destroyed by the pest. In both cases, involved farmers were not aware of the occurrence

of the Common Cockchafer larvae, despite considerable flight 1986. As a consequence of the 1994-flight infestation areas have expanded to include new regions. However, further damage was recorded only in a single case, namely on a sportsground in Sonnenbühl-Undingen. While searching for appropriate experimental sites, grub densities were assessed in 1995 on two meadows. They both proved to have a grub density reaching roughly 20 grubs/m$^2$ (3/4 of them as 3rd larval instar!).

2. Western Kraichgau (three-years-generation):
Only two months before the 1989-flight started damage by the white grubs were reported to our institute, indicating the infestation of three different parts in the area. Unfortunately, growers needed at least two generations of the Common Cockchafer to recognise the real causes for the retarded growth of their apple trees. In spite of the increasing spread according to the 1992 and 1995-flight respectively. The weather conditions between 1989 and 1993 (mostly dry and warm) have accelerated the larval development of less than 2% of the initial larval population inducing an early flight already in 1994. Biological control trials were initiated in 1989, using *Beauveria brongniartii* (Sacch.) Petch. The results obtained did not show any effective control. As a consequence of this experience two insecticides were tested on infested edges of three forests in the region. SCHNETTER will report on this subject tomorrow.

3. Kaiserstuhl (three-years-generation):
Since the unexpectedly heavy flight of 1991, a series of surveys and trials are initiated by our institute. The infested area seemed to be restricted to the northern parts of the region, but not beyond the county of Freiburg. There only very few farmers have noticed White Grub infestation. A possible explanation for this restriction of the invasion can be the soil types. Soils of the infested area can be globally characterised as degraded Loss including sandy loam. These soils are known to maintain soil humidity effectively in a dry summer. More shallow stony soils, flat or extremely heavy dominate towards the South. In those sites drought may have stopped the egg and larval development. Similar to Kraichgau only less than 2% of the larval population succeeded to reach the adult stage in 1992 and to emerge 1993. The situation has completely changed after the 1994-flight. In this year from the earliest infestation areas an average of 70 white grubs/m2 were recorded, with a maximum of 288 larvae/m2 in a vineyard.
Surveying the forestry edges of the more southern parts of the Kaiserstuhl region for adults of the Common Cockchafer showed the whole region to be infested. Even in those aeras where we have found only single specimens grub damage has been recorded from grapevine nurseries in 1995. These findings correspond with the warnings of ZELGER (oral communication).

As a conclusion, forecasting the development of the pest population must have the first priority. It is quite essential to follow the population cycles of the different Common Cockchafer populations in the state. To establish such a forecasting system, precise records on the previous outbreaks are essential. Since, however, these are not available, we only can make assumptions be used as to the actual phases of the cycles.




LfP, Stuttgart

Bearbeiter: Fröschle und Monger (nach Lüders, geändert)

Figure 1: Spread of the Common Cockchafer (*Melolontha melolontha* L.) in Baden-Württemberg

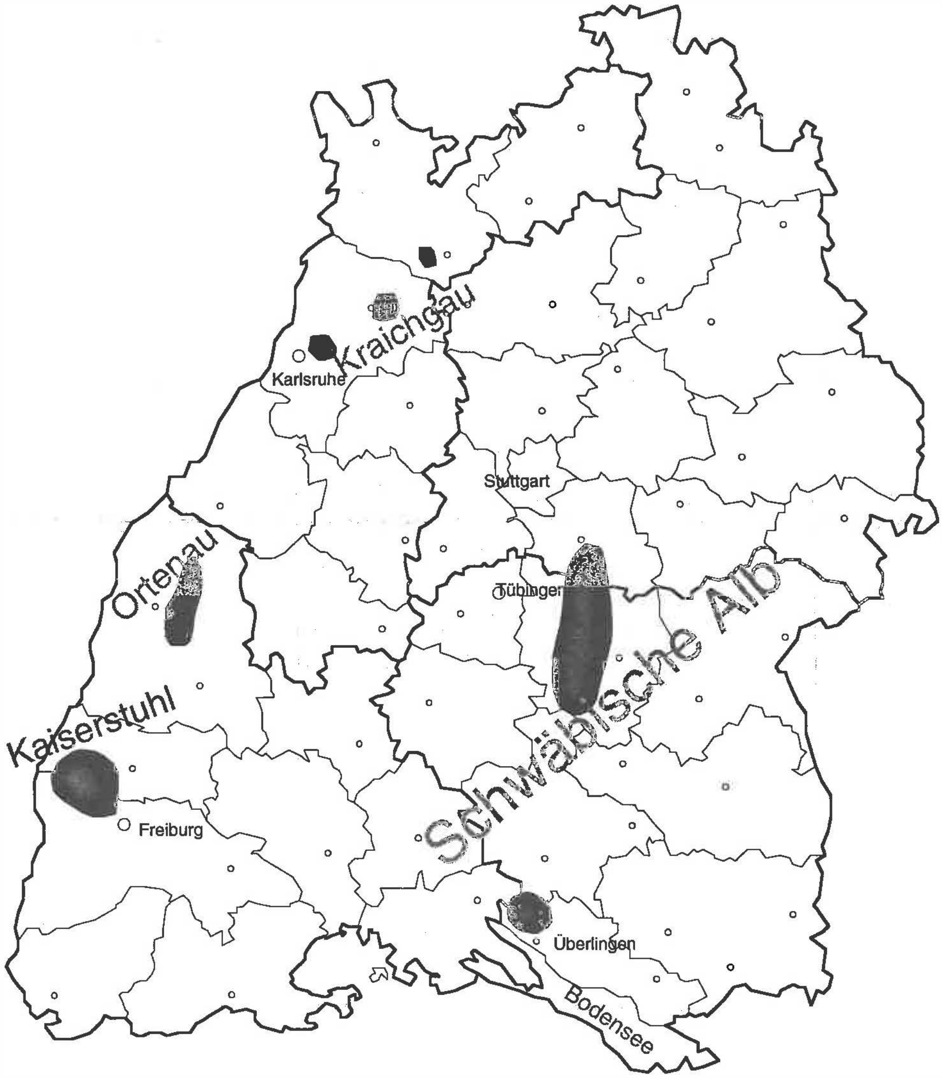



Figure 2: Regions with severe White Grub damage in Baden-Württemberg

# DISTRIBUTION ET GRADATION DE *MELOLONTHA MELOLONTHA* EN VALLE D'AOSTE

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RESUME

Depuis1987 sur le fond de la Vallée d'Aoste, de Morgex à Verrès (60 Km), des sondages sont effectués tous les 100 m pour vérifier l'évolution de la population de larves de *Melolontha melolontha L.* Tous les trois ans, en correspondance des L2, il est procédé à des sondages en travers de la Vallée (919 au total en 1993).

Ces contrôles ont permis de vérifier que:
- l'aire de diffusion est restée la même ≈9000 ha;
- la densité moyenne de L2 était de 32/m² en 1987, de 11/m² en 1990, de 12/m² en 1993;
- que la présence de larves mycosées est extrèmement faible: 0,38%.

## INTRODUCTION

Depuis toujours le hanneton cause de sérieux dommages aux cultures agricoles de la Vallée d'Aoste (Abbé Henry, 1644) mais ces dernières années l'arboriculture a été particulièrement touchée: plus de cent mille pommiers ont péri. Pour suivre l'évolution de ce parasite depuis 1987 sur le fond de la Vallée, de Morgex à Verrès (60 km) des sondages sont effectués toutes les années.

## BUTS

- vérifier l'aire de diffusion de ce parasite;
- vérifier la densité de la population larvaire;
- vérifier la régression dans le même cycle;
- comparer la densité des différents cycles;
- rechercher et établir l'éventuelle présence d'auxiliaires.

## DISPOSITIF

- moyens: pioches Blaisinger (lame de 16 cm), photographies aériennes;
- méthode: creusage d'une tranchée large de 17 cm et longue de 1 m, correspondant à 1/6 de m² (Chessel *et al.*,1984);
- distance des sondages: 100 m sur l'axe du fond de la Vallée et tous les 3 ans, en correspondance des stades larvaires L2, même sur les perpendiculiaires à l'axe de la vallée. Au total environ 920 sondages pour L1 et L3 et 2000 pour L2;
- unité territoriale 22 photographies aériennes.

RESULTATS

- Aire de diffusion de Morgex (1000 m) à Pont-Saint-Martin (343 m) pour un total de 7.830 ha.
  Faute de personnel, les zones de Verrès, de Challand et de Pont-Saint-Martin n'ont pas fait l'objet de sondages systématiques mais seulement de contrôles de la présence du parasite. L'aire de diffusion n'a pas varié depuis 1987; (fig. 1)
- La surface et les cultures concernées est ainsi repartie:

| | |
|---|---|
| prairies permanentes | 7.380 ha |
| vergers | 400 ha |
| vignobles | 50 ha; |

- En 1987 la densité moyenne de larves L2 par culture était:

| | |
|---|---|
| prairies | 42 larves/m² |
| vergers | 29 larves/m² |
| vignobles | 0,1 larves/m²; |

- La densité moyenne de la population larvaire (L2) a subi des fluctuations importantes dans son ensemble (fig. 2) et dans chaque unité territoriale (fig. 3). Voir graphiques;
- le nombre de larves momifiées par la moisissure *Beauveria brongniartii* repérées pendant les sondages est extrêmement faible (0,38% en 1993);
- l'examen de 16 lots de larves provenant des sondages et contrôlés pendant la quarantaine par P. Robert (I.N.R.A.) a donné les résultats suivants: la mycose à *Beauveria brongniartii* est présente en 14 lots (87%) et 27 larves (17%) tandis que les autres germes comme *Nosema, Bacillus popiliae* ou *Metarrhizium* sont assez rares.

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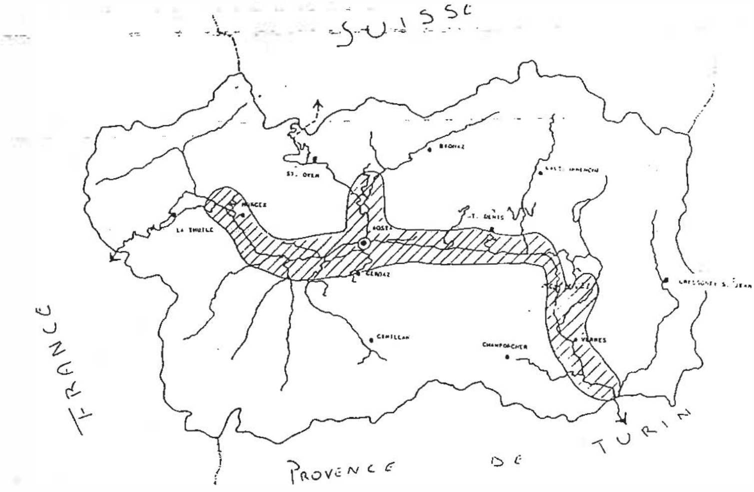
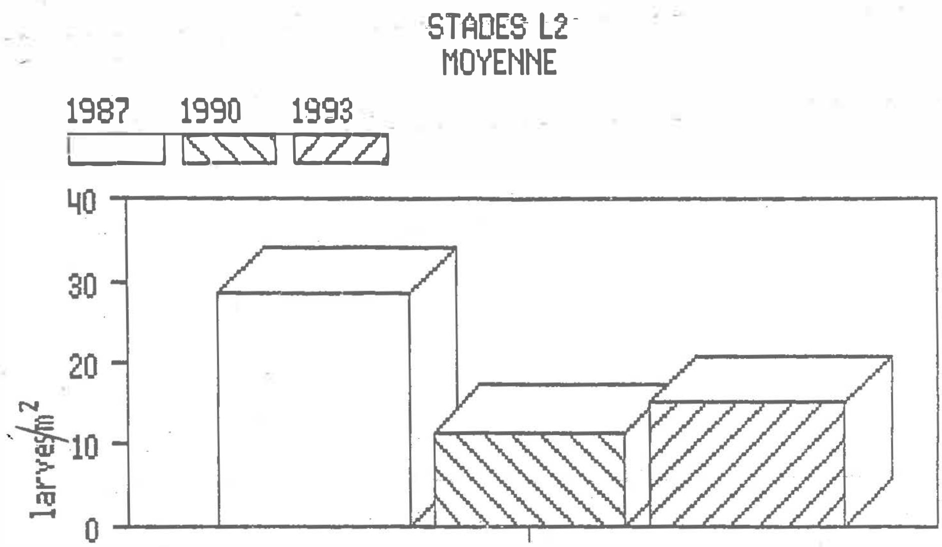
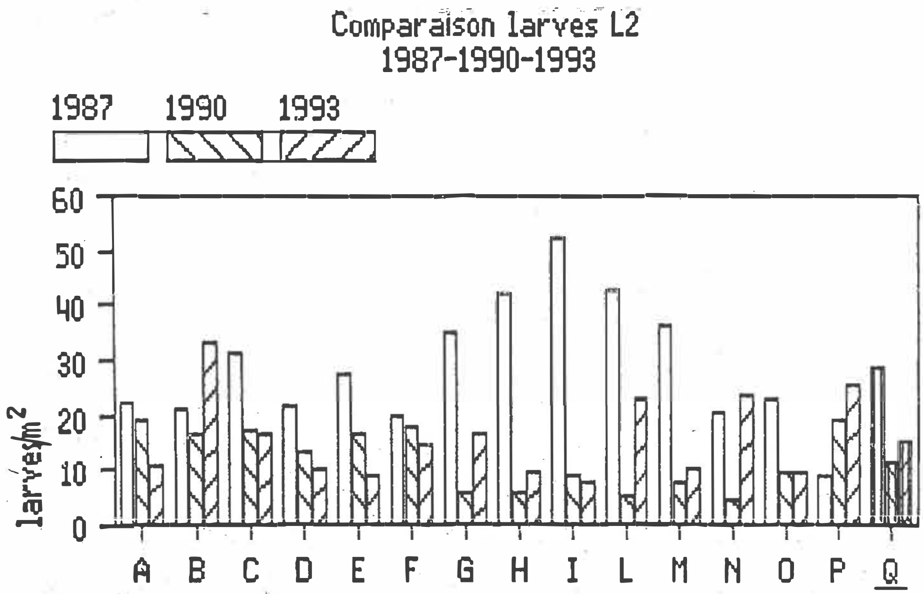



Fig. 1 - Aire de diffusion du hanneton en Vallée d'Aoste (Italie)




- Figure 2 -




A B...P: unités territoriales se rapportant à une photographie aérienne
Q: moyenne

— Figure 3 —

# Population development and dispersal of Melolontha and other Scarabaeidae in the Netherlands during the past ten years

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## Abstract

The grubs of *Melolontha* species cause nowadays considerable damage in The Netherlands. They feed on roots and other underground parts of a very wide range of plants. Serious damage is also inflicted by two other Scarabaeids: *Amphimallon solstitiale* and *Phyllopertha horticola*, which are also polyphagous, but feed mainly on grass roots. An extensive survey was done over the last ten years, including observations of the hatching periods of adult beetles and the course of the damages over the last six years. An increasing damage has been observed in this period. In 1993 a severe pest of *Melolontha* was recorded. Thusfar, control of the big grubs of this species is problematic. It is argued that further research is necessary to develop effective control programs based on better knowledge of the biology of the species concerned.

key words: Scarabaeidae, *Melolontha*, *Amphimallon*, *Phyllopertha*, outbreaks, population developments, damage, inventarisation, locations, flight periods, control

## Introduction

Since the early eighties the Plant Protection Service received complaints about serious damage to underground plant parts caused by larvae or grubs of Scarabaeidae. The main problems are caused by *Melolontha melolontha* (L.), *Amphimallon solstitiale* (L.), and *Phyllopertha horticola* (L.) (De Goffau, 1990). From history we know several outbreaks of *Melolontha* (Anonymus,1826, Ritzema Bos, 1882, Verver, 1870) and *Phyllopertha* (Sorauer, 1954) in Europe. As far as we can trace outbreaks of *Melolontha* sp. last for about 10 years and take place with intervals of fourty to fifty years. In recent years in the Netherlands again problems arose caused by these grubs. Plants, trees and grassfields die off by fretting. If birds discover the place of these tasty larvae in the ground, they cause secundary damage by foraging. We do not have the right treatment, neither chemical, nor biological. Older big *Melolontha* larvae, causing the most extensive damage are not sensitive enough for the registrated chemicals. Research on chemical control has been terminated nowadays in the Netherlands. Biological control experiments with eelworms have been done by the Research Institute for Plant Protection (IPO-DLO) at Wageningen, but they proved not effective enough against the bigger grubs under field conditions.

To handle these problems, we consider it essential to study the biology of the species concerned in detail. Observers, mainly officials of the Plant Protecting Service, collect all available data from different sources. This acquired information will help to find new methods to control these insect pests.

## Material and methods

Special registration cards with inquiries about damage, presence and location have been developed. In cases of beetle flights it was asked: observing date, point of time, area, municipality, and soil use, like type of nursery, sportfield or privately-owned garden or nature area and finally defining local vegetation and soil-type. Concerning the larvae the list of questions was similar with the following extensions: extend or gravity of damage, damage in previous years, north/south declivety and depth of occurence in the soil. All beetles and larvae were identified to uphold the required scientific level. Unfortunately we could not distinguish *Melolontha melolontha* (L.) and *Melolontha hippocastani* F. in the larval stages; so the term *Melolontha* sp. is used.

We have received data from arboriculture (De Goffau & Alkemade, 1994), orchards (De Goffau & Das, 1994), sport fields, farms, parcs and privately owned gardens. All observers received a reply with identification and further details.

An internal report has been composed every year from the collected data since 1989.
The data of each year in the research period have been recorded in tables and each verified observation was plotted on a map. It should be kept in mind that the presence of the grubs is only noticed when real damage appear above groundlevel.

## Results

Locations
Separate maps have been prepared of the locations of damages for each year (fig. 1 shows the damages in 1994) and from the locations of the observed beetle flights in The Netherlands (fig. 2). These maps provide a good overview of the locations with problems caused by Scarabaeids.

It is remarkable that, up to now, serious damage was only reported from sandy soils with a low groundwater level; *Melolontha* grubs, however, can also develop in loamy and clayey soils (Sorauer, 1954). In the growing-season the grubs were found from just under the sods to 30 cm deep depending on the depth of the roots of the plants, trees or shrubs. The grubs can actually kill turf and herbaceous plants, but also trees and hedges die. Especially grubs of *Melolontha* are very polyphagous; however, apparently they do not eat roots of *Bellis perennis* L. (family Compositae) and *Helleborus* sp. (family Ranunculaceae). It is evident now that also regions that used to have no problems, have to cope with recurrent damage. The explanation of this is that wet or marshy soils are well drained nowadays and the grubs can thus develop unhindered in the dry soil. We found that for their food the beetles need trees or at least shrubs in the neighbourhood.

Considering the high number of big *Melolontha* larvae in 1993 we expected many beetles in May of 1994. This has not happened; the larvae kept on feeding in the spring of 1994. Later on in the hot and dry summer, with temperatures of about 30 °C, they disappeared somehow. It is known that the larvae stay on the best depth for the most favourable temperature and humidity. At about 28 °C they die (Sorauer,1954). Yet in the summer 1994, deep underground, there were found at last just pupating larvae; the beetles will hatch a year later than expected. Evidently these larvae did not grow sufficiently in the 1993 autumn; it is known that in the Netherlands *Melolontha* spp.

indeed have a three or four years life-cycle.

In some cases other species caused identical problems, for example grubs of *Serica brunnea* (L.) that feed on roots of trees (see fig. 1).

Extent of damages

The number of damages over the years are showed in fig. 3. Problems with *Melolontha* spp., which have a three to four years life-cycle in the Netherlands, have increased since 1981. The number of real damages rose slowly but steadily, interrupted in some years. The damage numbers of *Amphimallon solstitiale*, which has a life-cycle of two years, showed an irregular pattern over the years. *Phyllopertha horticola* with its one year life-cycle gave rise to the highest number of damages at first, but *Melolontha* spp. caused most of the trouble since 1990. An increase of all three species has been noticed in 1993, up to a spectacular level in the case of *Melolontha* (De Goffau, 1994). In 1994 there were slightly less problems on the whole, as compared to 1993.

Flight periods

An analysis of the flight periods of the beetles (fig. 4) proved to be useful; the more so as the eggs are laid in this period, followed by the hatching of the larva four to six weeks afterwards. The onset of adult emergence proved to be greatly influenced by the preceeding weather conditions, especially the temperature. The flight period of the *Melolontha* beetles almost overlapped the flight period of *Phyllopertha horticola*. The flight period of *Amphimallon solstitiale* was somewhat later in the year. In some years with a slight overlap by *Melolontha* flights (see fig. 4.). The *Melolontha* beetles flied between 20.00 and 23.30 hour with only a few exceptions. Reports concerning the less common *Amphimallon solstitiale* were between 20.45 en 21.15 hours. *Phyllopertha horticola* flied chiefly between 12.00 en 14.30 hour.

Reported damages all through the year

The number of the damages during the last 6 years are given in fig. 5. There were some problems in spring, but most damages became visible in late summer or autumn. Probably damage occurs at different times of the year depending on wether the grubs follow a three or four years life-cycle (see fig. 5). As discussed above the not sufficiently grown *Melolontha* grubs in autumn 1993 kept on feeding in spring 1994. It is also obious that the ultimate losses depend on the preceeding weather conditions. Under wet conditions the visible damage is less severe than after continuing dry weather. If not completely destroyed, the plants have the chance to form new roots after rainfall as happened in the late summer and autumn of 1993 with damaged turfs. Although especially trees will not recover sufficiently.

## Discussion

The data presented may be used for developing a strategy pertaining to the control of Scarabeid beetles. More information is however necessary.

It is advisable to examine more in detail which plants are especially affected by the grubs and which are not.

An outbreak of *Melolontha* grubs in 1993 in pastures appeared suddenly. Damage by larvae had already been supposedly present in the years before, but this was not noticed in the rough grassland. The number of grubs was, however, so high in 1993, about 100 per square meter, that the sods had been completely loosened from the soil. It has also happened repeatedly that the bad condition of vegetation was ascribed to several other causes, until the grubs were found after removing the dead plants, trees or shrubs. In view of the experiences in the past, in combination with the lack of good control, we can expect some years with serious damage.

Experiences of other countries concerning outbreaks and control possibilities should be exchanged. For succesful control it is needed to have at hand the right parasites, predators, pathogens or other issues for prompt delivery.

**Acknownledgements**

I wish to thank our district employees who collected the data actively over several years. Also the employees of the Department of Pest Control of the Ministry of Housing, Spatial Planning and the Environment, who furnished data about beetle flights. I am grateful to my colleague mr. G. Romeijn for his help to construct the figures.

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Damages caused by larvae in 1994

Beetle flights in 1994

Figure 1.

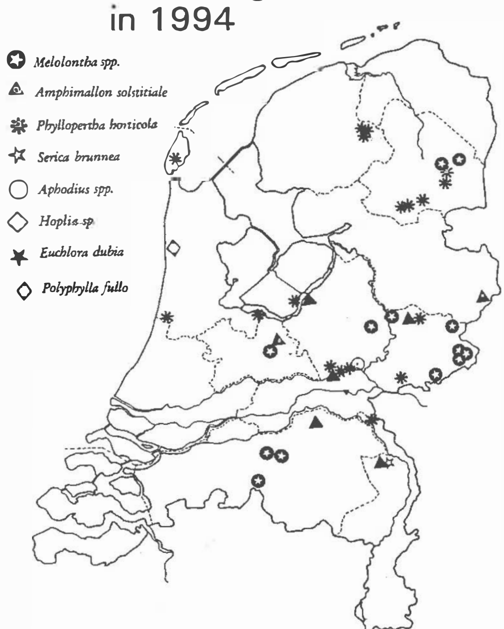


Figure 2.

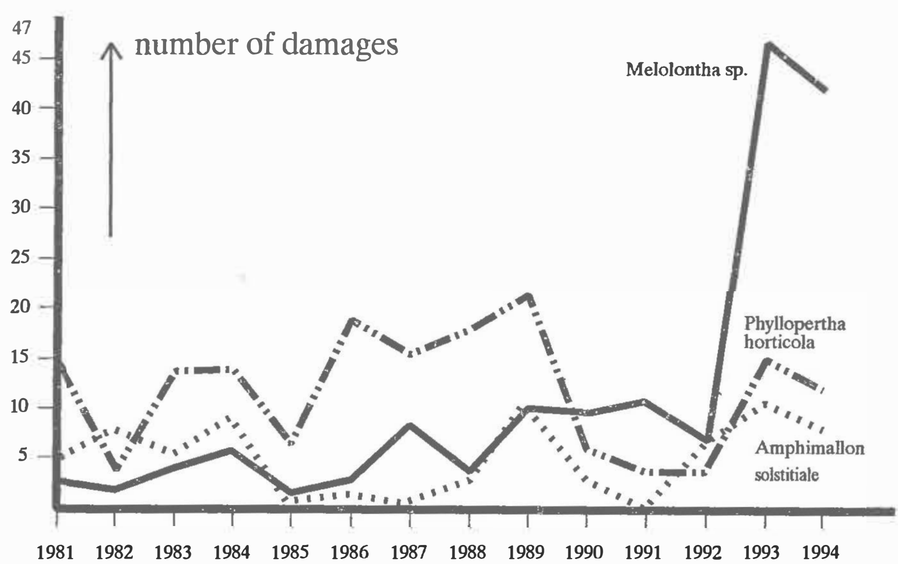


Figure 3.

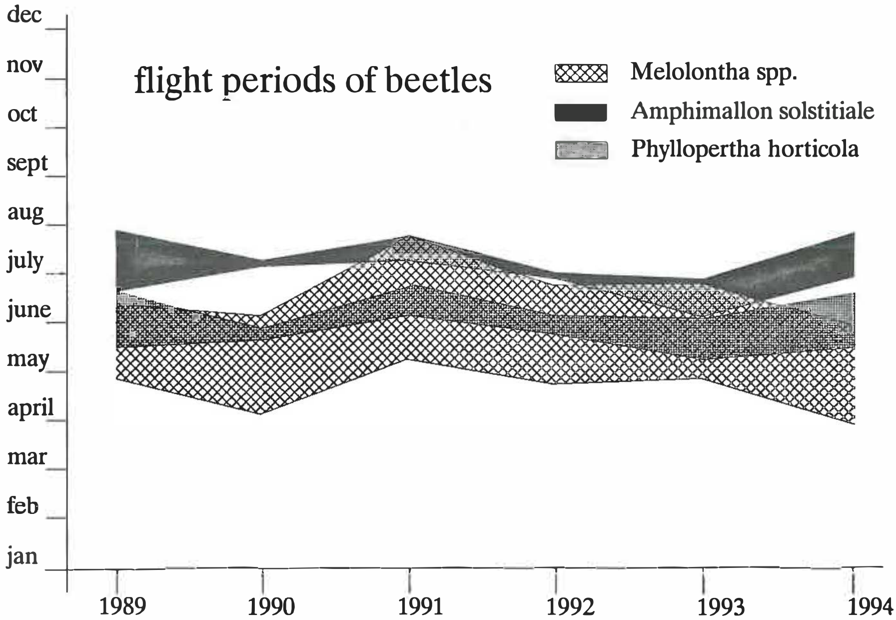


Figure 4.

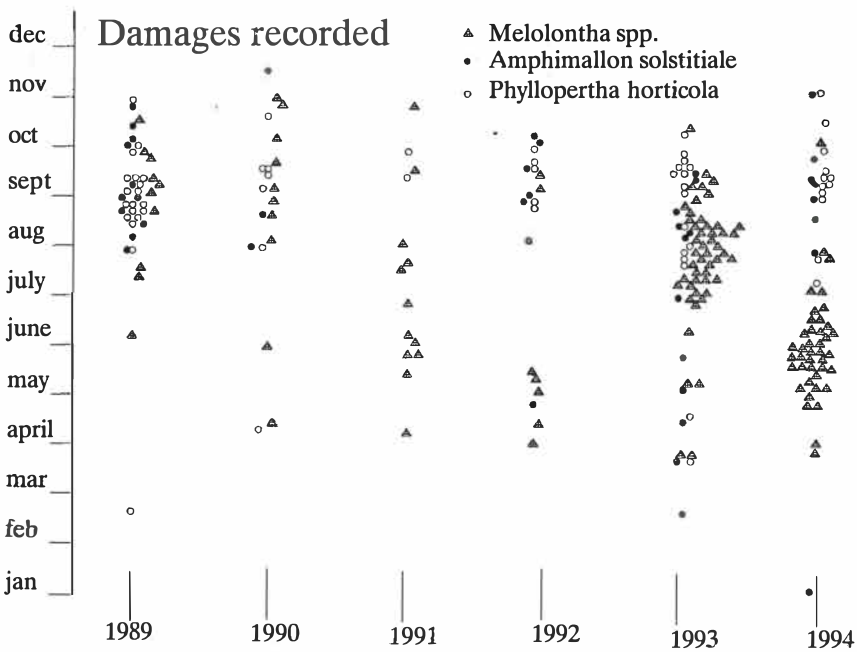


Figure 5.

# PRESENCE AND DIFFUSION OF THE COMMON COCKCHAFER (*Melolontha melolontha L.*) IN THE AREAS OF MEZZOCORONA AND SAN MICHELE a/A IN TRENTO PROVINCE.

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## SUMMARY

Presence and diffusion of the common cockchafer (*Melolontha melolontha* L.) in Trentino, in Mezzocorona and San Michele a/A areas.

In Trentino the first centres of infection of the new presence of the common cockchafer (*Melolontha melolontha* L.) were reported in 1987. Since then some precise controls have begun to evaluate the population's density and the diffusion of this plant pest. The affected area increased from two initial centres of infection in 1987 to about 100 ha in 1989-90 and with the flight of 1991 it reached about 150 ha. With the flight of 1994 the affected area increased to about 250 ha although the population had decreased.
As for apple trees and young vineyards, the greatest part of damages are caused by the larval activity of this Coleoptera species, which causes heavy damage on the root system.

## INTRODUCTION

The Trentino region has historically always been an area affected by periodic infestations of the common cockchafer. During past decades attention was always focused on the adults; in fact people had resorted to manual collection by percussions as a strategy of control (Bollettino Agrario 1886). The last reports of enormous populations date back to the period immediately after World War II; in recent times we have been observing a return of this coleoptera species, and for the first time there has been a shift in the interests towards the larvae, which may cause substantial damages to the roots of apple trees and young vines (until 2-3 years of age), thus threatening in certain instances the main crops of our area. The main problems, however, arise in the case of apple trees, which due to their superficial root system limited to the upper 20-30 cm of the soil are always, irrespective of age and rootstock, susceptible to attack by the larvae of the cockchafer. The vines, on the other hand, are not affected by the presence of the larvae once they have reached the age of 2-3 years, because their root system reaches layers deeper than those explored by the larvae of the cockchafer.

## MATERIALS AND METHODS

From 1989 samplings were carried out in the areas affected by the presence of the cockchafer; the observations were made by counting all the adults and larvae present in holes 50 cm x 50 cm wide and 50-70 cm deep. 4-6 holes were dug per hectare with the soil being sieved in the field.
In 1991 and in 1994 also visual controls of the flight were carried out, giving an evaluation of the duration and intensity of both the nutrition flight (towards the woods) and the oviposition flight (return flight).

## DISCUSSION

### Observations until 1988

The first reports of the new presence of this pest date back to 1987 when two foci of infection of an area of ca. 1 ha each were detected in the region of San Michele a/A showing the first plants suffering from the presence of the larvae of the cockchafer. The flight of the adults and with it the spreading of the population took place in 1988.

### Situation in 1989-90

During 1989, in correspondence with the period of major larval activity, there were increased reports of foci of infestation and of casual damages on apple trees and young vines, in an area of ca. 100 hectares in the region between San Michele a/A, Mezzocorona and Cadino, located mainly on the orographic left of the river Adige.

| Situation in autumn 1990: adults | | |
|---|---|---|
| **Zone** | **infested area** | **average presence/m²** |
| **1st focus** | **100 hectares** | **25 adults** |
| **areas of recent expansion** | **15 hectares** | **6 adults** |

In autumn 1990 accurate controls were carried out in order to evaluate the presence of adults in the affected area and the nearby surroundings. The result of these investigations revealed a mean population of 25 adults/m$^2$ in the area of the first focus (orographic left of the river Adige), with values ranging from a minimum of 5 to a maximum of 55 adults/m$^2$. Further controls carried out in the nearby area situated on the orographic right of the river Adige showed an average presence of 6 adults/m$^2$; this latter area was also regarded as a possible area of expansion with respect to the flight of the following year. During these investigations it clearly emerged that the most consistent populations were present in sandy soils, whereas the least numerous were present in soils with a more compact structure.

### Observations of the flight in 1991

Starting from the last decade in March periodic controls in the evenings were carried out aimed at monitoring the flight of the cockchafer. This date for the beginning of the observations was chosen because of the precocity of the year and because of the beginning emergence of adults, which could easily be noticed by the typical round-shaped holes particularily in the sandy soils. The flight towards the woods started the 31st of March, reached its peak between 6 and 10 April and continued approximately until 12 May. The preferred direction during this flight was towards the woods, but a part of the population remained also in the orchards feeding on cherry and apple trees; there was not the least damage on peaches. In the woods beeches and oaks were the preferred species. The return flight started around 8 April, reaching a first peak at mid month and continuing until 22 - 23 May.

It should be noted that during the period of the flight (precisely beginning from 17 April) there was a sudden fall of temperature to mean values around 5-7°C for a rather prolonged period. The flight was interrupted for 6-7 days and resumed as the temperatures rose again. Also the rains during May interfered with the intensity and duration of the return flight which reached a last peak around mid May.

**Situation after the flight in 1991**

During early summer the first controls for the evaluation of the oviposition were carried out, but only in September and October 1991 the new population present in the typical areas and in those of new expansion was monitored. 10 samplings were performed in the area of the focus and 15 in the nearby area of possible expansion.

| Situation in autumn 1991: larvae 1st year | | |
|---|---|---|
| **Zone** | **infested area** | **average presence /m²** |
| **1st focus** | **100 hectares** | **26 larvae** |
| **areas of new expansion** | **50 hectares** | **33 larvae** |
| **adjacent zones** | **100 hectares** | **4 larvae** |

The results indicate a mean population of 26 larvae/$m^2$ in the area of the first focus (orographic left of the river Adige) with values ranging from a minimum of 5 to a maximum of 99 larvae/$m^2$. The areas of new expansion show a mean population of 33 larvae/$m^2$ in the zone located on the orographic right of the river Adige (ca. 50 hectares) with a minimum of 12 to maximum of 62 larvae/$m^2$. In order to assess further expansion, the controls were extended to about another 100 hectares adjacent to the infested area, where however the population density was low (on average 4 larvae/$m^2$).
During the years '92-'93 there were several reports of damages both in the area of the first focus and the areas of new expansion.

| Situation at the beginning of spring 1994: adults | | |
|---|---|---|
| **Zone** | **infested area** | **average presence/m²** |
| **1st focus** | **100 hectares** | **19 adults** |
| **areas of new expansion** | **50 hectares** | **15 adults** |
| **adjacent zones** | **100 hectares** | **3 adults** |

The further assessment of the adults, carried out on 24 March 1994, showed the same situation as the previous control on the larvae. The various samplings gave as result a mean population of 19 adults/$m^2$ in the area of the first focus, with a minimum of 4 to a maximum of 74 adults/$m^2$. The areas of new expansion presented a mean population of 15 adults/$m^2$ with a minimum of 4 and a maximum of 31 adults/$m^2$. In the areas adjacent to the infested zones a low population density was detected (3 adults/$m^2$).

**Considerations**

The events of 1991 can give place to the following considerations:
- the adverse climatic conditions exactly in the moment of the flight probably had a detrimental effect on the presence and on the new diffusion of the cockchafer; with more regular climatic conditions both the population density and the infested area could presumably have been higher.
- In the typical zones the population remained constant and in some cases slightly decreased.
- The area located on the orographic right of the river Adige (between river Adige and the motorway), which was less infested during previous years, showed an increased population density after the new flight; thus the area of new expansion can be estimated of comprising about 50 hectares.

- Analyzing the population densities a gradient of infestation can be observed; in fact both on the right and on the left side of the river Adige the population decreases with increasing distance from the river banks. This indicates that the more the soil is sandy, the more it is suitable for the cockchafer.

**Observations of the flight in 1994**

The observations were carried out beginning from mid March.
The flight towards the woods started on 23 March, it remained weak until the end of the month and increased afterwards reaching its peak between 12 and 20 April. The flight ended around mid May.
During the flight the preferred direction was towards the woods, however between 18 and 20 April a great quantity of adults stopped for feeding in the orchards and vineyards. During those days in fact the sky was cloudy and there were slight precipitations.
The return flight started on 25 April, reached a first peak from 2 to 5 May and continued until 20 May.

**Situation after the flight in 1994**

During 1995, which corresponds to the period of the highest larval activity, there were only a few reports of damages.

| Situation in autumn 1995: larvae 2nd year | | |
|---|---|---|
| **Zone** | **infested area** | **average presence/m²** |
| **1st focus** | **100 hectares** | **8 larvae** |
| **areas of new expansion** | **50 hectares** | **5 larvae** |
| **adjacent zones** | **100 hectares** | **5 larvae** |

At the beginning of October 1995 the area affected by the cockchafer was monitored.
In the area of the first focus the mean population was 8 2nd year-larvae/$m^2$. The areas of new expansion and the adjacent zones (100 hectares of further expansion) presented a mean population of 5 larvae/$m^2$ with a minimum of 0 and a maximum of 17 larvae in both areas.

**Considerations**

According to the events after the flight in 1995 the situation can be summarized as follows:
- In the area of the first focus and in the areas of new expansion the population decreased, although still present at a mean density/$m^2$ which constitutes a hazard for the young plantations.
- In the adjacent areas the population density slightly increased and affected further 100 hectares.

It is not clear whether the decrease of the population in the infested areas was due to natural causes or due to the nets, which by covering the foci of infestation reduce the number of adults at the time of the flight.

| Diffusion of the cockchafer at Mezzocorona and S.Michele a/Adige | | |
|---|---|---|
| **year** | **affected area** | **mean population/m$^2$** |
| **1990** | **100 ha** | **25 adults/m$^2$** |
| **1993** | **150 ha** | **13 adults/m$^2$** |
| **1995** | **250 ha** | **6 larvae/m$^2$** |

This table summarizes the diffusion of the cockchafer from 1990 to autumn 1995. It can be seen that the affected area has increased although the population density has declined.

**Existing species**

During the flights the presence of two morphologically different types which seem to be *Melolontha melolontha L.* and *Melolontha hippocastani F.* was observed. The two species were often observed mating, but there is no proof of fertilization having taken place. It should be stressed that in our region the flights occur in three-year cycles and that the flight intensity in the years between the flights is insignificant; moreover, from the numerous soil-samplings emerged that the observed larvae were of the same age except a very few individuals which were of different development stages.

**Methods of control**

The farmers sprayed common phosphoric esters approved by Italian law for soil treatments. The results were little satisfactory: although a plant can be saved, there is absolutely no impact on the population density. For this reason also in our region resort was taken to the fungus Beauveria brongniartii, which was kindly supplied by the Agricultural Research Centre Laimburg (BZ). In 1989 the treated area amounted to 10 hectares, whereas in late summer 1991 the area extended to 80 hectares.
It seems however that by covering the soil with plastic nets the decline of the population occurs more rapidly. In the region 12 hectares were covered in 1991 and 25 hectares in 1994.

## CONCLUSIONS

The experiences realized in our region about the cockchafer are interesting both from the experimental point or view as for the applicative aspects in the field of rural production. This is one further experience which proves on one hand the great limits of the chemical means of control as compared to the force of nature, and on the other demonstrates that for solving any problem in the field it is essential to have knowledge about the basic processes, constant controls, and a tight collaboration between farmers, advisors, researchers and politicians, since only from a valuable and productive cooperation valid approaches for the solution of such vast problems can arise.

## ACKNOWLEDGEMENTS

We express our thanks to:

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-the colleagues at the Istituto Agrario di San Michele a/A and at the technical advisory service ESAT for their great help during the periods of the controls in the open field.

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# PROBLEMS OF THE OCCURRENCE AND MANAGEMENT OF MELOLONTHINAE IN POLISH FORESTRY

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In this paper we would like to present problems of the occurrence of the most important species of insect pests feeding on the roots of forest trees and shrubs, the method of prognosis and the method of control in Polish forestry

## PEST OCCURRENCE

Insect pest living in soil and feeding on the roots of forest trees and shrubs in Poland as well as the importance of respective species are presented in Table 1. The most important are the following species: *Melolontha melolontha*, *Melolontha hippocastani* and *Amphimallus solstitialis* (Woreta 1994). These three species have the greatest participation in the structure of insects occurrence over last years (Fig.1). *Melolontha* sp. occur practically over the whole territory of the country (Fig.2). Basing on the prognostic data from 1956-1990, seven regions of the most frequent occurrence of *Melolontha* sp. in Poland were found (Śliwa 1993). The greatest threat from white grubs concerning mainly nurseries and young plantations, but all forest tree species can be attacked by these pests.

The areas of forest nurseries and plantations threatened by insect pests of roots between 1956-1995 are given in Fig.3. The greatest area threatened by soil pests recorded in 1960 covered more than ten thousand hectares. In the following years that area succesively decreased and attained the level of 500 hectares in 1980-1990.

Since 1990, the situation has significantly changed and the area threatened by soil pests has succesively increased to reach the level of 1645 hectares in 1995. Appart from a higher activity of Melolonthinae, those changes result from the afforestation of large areas of non cultivated lands abandoned by agriculture, where soil pests have comfortable conditions for their development. Such was the situation in 1960's and at present it develops in a similar way.

Fig.4. illustrates the proportion of the respective types of soil threatened by insect pests of roots in afforested area in 1995. The Figure shows that mainly the lands abandoned by agriculture are subject to afforestation. It can be forseen that the problem of the threat by insect pests of roots will become more serious because still more lands not cultivated by agriculture will be afforested.

In the future, the area forseen for afforestation will be 1 300 000 hectares. Taking into account that only 10% of this area will be threatened by pests of roots, it may be easily assumed that the area which must be protected is 130 000 hectares. It is evident that the problem of white grubs is very serious.

**PROGNOSIS**

The prognosis of the threat by pests of roots refers usually to (collective work 1988):

- the areas which will be afforested or reforested
- the areas in which nurseries will be established
- the areas of existing nurseries

Each year usually in September the control pits are dug out and white grubs are counted.

The control pits measurements are 1 x 0,5 m and their depth depends on the level of insects occurrence.

The following number of pits are required (collective work 1988):

- nurseries to 1 ha: at least 2 pits for each 1000 m
- nurseries greater than 1 ha: 15 pits for each hectare
- afforestation and reforestation areas: 6 pits for each hectare.

For example, the approximate critical numbers of white grubs are:

for nurseries, independent of insect age: *Melolontha* sp. - 0,5 indiv. per pit, *A. solstitialis* - 1 indiv. per pit.

for plantations, depending on insect age and type of forest soil:

*Melolontha* sp. - 1 to 4 indiv. (two-year-old) per pit, *A. solstitialis* - 1 to 8 indiv. (two-year-old) per pit. In dry soil the critical numbers are lower.

If the numbers of white grubs found in control pits are higher than that of the established approximate critical numbers, the protection is needed.

**MANAGEMENT**

The plantation of forest trees in areas threatened by pests of roots without protection treatments is practically not possible. As far, the application of chemicals is the main method of protection against these pests. At present no better means are known in a situation when trees must be planted and the quick control of pests is needed.

In Poland the following insecticides against soil pests are mainly used in surface treatments before planting (Głowacka 1995, Malinowski 1995):

- Basudin 10 G and other products containing diazinon as active ingredient at a dose of 80-120 kg/ha.
- Dursban 480 EC and other products containing chlorpirifos as active ingredient at a dose of 2,5-3 l/ha in 450-600 l of water
- Marshal SuSCon 10 CG containing carbosulfan as active ingredient at a dose of 80-120 kg/ha

Marshal suSCon 10 CG is a special controlled release formulation with active ingredient concentration of 10% which controls white grubs during 2 years (Malinowski, Mazur 1995). According to Polish classification, such products as Basudin and Marshal suSCon 10 CG belong to the third class and Dursban 480 EC to the second class.

Another method in reducing white grubs population is the aplication in the hole of the following granular insecticides during or after planting using special applicators:

- Marshal suSCon 10 CG at a dose of 10 g/plant
- Furadan 5 G containing carbofuran as active ingredient or other analogous product at a dose of 4-9 g/plant
- Counter 5G containing terbufos as active ingredient at a dose of 5-10 g/plant

The products, Furadan 5G and Counter 5G are rarely used because of their toxicity to higher animals.

- If nessessary, Dursban 480 EC may be used during growing season at a dose of 5 l/ha in 2000 l of water (surface treatment).

The development of new methods of white grubs management, more safe for the environment is needed.

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Table 1

**THE MOST IMPORTANT INSECT PESTS LIVING IN SOIL AND FEEDING ON ROOTS OF FOREST TREES AND BUSHES OCCURING IN POLAND**

| Species | Importance |
|---|---|
| *Melolontha melolontha* L.<br>*Melolontha hippocastani* F.<br>*Amphimallus solstitialis* L. | Great importance in nurseries and plantations |
| *Phyllopertha horticola* L.<br>*Anomala aenea* Deg.<br>*Agrotis* sp. | Local importance in nurseries |
| *Serica brunnea* L.<br>*Polyphylla fullo* L.<br>*Elateridae*<br>*Tipulidae*<br>*Curculionidae*<br>*Gryllotalpa gryllotalpa* L. | Small importance, sporadic occurrence on small areas |

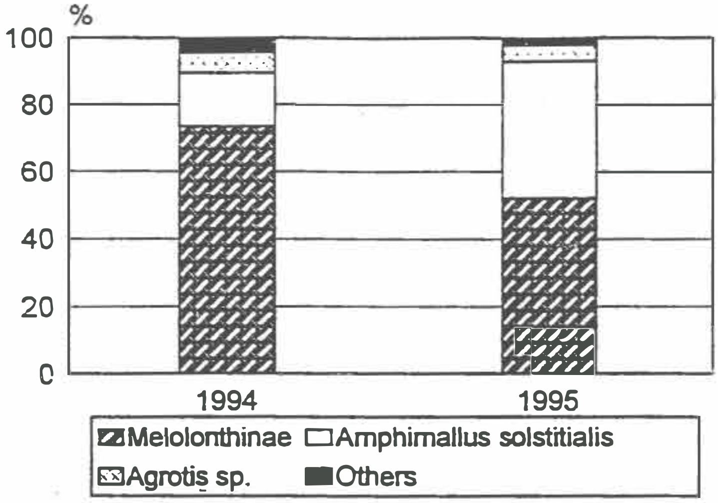


**Fig.1. Part of the respective groups of insect pests feeding on roots of trees and shrubs in the structure of insects occurrence in 1994 and 1995.**

**Fig.2. Regions of the most frequent occurrence of Melolontha sp. in Poland (based on prognostic data from 1956 - 1990).**

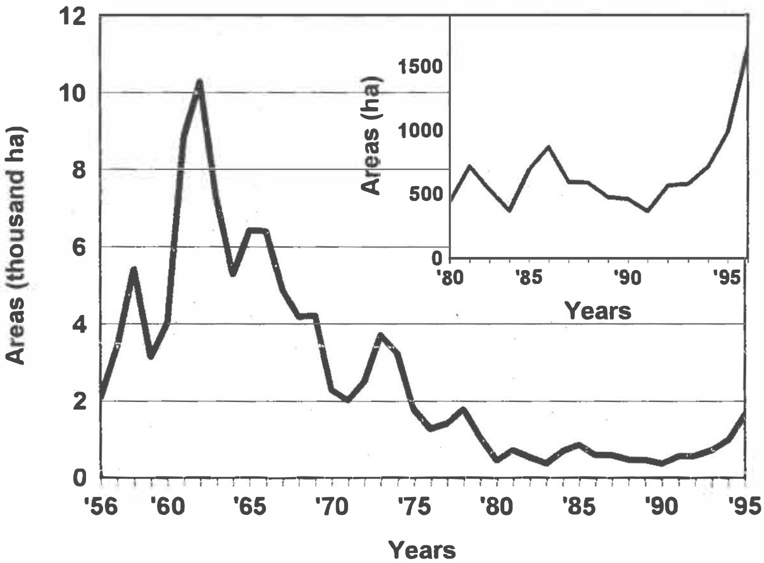


Fig.3. The areas of forest nurseries and plantations threatened from insect pests of roots.

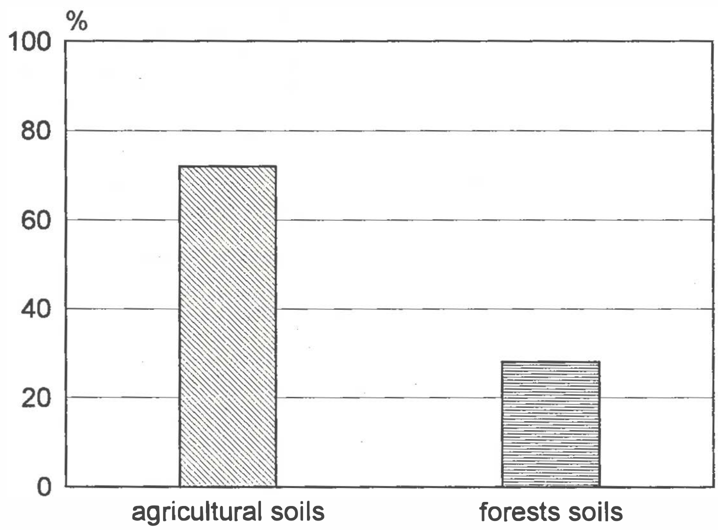


Fig.4. Part of the type of soils threatened from insect pests of roots in afforested area in 1995.

# ON THE OCCURENCE OF THE COCKCHAFER (*MELOLONTHA MELOLONTHA* (L.)) DEPENDENT ON THE PRESENCE OF DANDELION (*TARAXACUM OFFICINALE* WIGGERS)

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**Abstract**

According to SCHWERDTFEGER and DARUP (1955) the abundance of the beetles had to be expected to significantly increase at the end of the seventies. Taking into account this forcast several investigations on the population dynamics of the chockchafer were proposed (SCHÜTTE, 1976).
The investigations from laboratory verify that the roots of dandelion are the prior feeding plant for grubs as HUBER (1964) followed from his field observations before. The beetles lay their eggs near dandelion plants providing the grubs with the appropriate feeding plants.
The plant protection offices stated that no gradation of the cockchafer has been recorded during the last 57 years in Schleswig-Holstein. The maybug could propagate and caused some harms but not gradation was recognized in the other northern countries of Germany either. In the alpine area damages were also due to this species and it was controled by insecticides here and there.
The tendency of increasing abundance from north to south was not only true for the maybug but for dandelion too.
The beetle´s importance revealed by the numbers of publications written within the last 30 years also differed between the Europaen regions. The number of publications from the alpine area has been steady between 1965 and 1985 and increasing afterwards. In 1966, 1981 and 1988 severe damages were due to the maybug there. In Germany where no tendency could be recognized damages were recorded in 1977, in the eigthies and in 1994.
On the other hand in France, East- and South Europe the frequency of publications has steadily been deacreasing till now. High abundances have not been recorded from those regions. It seems to be obvious that the interest in the cockchafer diminished in the latter regions like in Schleswig-Holstein.

## Introduction

Exactly 40 years ago SCHWERDTFEGER and DARUP (1955) made known that the period the cockchafer needs for one gradation is 40 years long. This period was estimated from data of the east and the middle of Germany, where people had been suffering from the maybug as long as they could rememder.
As their estimation based on data from 32 forest offices, it could be generally correct for other regions where the cockchafer appears. Therefore I used it for further planning of investigtions. The presumably right time to start the investigations of the population dynamics was at the supposed beginning of the next gradation, at the end of the seventies (SCHÜTTE, 1976).
The research concept was also influenced by findings and a statement of HORBER (1967). He stated that the grubs were beneficial on meadows and pastures up to an abundance of 50 each squaremeter, because they keep down dandelion plants.

Now, in 1995, my contribution to the ongoing discussion includes a report on the results of our investigations and a discussion of the timing and the research concept.

## 1 Summary on the empiric results of the end of the seventies

### 1.1 Results of laboratory and field investigations on the dependence of the cockchafer grubs on dandelion (HAUSS et al., 1976)

The roots of dandelion were peferently fed compared to other tested momocotyledones and dicotyledones.
The weight of the grubs feeding on dandelion was significantly the highest and the pree-imaginal development was the shortest.
The mortality was lowest with grubs feeding on dandelion campared with controls.

### 1.2 The significant preference of dandelion was confirmed by the results of investigations concerning the providence of the beetles (SCHÜTTE et al., 1985)

Appetent female beetles perceiving ordorous substances of dandelion were stimulated to lay their eggs.
Flying and egg-laying beetles clearly prefered dandelion beds in field experiments in tents.
The abundance of the grubs was reduced to 55% on plots where dandelion was reduced to 12% of its abundance by spraying the herbizide "2,4-D+MCPA-Ester". This reduction of the abundance of the grubs was noticeable because the plot´s size was only 20x20 m and dandelion was highly abundant in the surrounding area.

### 1.3 Mode of orientation of egg-laying beetles seeking for feeding trees and suitable sites for egg-laying (SCHÜTTE et al., 1985)

The general conviction that maybugs fly towards the highest silhouettes they can perceive has to be supplemented. They also orientate with the help of odorous substances from trees and other feeding plants. Furthermore especially the female beetles seeking for places to lay their eggs did not fly to high silhouettes. When different horizonts were imitated with silhouettes most of the eggs were layed in beds where the imitated silhouette was lowest. One can genrally conclude that the very low silhouttes of plants are a good signal in order to find appropriate places for egg-laying.
Signals for the perception of new egg-laying places may also be the ordorous substances of dandelion. Only females with appetence to lay eggs answered with high activity to the smell of dandelion.

## 2 Maybug records at plant protection offices in Germany during the last three decades

### 2.1 Schleswig-Holstein

Following the maximum of the last gradation in 1938, when 200 t living maybugs were collected in this northernmost German country, only locally restricted and minor occurences were reported in 1976, 1981, 1982, and 1984 (HASE, 1984). The maybug records of 1976 were of special interest, as they represented the first reports since the last gradation. However, according to HASE (personal communication) and the Pflanzenschutzamt (plant protection

office) Schleswig-Holstein, these maybug records are of minor significance as no harms have been reported until 1995.
To summarize the records in Schleswig-Holstein until 1995: 36 years of no high abundance of flying beetles, 41 years of no high density of the grubs, and 57 years no alarming increase in population density which would have called for control.

2.2 Niedersachsen, Nordrhein-Westfalen, Mecklenburg-Vorpommern and Sachsen-Anhalt

In 1984 the Landwirtschaftskammer (office of agriculture) Hannover announced the occurence of maybugs in the area of Peine, Gifhorn, Diepholz. In the Nienburg area, barley, sugar beet and potatoes were infested and damaged. In the Teutoburger Wald, spruce trees and rhododendron get harmed. Two years ago, a strawberry field of 2 hectares was heavily infested and had to be ploughed. According to HENSELER, Bonn (personal communication), the occurence of maybugs in Mecklenburg-Vorpommern and Sachsen-Anhalt varied locally and from year to year, and it was hardly more than sporadically.
To summarize the records in these northern countries in the last 30 years: Beginning in the eigthies, maybugs were sporadically reported at small locations and very limited in time. Their occurence never caused any significant hazards, although they were sometimes abundant enough to perform some experiments on their possible control using insecticides.

2.3 Baden-Württemberg and the alpine area

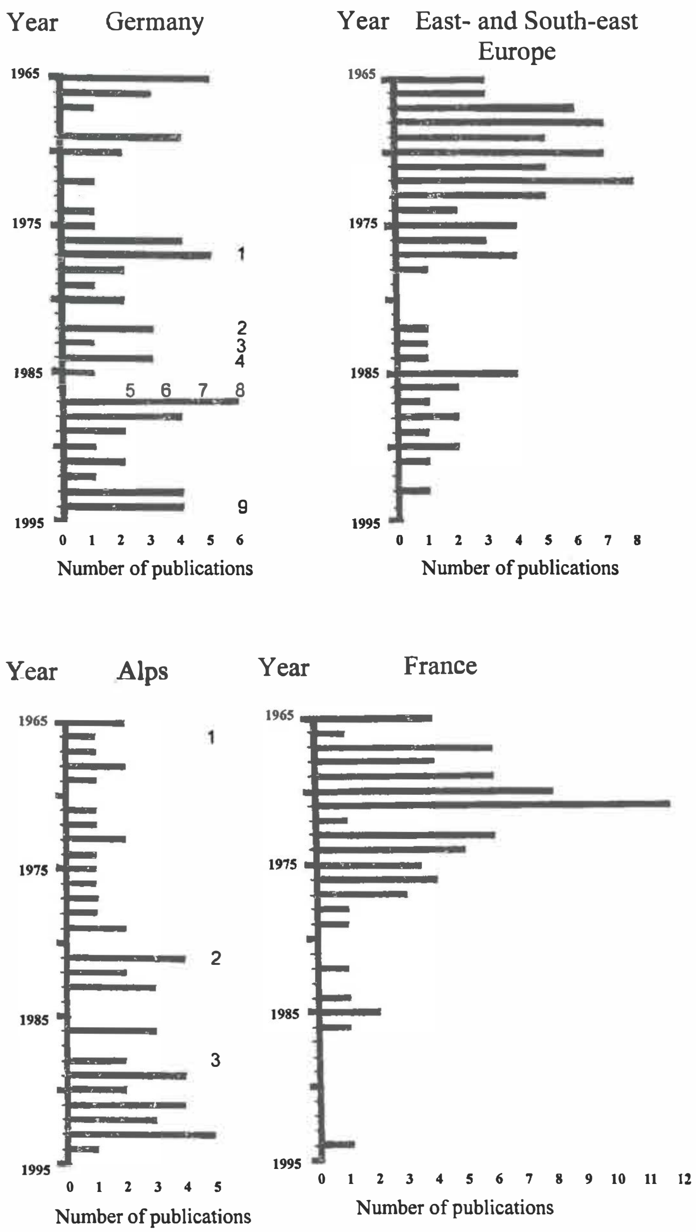
In 1974, when the experiments began, it was well known that the maybug was abundant and sometimes even harmful in the alpine area. We started our investigations with the collection of bugs and grubs at several plots near the Bodensee in the alpine area, which were transferred to Kiel for further experiments. It was obvious that the maybug population density in some places was quite high in the alpine area in 1974. At the same time hardly any specimen could be found in the northern parts of Germany. For Baden-Württemberg, damages in strawberry fields were reported near Rastatt for the first time in the beginning of the eighties. In 1989, apples, strawberries, raspberries, vine and sugar beet were damaged, and since 1992 young vine plants, meadows, ski-runs and a camping lot have been affected (FRÖSCHLE, 1994).

To summarize the records in this southern part of Germany: In the alps, abundant and harmful populations were already reported in the seventies. In the beginning of the eigthies an increasing and sometimes harmful population density, which seemed to become harmful to agriculture, has also been noticed in Baden-Württemberg.

## 3 Number of maybug related publications in four different regions

In an effort to gain some information about the occurence of the two maybug species in Europe, I analyzed data from from the literature database DIMDI (Deutsches Institut für medizinische Dokumentation und Information, Cologne, Germany). 320 publications on maybugs were counted for the 1965-1995. 30 of these were discarded because they were not related to Europe. The remaining 290 articles were assigned to the following four regions:

1) Germany
2) France
3) Alpine area (Switzerland, Austria, Italy)
4) Central and South Europe (Hungary, Poland, Russia, Rumania, Czechia)




Figures 1-4 show the number of publications in each region for every year. Articles with a number are reporting a new increase of maybug populations after some years of rest. (The numbers also compute the increases for each region).

3.1 Germany
It is remarkable, that a reappearance of maybugs was reported at four different locations. While the first report dates back to 1977, another three came at the beginning of the eigthies and the last one in 1994. Generally, the number of publications per year is equally distributed throughout the 30 years examined, with a slight increase during the last eight years.

3.2 France
In contrast to figure 1, a reappearance of maybugs was not even once reported in France. The articles are not equally distributed, most of them were published between 1965 and 1977, and only 4 articles appeared during the last decade.

3.3 Alpine area
Three articles, publicated in 1966, 1981 and 1988 reported a reappearance of maybugs. There is no special event or maximum like in Germany, but the bugs seemed to increase once in a while and independently. Regarding the overall distribution of publications a higher share in 1986-1994 is noteworthy.

3.4 Central and South Europe
It must be taken in mind that this area represents many and some very big countries with different climates. Initially, a summary of this data was not planned but turned out to be necessary, as the number of articles per country was too low. A similar picture as for France emerged for the whole area: A reappearance of maybugs has never been reported, and the overall number of publications was higher in the first decade from 1965-1975.

**Discussion**

Unfortunately it is not possible to find reports on the abundance and occurence of dandelion like for the maybug. So one can only take into account regions with a well known high abundance of dandelion in order to verify the dependence of the maybug in nature and cultivated land.

Within the last decades in Schleswig-Holstein, the northernmost German country, meadews and pastures more and more disappeared. Very early compared to other regions in Germany farmers specialized themselves and reduced rotation to only very few crops (wheat, barley oilseed-rape no cattles and pastures). Even in the valleys, the former areas of cockchafer damage, meadows disappeared and the stock of cattles decreased. Dandelion has only been found on small plots there during the last 20 years. Due to the low abundance of its main feeding plant, the cockchafer could not appear on a population level which could have caused a gradation.

Sites with dandelion covering the main part of the ground could not be found in the other northern countries in Germany either. But there have been some more valleys which have here and there been suitable and thus coverded with different proportions of dandelion. There have been some damages from the maybug but only very limited in time and space. According to the records especially from Niedersachsen and Nordrhein-Westfalen no real gradation occured because the conditions were not right.

The conditions in East- and South Europe and in France do not seem to have differed much from the latter mentioned countries in Germany. At least from the frequency of publications on the maybug one can conclude that the control of the cockchafer has been of no interest in these regions during the last 20 years period. One of the 4 publications written during this period (ROBERT et al.,1986) mentions that the grubs have been a severe pest problem till 1950. This underlines the conclusion drawn above. After 1950 grubs have only been harmfull on extensively used monoculture grasslands.

In France the abundance and occurrence of maybugs have been monitored from 1980 to 1985 and by that investigations three newly endangered areas were recognized:

1. near Limousin (north of Bordeux)
2. in the Lorraine (mountains near Nanci)
3. in Aquitane

But gradations have been observed in none of the three regions.

In the alpine area dandelion has been quite frequent on meadows only a few years ago. On the plots I visited it mainly completely covered the ground.
Another interesting observation was made on a flight to München. One could see many fields and grounds covered by flowering dandelion and it was possible to distinguish it by the different colour from flowering oilseed rape.
Severe harms are only to be expected when dandelion or meadows and pastures covered with dandelion make up a bigger proportion of cultivated land than today. This was true on some vine and fruit cultures near the Kaiserstuhl I was shown by Dr. FRÖSCHLE from Stuttgart. Some years ago farmers switched to another strategy of control enabling plants to cover the ground between the vine and fruit rows. Beside other typical plant species dandelion came up there again. Knowing about the the maybug laying ist eggs near dandelion the problems that occured are not a surprise.
Especially when dandelion roots have already been destroyed, the grubs begin to damage cultivated plants.

To summarize the results of our investigations and observations there is a correlation between the abundance of dandelion increasing from north to south and the occurence of the cockchafer depending on dandelion.

**Closing remarks**

1. The idea to start investigations on the population dynamics of the maybug in 1976 has been confirmed, not only by the occurence and propagation ofthe maybug even in Schleswig-Holstein.
2. The dependence of the grubs on dandelion, which has been postulated by HORBER (1967) could be verified in laboratory in tents and in field. Furthermore could be shown that the cockchafer preferenty lays its eggs near dandelion plants providing the grubs with the best resource. Therefore the abundance of the two species is correlated.
3. A deliberate integrated control of the cockchafer cannot be done without taking into account the proof correlations mentioned above. It will be necessary to control dandelion in order to fight the cockchafer.

This synopsis on the occurence of dandelion and the maybug during the last 30 years illuminated that the conditions for propagation of the two have been changing. During the last decade in some regions the abundance of dandelion did not only increase on sites where the herbicide management has been changed. Meadowes and pastures partly covered by dandelion have become more too. Therefore the conditions for a gradation are much better now than in the last 20 years. It is time to impede surprises with the help of prognostic investigations and to find appropriate remedies preventing gradations of both species.

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# Parasitoids, pathogens and biological control

# OCCURRENCE AND BIOCONTROL OF GRASS GRUBS, ESPECIALLY OF *MELOLONTHA MELOLONTHA*

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## Abstract

Cockchafers (*M. melolontha* L.) in The Netherlands have been recorded in the beginning of the century as harmful insects on a wide range of crops. After 1914 they disappeared to reappear again in the late forties until the mid fifties. Damage has been observed a.o. in potatoes, various legumes, fruit trees, ornamentals and grassland. In recent years an outbreak occurred, again on the same types of crops.

## Population monitoring

The nearly nocturnal lifestyle of the adult is difficult to monitor. In Fig. 1 counts are given, based on counts of adults attracted by the light of streetlamps. Regularly, on days with perfect flight conditions, 40 streetlamps were checked from 21.30 onwards. During 60 seconds an estimation was made of the total adults flying in the light of an individual lamp. In this way it was tried to give an overview of the flight intensity of the chafer and its abundance during the last three years. It is not possible to give an estimation of the population from these figures. It merely reflects the abundance during the years of flight. In The Netherlands there seems to be a periodicity of about 50 years. A three year life cycle is observed in The Netherlands.

## Damage

First damage was found in a sportfield near Eindhoven, in 1992. A year later much damage was recorded from the eastern part of the country, especially on grassland. Several hectares have been damaged, not only primary damage by the grub but also secondary damage by predators like crows. In the autumn of 1993 severe damage has been recorded from tree nurseries, especially in *Quercus*, *Fagus* and *Carpinus*. In a few cases severe damage was done in apple orchards.

## Biological control

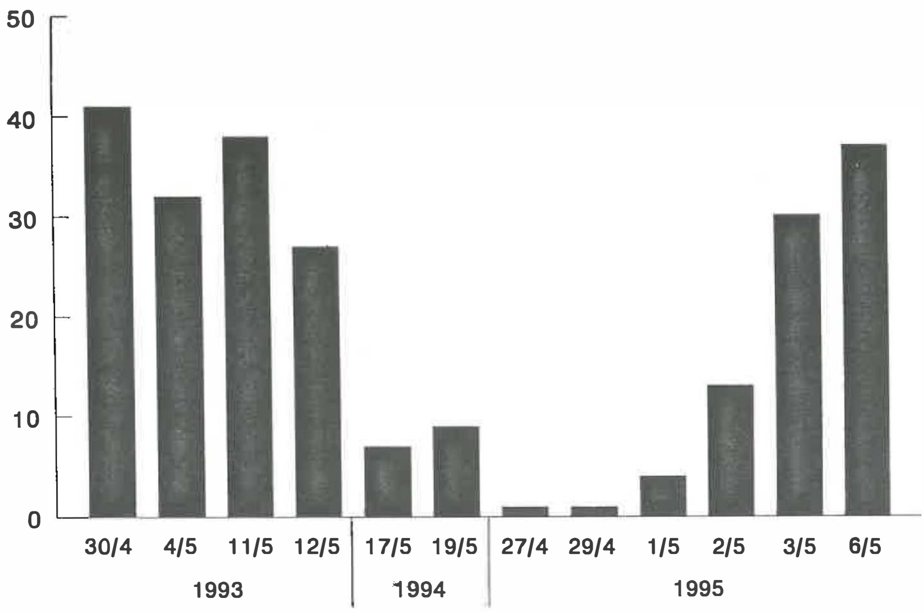
Several experiments have been carried out on biological control with nematodes. In bioassays some results were reached with *Steinernema glaseri*.

This nematod reached a parasitation of 38%. Infective juveniles, reared from these larvae were used again in further trials. This S1 generation was able to kill 67% of the grubs in bioassays, the S2 reached 70%. Later trials performed even better (Tab. 1). Field trials with *S. glaseri* and *Heterorhabditis* sp. failed. In 1994 field trials with S. glaseri were carried out to identify the limiting factors for nematodes in grassland areas. It was found that nematodes disappear from the soil in a very short time at a dose of 250.000/m². The reason is unknown. Expected causes may be dissication, predation by nematod eating Collembola and Nematoda, fungi etc. Another death cause is the influence of daylight, UV being the key factor. For this reason field work has been carried out after sunset.

Tab. 1. Performance of *S. glaseri* in bioassays against *M. melolontha*

| | life | dead | % dead |
|---|---|---|---|
| blanco | 29 | 1 | 3.3 |
| *S. glaseri* #326 | 9 | 21 | 67.7 |
| *S. glaseri* S-1 | 3 | 27 | 90 |
| *S. glaseri* S-2 | 0 | 30 | 100 |

**Fig. 1. Adult counts of M. melolontha on 40 streetlamps between 21.30 and 22.30 hour**



# TIPHIA FEMORATA, A PARASITE OF THE GARDEN CHAFER (***Phyllopertha horticola*** L.) and its possibilities for biological control


## Abstract

During studies on the biology and biological control of the garden chafer (*Phyllopertha horticola* L.) on a golfcourse near Arnhem (The Netherlands) the parasite *Tiphia femorata* (F.) was found in large numbers. In the early years of our study these parasites remained unnoticed despite intensive monitoring of the host population in this area. Influence of pollen of wild carrot (*Daucus carota* L.) on egg production of the parasite has been studied.


## Introduction

Garden chafers are among the most serious turfgrass pests in the Netherlands. Turfgrass-infesting white grubs feed on the roots of grasses. Pruning of roots below the soil surface is the primary cause of turf damage. When this damage is followed by extremely dry weather the loosened turf dries out and dies. In late summer and autumn grubs are large enough to be attractive to larger predators like crows, foxes and badgers. These predators, in search for grubs, cause secondary damage which in most cases is more destructive than the primary damage of the grubs themselves.

## Biology

*Tiphia* species are known as ectoparasites of scarabaeid larvae. Adults are free living, solitairy wasps, morphologically adapted for searching their hosts in the soil. They are slender wasps with strong, dentated fore femora which are specialized tools for digging. On a golfcourse near Arnhem a few adults of *T. femorata* were recorded in 1990, parasitizing garden chafer. The local distribution was restricted to an area of about 1000 $m^2$. In 1991 and 1992 yellow pan-traps have been placed for monitoring the flight period of the parasite. Gravid females of *T. femorata*, in search for the grubs, dig into the ground and follow the alleys of the grubs. As soon as a grub has been located, it is paralized by a sting. Once paralized, an egg is attached ventrally on the intersegmental membrane. In most cases eggs are laid between the 8th and 9th segment, in some cases they are layd between the 7th and 8th or 9th and 10th. L1 of the host larva has not been found parasitized, whereas L2 and L3 are not discriminated in egg laying.

The flight period of *T. femorata* starts in early August and ends in the beginning of September The flight was monitored with yellow pan traps (40x50 cm). The flight shows protandry with a breaking point at the 16th of August (Fig. 2). Later, on the 23rd of August again more males are present whereas females are less abundant at that moment, maybe caused by egg laying activity and being mated already. At the end of the flight period females are more abundant than males. Flight activity was not measured but the impression is that on warm,

shiny days the flight starts at 10h AM and ends at 5h PM. Mating was observed during the entire day and seems to be preferably done on the umbels of wild carrot. Not only mating takes place on the flowers of umbelliferes. Adults, both males and females are actively searching for pollen on the umbels. It is known that *Scolioidea*, where *Tiphia* belongs, preferably feed on nectar and pollen of umbelliferes, apparently for survival and as a protein source for egg production. Females spend more time (average of 4 minutes) on a single umbel than males (average of 1 minute).
From the end of September parasite pupae were found on a depth of about 30 cm. Parasitized grubs, which are provided with an egg tend to go deeper in the soil than unparasitized grubs, providing an overwintering site for their parasite. The full grown parasite larva forms an earthen cell in which they spin a light brown cocoon.

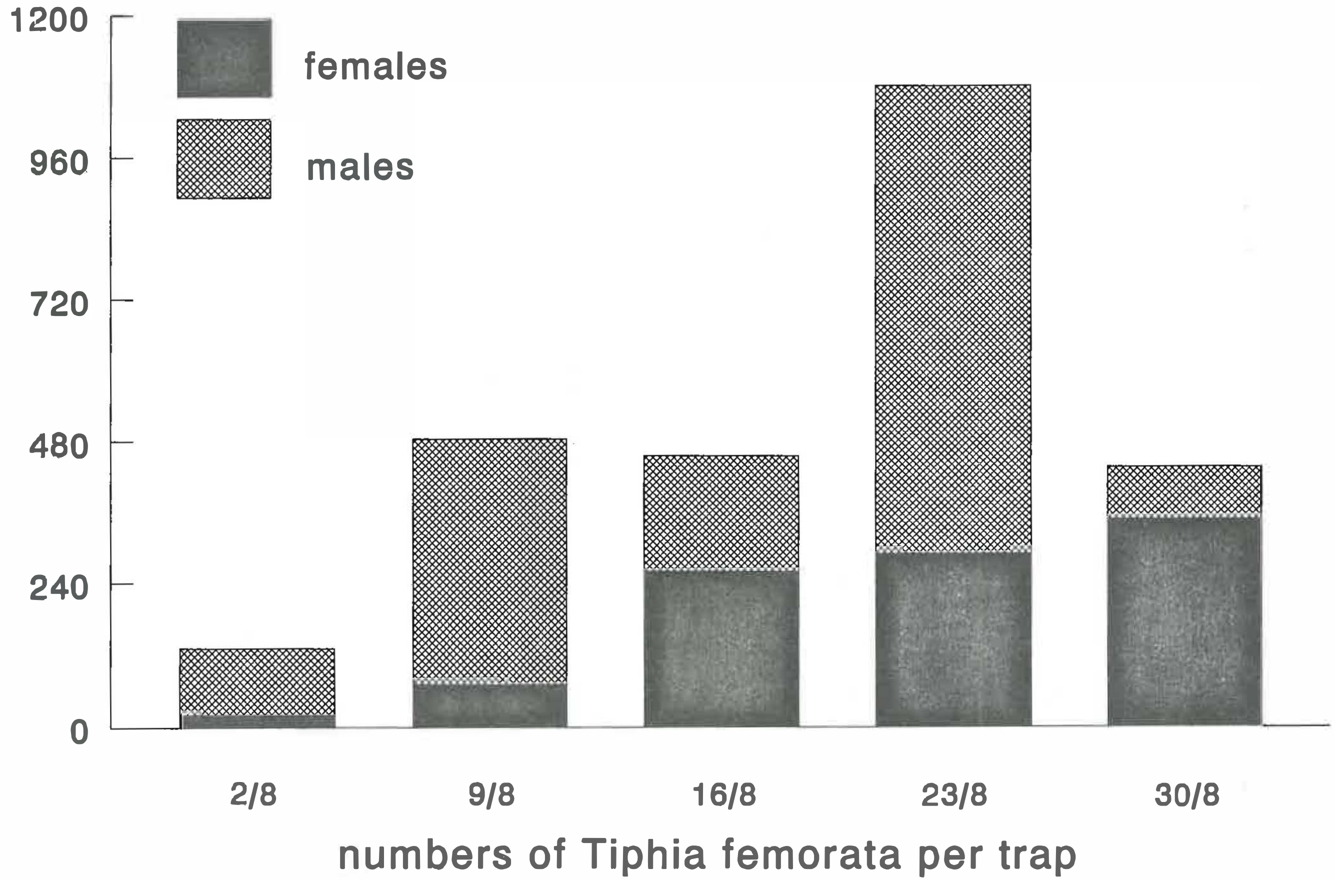
Preference

Pots with cut flowers of umbelliferes, wild carrot (*Daucus carota*), hogweed (*Heracleum sphondylum*) and upright hedge-parsley (*Torilis japonica*) were placed in the experimental area of the golfcourse. No naturally occurring umbellifers were found on the golfcourse. Around the study area wild flowers were checked for flying adults of *T. femorata*. The flowers of umbelliferes appeared to be highly attractive to the parasite. Visits without feeding on *Linaria vulgaris* was observed three times; in these cases the parasite was able to reach the inside of the flower through a hole, probably cut out by curculionid beetles. On other flowers no visits of *T. femorata* were observed (Table 1).

Table 1. Incidence of flower visiting by *T. femorata* on flowers present on the golfcourse Papendal in August. - no visits; + visiting; ++ visiting and feeding.

| | |
|---|---|
| *Achillea millefolium* | - |
| *Calluna vulgaris* | - |
| *Hieracium umbellatum* | - |
| *Galium hercynicum* | - |
| *Tanacetum vulgare* | - |
| *Linaria vulgaris* | + |
| *Hypochaeris glabra* | - |
| *Hypericum perforatum* | - |
| *Melandrium album* | - |
| *Solidago virgaurea* | - |
| *Senecio sylvaticus* | - |
| *Chamaenerion angustifolium* | - |
| *Anthemis arvensis* | - |
| *Teucrium scorodonia* | - |
| *Teesdalia nudicaulis* | - |
| *Hieracium auricula* | - |
| *Hieratium pilosella* | - |
| *Heracleum sphondilum* | ++ |
| *Torilis japonica* | ++ |
| *Daucus carota* | ++ |
| *Cirsium arvense* | - |
| *Cerastium arvense* | - |
| *Trifolium repens* | - |

Fig. 2. T. femorata in yellow pantraps 1991



# *Bacillus popilliae*: a difficult pathogen

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**Abstract**
A new isolate of the milky disease bacterium *Bacillus popilliae* (BP), BP subsp. *melolontha* HD-1 is described. Vegetative growth on complex media supplemented with activated charcoal occurs at a temperature optimum of 30°C, but sporulation cannot be observed. Infection studies with the new isolate and other BP strains applying sporangia *per os* demonstrate a specific infection of *Melolontha* grubs when feeding at least $2x10^8$ spores per grub. While vegetative cells administered *per os* are not infective, they unspecifically cause milky disease by injection. These experiments demonstrate that infectivity and specifity of BP is correlated with the ability of the germinated cells to penetrate the gut epithelium. Further, the composition of the parasporal crystal proteins of the new isolate and different other BP strains and their plasmid patterns are compared.

**Key words**
*Bacillus popilliae*, milky disease, *Melolontha melolontha*, *Melolontha hippocastani*, crystal toxin

**Introduction**
*Bacillus popilliae* (BP) was first described by Dutky (1940) as a pathogen of Japanese beetle larvae (*Popillia japonica*). It is a spore forming, catalase negative, facultative anaerobic bacterium, which forms a parasporal protein crystal during sporulation. Both, spore and crystal remain in the sporangium after sporulation, autolysis, which is common for many *Bacillus* species, does not occur.
BP is an obligate pathogen of Scarab grubs (Bulla *et al.* 1978, for review see Klein & Jackson 1992). If sporangia are ingested by the grub, the spores germinate in the gut lumen, penetrate the gut epithelium, and proliferate and sporulate in the hemolymph (Splitstoesser *et al.* 1978; Kawanishi *et al.* 1978). The final cause for the death of the grub is not known, but fat body depletion (Sharpe & Detroy 1979) and general exhaustion are considered to be important factors. Because of the milky white appearance of the diseased grubs, the infection caused by BP is called milky disease. A BP subsp. *melolontha* isolated from the German cockchafer *Melolontha melolontha* was first described by Hurpin & Vago (1958).

**Materials and methods**
Grubs were field collected in different areas of Baden-Württemberg. They were placed in 36mm x 83 mm culture vials and were fed with carrot slices. BP subsp. *melolontha* HD-1 was isolated from a diseased grub (Krieger *et al.* 1994). BP subsp. *melolontha* ZUR (former *Bacillus fribourgensis*) was obtained from G. Benz, Zürich, Switzerland. The other BP isolates were provided by D.W. Dingman, New Haven, Connecticut, USA. Vegetative cells of BP were cultured on MYPGP-medium (Dingman & Stahly 1983), J-medium (St. Julian et al. 1963) supplemented with 1.5 % yeast extract and 0.2 % trehalose, or Columbia agar supplemented with 8 % sheep blood. Agar plates or liqid media containing 5 µg/ml vancomycin were used. Numbers of sporangia and vegetative cells were determined by counting in a Neubauer counting chamber. Spore germination rates were calculated by plating a known number of sporangia onto agar plates and counting the colonies developping from these sporangia after six days of incubation at

30°C. Gut juice was obtained by decapiting the grubs. The gut juice was centrifuged and the supernatant was sterile-filtered with Millipore 0.2 µm pipette tip filter units. Sporangia were incubated in the gut juice for 1 h at 30°C. Sporangia (spores and crystals) were obtained by injecting 1000 to 10,000 vegetative cells suspended in physiological sodium chloride solution into the hemolymph and re-isolating the sporangia from the dead grub. In order to test selectivity, at least 20 grubs were injected with every BP isolate. For the determination of the stage of disease, a droplet of hemolymph was taken from the grubs and controlled by light microscopy (1000x magnification). Feeding experiments were conducted by offering infested carrot slices to the grubs. 10 to 15 grubs were used for every test dose of sporangia.
SDS-polyacrylamide gelelectrophoresis (SDS-PAGE) was conducted with the Pharmacia PhastSystem in 7.5 % gels under denaturing conditions. Gels were dyed with Coommassie Brilliant Blue in the Pharmacia Development Unit. Antibodies were produced in rabbits against SDS-PAGE purified protein (only the 80 kDa band). Gels were blotted in the Pharmacia PhastSystem Blotting Unit. Immunological detection was conducted with an anti-rabbit horse raddish peroxidase conjugate purchased from Sigma. Plasmid preparation followed standard alkali lysis or dodecyl sulfate lysis protocols. Prior to lysis, vegetative cells were incubated with 5 mg/ml lysozyme at 37°C for 30 min. Plasmids were seperated in 0.8 % agarose gels.

## Results

**Infectivity and selectivity** When sporangia of BP were fed to the grubs of *Melolontha melolontha* or *Melolontha hippocastani*, milky disease was caused only by BP subsp. *melolontha* HD-1 and BP subsp. *melolontha* ZUR. These isolates originated from diseased *Melolontha* grubs. No infection occured when feeding sporangia of other BP strains isolated from *Popillia japonica*, *Cyclocephala hirtae*, *Cyclocephala borealis*, *Phyllophaga* spp., *Phylloperta anxia* and *Amphimallon majalis*.

| number of sporangia | infection rate *per os* |
|---|---|
| 0 (control) | 0 % |
| 2 x $10^6$ | 0 % |
| 2 x $10^7$ | 8 % |
| 2 x $10^8$ | 20 % |
| 2 x $10^9$ | 25 % |

Table 1: Number of sporangia applied *per os* and infection rate for third instar grubs of *Melolontha melolontha*.

When feeding vegetative cells to *Melolontha* grubs, no milky disease could be observed even with those isolates, that were pathogenic when sporangia were ingested. However, injection of vegetative cells directly into the hemolymph of *Melolontha* grubs caused milky disease with all tested BP isolates (data not shown).

**Growth and spore germination** Vegetative cells of the new BP subsp. *melolontha* HD-1 isolate grew on all tested media (GSJ-medium, MYPGP medium, Columbia blood agar), but no sporulation occured. Activated charcoal added to a concentration of 0.1 % promoted the growth of vegetative cells. The optimal growth temperature was 30°C (see table 2). The new BP isolate subsp. *melolontha* HD-1 has the same growth characteristics as the known BP subsp. *melolontha* ZUR isolate. While growth of the subtropic BP subsp. *cyclocephala* is still observed at 37°C, the two isolates from *Melolontha* do not grow at higher temperature. Sporulation of BP subsp. *melolontha* HD-1 *in vitro* could not be observed. The germination rate of BP subsp. *melolontha* HD-1 was generally very low. Only about 1 % of the spores germinated. Treatment with gut juice from grubs only slightly improved germination (see table 3).

Table 2: Growth of BP isolates on GSJ medium supplemented with 0.1 % activated charcoal at different temperatures (BPM: BP subsp. *melolontha* HD-1; BPF: BP subsp. *melolontha* ZUR, BPC: BP subsp. *cyclocephala*. - no; + little, ++ good; +++ very good growth observed).

| | growth observed after | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 days | | | 4 days | | | 10 days | | |
| isolate | 23°C | 30°C | 37°C | 23°C | 30°C | 37°C | 23°C | 30°C | 37°C |
| BPM | - | ++ | - | + | +++ | - | +++ | +++ | - |
| BPF | - | ++ | - | + | +++ | - | +++ | +++ | - |
| BPC | - | ++ | ++ | + | +++ | ++ | +++ | +++ | ++ |

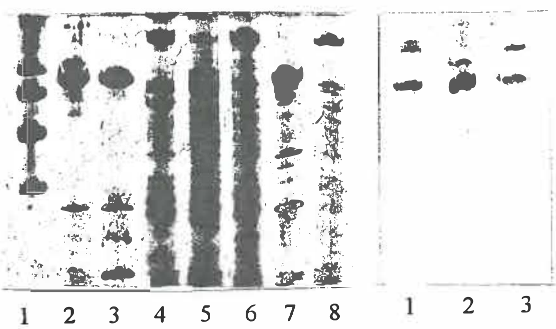
Table 3: Germination rates of sporangia

| | | untreated sporangia | | gut juice incubated sporangia | |
|---|---|---|---|---|---|
| medium | sporangia plated | colonies observed | germination rate | colonies observed | germination rate |
| Columbia | $7.5 \times 10^4$ | 610 (± 20) | 0.8 % | 950 (± 10) | 1.3 % |
| GSJ | $7.5 \times 10^4$ | 580 (± 10) | 0.75 % | 900 (± 40) | 1.2 % |

**Biochemical characterization** All examined BP parasporal crystals contained one or two major protein bands at 150 kDa or 80 kDa respectively. Whether only one of the two bands or both bands together could be observed seemed to depend on the time the sporangia remained in the decaying grub. Generally, the parasporal crystal of sporangia isolated from still living or freshly died grubs contained only (or mostly) the 150 kDa protein, while the major compound of "older" parasporal crystals was the 80 kDa protein (fig. 1a). In western blot analysis of the parasporal crystals, both the 150 kDa protein band as well as the 80 kDa band were detected by antibodies developped against the 80 kDa protein (fig. 1b).

In the examined BP strains, very variable plasmid numbers and sizes were observed. The new isolate BP subsp. *melolontha* HD-1 possesses three plasmids (fig. 3), which exhibit a quite different pattern than the three plasmids observed in BP subsp. *melolontha* ZUR. BP subsp. *popillia* had three plasmids, while only one plasmid was present in BP subsp. *amphimallon*, BP subsp. *polyphaga*, and BP subsp. *phylloperta*.

Figure 1: (a) SDS-PAGE and (b) western blot analysis of protein crystals of BP isolates.




(a) lanes 1 to 8: protein standard 92.5 kDa, 65 kDa, 45 kDa 29.5 kDa; BP subsp. *amph.* (old), BP subsp. *mel.* HD-1 (old) and BP subsp. *mel.* HD-1 (fresh), BP subsp. *mel.* ZUR (fresh), BP subsp. *pop.* (fresh), BP subsp. *cycl.* (old) and BP subsp. *phyllop.* (fresh). (b) lanes 1 to 3: BP subsp. *mel.* HD-1, BP subsp. *amph.* and BP subsp. *mel.* ZU.

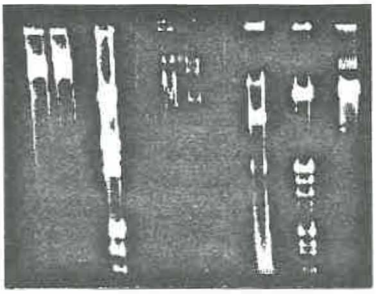



Figure 2: Plasmid analysis of different BP strains. Lanes 1 to 8: BP subsp. *polyph.*, BP subsp. *phyllop.*, lambda standard (not circular, only serves for orientation), BP subsp. *mel.* ZU, BP subsp. *popillia*, BP subsp. *mel.* HD-1, lambda standard, BP subsp. *amph.*

**Discussion**

Only very high numbers of sporangia ($10^8$ to $10^9$) caused milky disease in grubs of *Melolontha melolontha* with an infection rate of 20 % to 25 %. These results correlate well with the results of St. Julia et al. (1978), who found infection rates of 20 % when feeding $1x10^8$ dried sporangia of BP subsp. *popillia* to grubs of *Popillia japonica.* According to Klein & Jackson (1992), when spores are administered *per os*, there is very little cross infectivity between the strains of BP isolated from one Scarab species for other species. However, we could demonstrate cross-infectivity of different BP strains upon the injection of vegetative cells. From these results we conclude, that the penetration of the gut epithelium is the crucial step in infectivity and selectivity, and that the parasporal crystal plays an important role in pathogenicity. Homologies between the BP subsp. *melolontha* HD-1 crystal protein and some *Bacillus thuringiensis* toxins (Cry II A and CryII B) have been demonstrated (Zhang *et al.* 1994) and give an evidence for a possible cytotoxic or cytolytic effect of the BP crystal protein.

Our results of 1 % spore germination of BP subsp. *melolontha* HD-1 on solid media are consistent with Bulla & Costilow (1978), who report a germination rate of 1 % of an untreated spore population of BP. Sporulation *in vitro* was not observed during our studies. As an obligate pathogen, BP has very special nutritional requirements. Efforts have been taken for a long time to produce infective spores *in vitro*. Rhodes *et al.* (1965) found a sporulation rate of 0.1 % to 0.3 % on solid media supplemented with yeast extract and different carbohydrates. Lüthy *et al.* (1970) and Ebersold (1976) rarely observed *in vitro* sporulation in cell culture systems. Generally, the infectivity of the obtained spores was extremely low.

No differences in the protein composition of the parasporal crystal could be observed with different BP isolates. In contrast, plasmid patterns were very variable. Dingman (1994) examined the plasmid patterns of different BP strains and discussed the occurence of correlated plasmids for isolates from closely related beetle species. For plasmid patterns of other BP strains presented by Valyasevi *et al.* (1990) and Dingman & Stahly (1979), the investigations demonstrated only coincidential plasmid patterns but different sizes of plasmids.

Thus, from a taxonomical point of view, neither the protein composition of the parasporal crystal nor the plasmids patterns of different BP strains can be considered of much importance. Perhaps the Random Amplified Polymorphic DNA (RAPD) analysis (Woodburn & Yousten 1994) will yield more information.

## Conclusions and perspectives

For the biological control of the German cockchafer *Melolontha melolontha* and *M. hippocastani*, the low infection rate due to the poor germination rate of spores renders BP a very ineffective control agent until now. Methods to improve spore germination have to be developped. In addition, only if an *in vitro* production of infective BP spores can be achieved, a commercially interesting product can be developped. A possible application field for BP may lie in the combination with other little effective pathogens like fungi or nematodes as described by Thurston *et al.* (1993).

Considering the protein crystal, much more investigations will have to reveal its role in pathogenesis. Although the reported homologies between the BP and some *Bacillus thuringiensis* crystal proteins are promising, a similar mechanism of toxicity (binding, pore formation and cell lysis) will have to be demonstrated. As bioassay *in vivo* with grubs is very difficult, new *in vitro* bioassay methods (e.g. with cell culture systems) will have to be introduced for this purpose.

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# Characterization of a *cry* gene from *Bacillus popilliae* subsp. *melolonthae* H1

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*Bacillus popilliae* is a Gram-positive bacterium. Its sporangia are known to be pathogenic for larva of Scarabaeidae insects, which are important pests in agriculture, foresty and husbandry. *Bacillus popilliae* can produce parasporal crystals by sporulation, which consist mainly of proteins. The function of spores and parasporal crystals by the infection to grabs is until now not clear.

To clone the crystal protein gene of *Bacillus popilliae* subsp. *melolonthae* H1, a strain isolated from an infected grub of common cockchafer (*Melolontha melolontha*), the sporangia of the strain were broken with different methods and the parasporal crystals were partially purified by gradient centrifugation. SDS-PAGE shows that the parasporal crystal of the strain contains a single 80 kD peptide component. This peptide was digested by trypsin and some of the proteolytic fragments were sequenced. Two of the above fragments were found to have sequence similarity with the amino acid sequence of *cry*IIA crystal protein of *Bacillus thuringiensis*. According the amino acid sequences of the above two fragments, primers were designed for a PCR reaction with the genomic DNA as template. In the above PCR reaction, a 283 bp long DNA fragment was obtained and was further used as gene probe. In the following cloning work, a 5.3kb EcoRI fragment was cloned into a *E. coli* plasmid pBC SK+.

After sequencing, the cloned 5.3kb fragment was found to contain a 2121bp open reading frame, which can encode a 79KD protein with 706 amino acids. This gene, designated as *cry*BP1, shows sequence similarities to the *cry* genes of *Bacillus thuringiensis*, especially to *cry*II genes with more than 30% amino acid identity.

The full paper will be submitted to a specialised journal.

## IS THERE A ROLE FOR *Serratia* spp. IN THE BIOCONTROL OF *Melolontha* spp.?

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### Abstract

Bacteria of the genus *Serratia* (Enterobacteriaceae) are known to cause disease in insects. They have also occasionally been isolated from dead larvae of *Melolontha* spp., but their effects on these insects have not been studied in detail. We recently carried out a series of experiments, screening isolates of *Serratia* spp. for their effects on larvae of *M. hippocastani*. Some isolates, taken from other insect species, consistently produced an antifeeding response in the treated larvae when applied by coating pieces of carrot with bacteria. This effect was permanent; once feeding had stopped larvae presented with fresh untreated food did not resume feeding. Nonfeeding larvae died prior to pupation. Strains of *Serratia* spp. were also isolated from soil in different areas of Europe and several produced the same antifeeding response. Other isolates, and *S. entomophila* isolated from the New Zealand grass grub (*Costelytra zealandica*), produced no ill-effects. These results indicate that an antifeeding effect and a pathological response can be produced when larvae of *M. hippocastani* are treated with specific strains of *Serratia* spp. The potential use of this antifeeding activity and the role of bacteria from the Enterobacteriaceae in regulation of natural populations is worthy of further study.

### Introduction

Larvae of the May beetles, *Melolontha melolontha* and *M. hippocastani,* live in the soil where they are continually exposed to large numbers of microorganisms. Large quantities of these are ingested as larvae feed on roots and organic matter. The soil, however, acts as an important reservoir for microbial pathogens of insects (Jackson *et al.*, in press) and thus it is not surprising that *Melolontha* spp. larvae have been reported as hosts for a large number of pathogenic microbes (Niklas, 1960; Keller, 1974, Albert *et al.,* 1988). The long term cyclic nature of May beetle outbreaks suggests that a delayed density-dependent mortality factor is operating within the population, which could be a pathogen or complex of pathogens. The most important pathogens which have been recorded from larvae of the May beetle are *Beauveria brongniartii* and *Rickettsiella melolonthae*. However, in many investigations dealing with diseases of larvae of May beetles a high incidence of unspecified bacterioses and other unknown mortality factors are listed (Niklas, 1958; Albert *et al.*, 1988). For example, Niklas (1958) found that more than 30% of all larvae held in his laboratory were killed by unknown nonsporeforming bacteria. To date, however, this group of bacteria have received little attention.

At present, both *Melolontha* spp. are expanding their range and building up to high numbers in several European countries. As the practical use of the fungus *Beauveria brongniartii* has often given unreliable results (Zimmermann, 1992), new measures are required to prevent pest damage in the short-term and, also, a long-term strategy for control of the pest needs to be developed. For both of these aims it appears necessary to look for new control agents. Bacteria of the genus *Serratia* have occasionally been implicated as pathogens of *Melolontha* spp. (Klein and Jackson, 1992). In New Zealand, strains of *Serratia* spp. containing a specific plasmid are pathogenic to another scarab pest, the grass grub (*Costelytra zealandica*) (Jackson *et al.*, 1993; Glare *et al.*, 1993) and have been developed as a microbial control product Invade$^{TM}$(Jackson, 1994). In this paper we report on preliminary tests with a range of bacterial strains from the genus *Serratia* against larvae of *M. hippocastani.*

## Experimental methods

*Insects and assay system*

Larvae of *M. hippocastani* were collected from forest sites near Heidelberg (L3) and Darmstadt (L2). In the laboratory they were placed individually in compartments in trays, fed regularly with pieces of carrot and moved to clean trays at approximately weekly intervals. Third instar larvae were fed regularly with approximately 1 gm fresh carrot, while 2nd instar larvae received approximately 0.5 gm. For each assay, pieces of carrot were coated with bacteria by rolling over the plate culture. Bacterial coated carrot was presented for the first feeding period only and thereafter fresh uncoated carrot. Consumption was estimated visually from the proportion of carrot remaining after each feeding period. All experiments were carried out in the laboratory in ambient summer conditions.

*Bacteria*

Larvae were treated with either standard strains of *Serratia* spp. taken from the AgResearch Insect Pathogen Culture Collection (AgRe IPCC) or soil isolates collected in Europe. The former included plasmid-bearing grass grub pathogenic and plasmid-free nonpathogenic strains of *S. entomophila* (Glare *et al.*, 1993) as well as *S. marcescens* strains originally isolated from insects and soil (Table 1). Bacteria isolated from the soil in Europe were tentatively identified as *Serratia* spp. on the basis of positive growth on CTA agar and production of DNase (Grimont and Grimont, 1984). Bacteria were streaked and cultured on plates of Nutrient Agar by incubation for 24hrs at 25$^{o}$C

*Experimental programme*

A series of experiments was carried out in the laboratory to examine the effects of the bacteria on *Melolontha* larvae. In all experiments the experimental unit was a row of five compartments in a tray.

Experiment 1. Third instar larvae were treated with *S. entomophila* strains 154+, and 154-, and *S. marcescens* 363 (Table 1). Ten larvae were used for each treatment and an untreated control with the layout being a randomised complete block design (RCBD) with four treatments and two blocks (i.e. 8 rows of 5 larvae). Larvae were examined for survival and feeding after 2 and 5 days and thereafter at 3-4 day intervals for approximately one month until all larvae had ceased feeding. Larvae were maintained for a further month in a darkened cupboard.

Experiment 2. Larvae were treated with *S. entomophila* strain154, *S. marcescens* strains 363, 505 and *S. liquefaciens* 256 and European environmental isolates *Serratia* S5 and F79 (Table 1). There was also an untreated control. Five 2nd instar larvae and five 3rd instar larvae were allocated to each treatment. Layout was 14 rows of 5 larvae arranged as a RCBD with seven treatments and two blocks (blocks 1 and 2 being 2nd and 3rd instar respectively) Larvae were examined and carrot replaced after 3 and 7 days and thereafter at weekly intervals for 5 weeks.

Table 1. List of species and strains of *Serratia* spp used in bioassays against larvae of *Melolontha hippocastani;*

| *Species* | *Strain no* | *Isolated from* | *Location* |
|---|---|---|---|
| *S. entomophila* | 154+[1,3] | *Costelytra zealandica* | Canterbury, New Zealand |
| *S. entomophila* | 154-[1,4] | Lab derived from 154+ | - |
| *S. marcescens* | 363[1] | *Paropsis charybdis* | Canterbury, New Zealand |
| *S. marcescens* | 392[1] | Grassland soil | Canterbury, New Zealand |
| *S. marcescens* | 257[1] | *Musca domestica* | (Univ. California, Culture Collection) |
| *S. marcescens* | 505[1] | *Hylamorpha elegans* | Valdivia, Chile |
| *S. marcescens* | 450[1] | *Hyponomeuta malineus* | (Pasteur Insitute, Culture Collection) |
| *S. liquefaciens* | 256[1] | *Bombyx mori* | (Univ. California, Culture Collection) |
| *Serratia* sp. | F79[2] | Grassland soil | France |
| *Serratia* sp. | S5[2] | Grassland soil | Switzerland |
| *Serratia* sp. | D6a[2] | Pine forest soil | Hessen, Germany |
| *Serratia* sp. | D23b[2] | Grassland soil | Hessen, Germany |
| *Serratia* sp. | D23d[2] | Grassland soil | Hessen, Germany |
| *Serratia* sp. | D47a[2] | Forest soil | Baden Württemberg, Germany |
| *Serratia* sp. | D84a[2] | Grassland soil | Bavaria, Germany |
| *Serratia* sp. | D128a[2] | Forest moss | Schleswig Holstein, Germany |

[1] Strains from AgRe IPCC; [2] Strains isolated by TAJ, this study; [3] 154+; plasmid bearing, grass grub pathogenic strain; [4] 154-, plasmid free, nonpathogenic strain;

Experiment 3. Second instar larvae were treated with AgResearch isolates, *S. marcescens* 363, 505, 392, 257, and 450 (Table 1). European environmental isolates included *Serratia* strains D6a, D23b, D23d, D47a, D84a and D128a. There was also an untreated control. Layout was as 19 rows of 5 larvae, with the rows arranged in a completely randomised design.

Four rows (20 larvae) served as controls, two rows (10 larvae) were allocated to each of the 363, 505, 450 and D84a strains, and one row (5 larvae) to each of the other strains. Larvae were assessed, and carrot replaced, after 3, 7 and 13 days.

**Results**

Experiment 1. Treatment with either grass grub pathogenic (154+) or nonpathogenic (154-) *S. entomophila* strains had no effect on feeding or survival of the larvae. Treatment with *S. marcescens* 363, however, reduced larval feeding, such that these larvae only consumed one third of the carrot consumed by the untreated in the first five days of the experiment (Fig. 1). Untreated larvae continued to feed for about one month until they entered the nonfeeding prepupal stage.

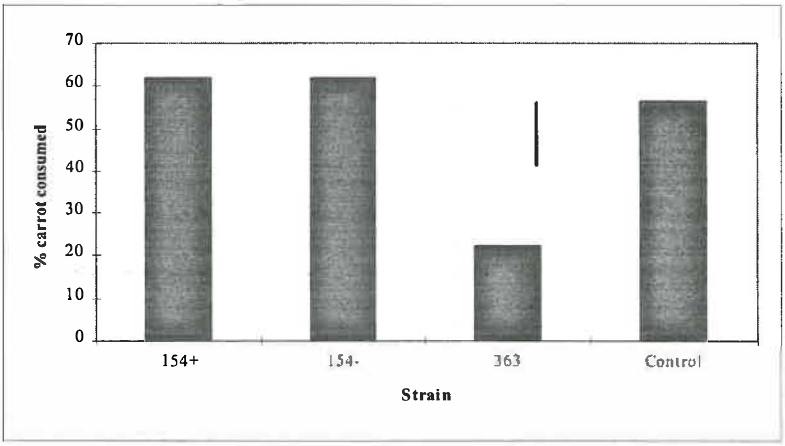


Fig 1. Effect of *S. entomophila* strains (154+,154-) and *S. marcescens* 363 on feeding by larvae of *Melolontha hippocastani* over 5 days (Experiment 1). Bar represents LSD(5%).

Larvae treated with strain 363 remained nonfeeding despite the regular offer of fresh carrot. After 2 months, 70% of control larvae were still alive and had pupated, while only 10% of larvae treated with 363 had survived.

Experiment 2. Strain 363 again reduced feeding to low levels within the first 3 day period and larvae did not resume feeding throughout the experiment (Fig. 2). A similar response was obtained with strains 505 and S5. Strains 154+, 256 and F79, on the other hand, had no effect on larval feeding. The 2nd and 3rd instar larvae showed a similar response to bacteria. After 35 days, high mortality had occurred among the non-feeding larvae treated with strains 363, 505 and S5 while among other strains and the controls, 80% or more of larvae survived (Fig. 2). Bacteria were isolated from the dead larvae and in 18/21 cases the reisolated strain corresponded in pigmentation and biochemical characteristics to the applied strain.

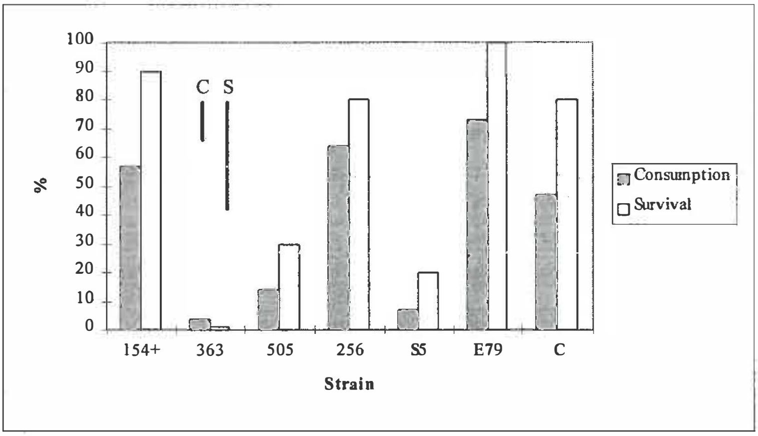


Fig. 2. Effect of *Serratia* spp. on consumption of carrot and survival of *M. hippocastani* larvae over a 35 day period (Experiment 2). Bars represent LSD(5%) for consumption (C) and survival (S).

Experiment 3. The repeated tests with *S. marcescens* strains 363 and 505 again produced a reduction in feeding (P<0.05) (Fig. 3), as did *S. marcescens* strains 450, 257 and 392 (P<0.05, 0.10 and 0.05 respectively) The environmental isolates D23b, D47a and D128a also caused reduced feeding(P<0.05, 0.10 and 0.05 respectively), while little effect was recorded following ingestion of isolates D6a, D23d and D84a.

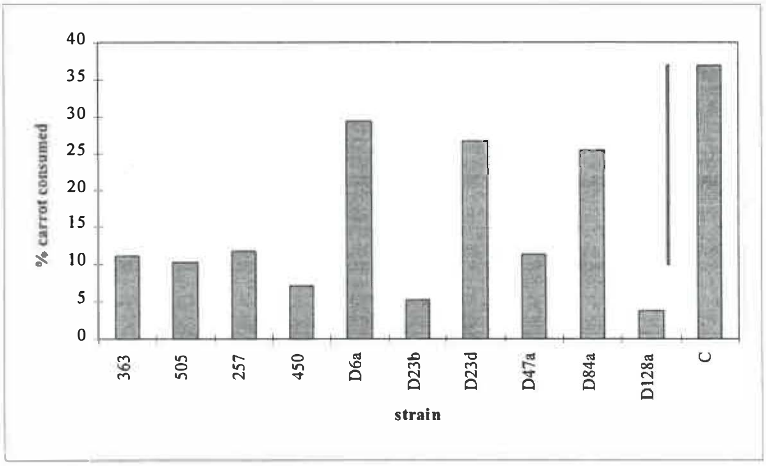


Fig. 3. Effect of *Serratia* spp. on feeding by *M. hippocastani* larvae over a 13 day period. (Experiment 3). Bar represents LSD(5%) for comparison of the control (4 rows of 5 larvae) with a strain for which one row of 5 larvae was treated.

## Discussion

*Serratia* spp. bacteria have frequently been isolated from dead and diseased insects by various researchers, but their role in pathogenesis has not always been clear as these bacteria are also found in the soil. In these tests, high doses of specific *Serratia* strains consistently produced cessation of feeding when ingested by larvae of *M. hippocastani*. This reaction was the same for both 2nd and 3rd instar larvae collected from two different sites.

Cessation of feeding suggests that some toxin is produced or a pathogenic effect is initiated. The response is more than just taste aversion as bacteria were only supplied in the first feeding period and thereafter fresh carrot was supplied. Reisolation of the applied strain from the dead larvae suggests that Koch's Postulates for infectivity have been met, but a mechanism for pathogenicity has yet to be established. In these tests high numbers of bacteria were ingested. It remains to be established what dose is necessary to initiate an infection or antifeeding response.

The role of bacterial-induced pathogenesis among *Melolontha* larvae in their natural habitat is unclear. Strains of *Serratia* spp. which caused cessation of feeding were found in soils of forests infested with *Melolontha* larvae. Septicaemic larvae are not often found in the field but this may be because they decay and disappear rapidly. In the laboratory, larvae frequently succumb to septicaemias (eg. Niklas, 1958), but the causal agent is seldom determined. Interestingly, cessation of feeding is one of the first steps in pathogenesis of amber disease in *Costelytra zealandica* (Jackson *et al.,* 1993), but the non-feeding *M. hippocastani* larvae did not show the gut clearance typical of this disease.

This study has demonstrated that specific strains of *Serratia* spp. can cause cessation of feeding and death in *M. hippocastani* larvae. The cause, significance and possible use of this activity need to be determined through further study.

**Acknowledgments**
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# Strain Selection and Epizootic Features in Microbial Grub Control with *Beauveria brongniartii* (Sacc.) Petch

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Key words: *Beauveria brongniartii*, *Melolontha hippocastani* F., incubation period, vertical migration, pathogens, *Rickettsiella melolonthae*, virulence, temperature, germination

**Abstract**
In the Upper Rhine Valley (Southwest Germany) near Karlsruhe and Mannheim the grubs of the cockchafer *Melolontha hippocastani* (Coleoptera: Scarabaeidae) cause high damage in forest stands with sandy soil. The three present populations have asynchronous flight years and reach different gradation levels with regard to population increase and infection rates. *Beauveria brongniartii* is the most common pathogen and appears throughout the years in the whole vertical migration range. The infection potential in laboratory bioassays depended on temperature, the dose of application and the larval state.

**Methods**
In infested forest stands the population densities were monitored by digging series of 10 pits of 0,25 $m^2$ in a random distribution (Burchard 1988). This was done at least once a year and in selected stands 4 times a year. Additionally the depth distribution in the soil was evaluated monthly. All cockchafers and instars were brought to the laboratory and reared individually at least for 3 month to state the infection level. Grain germlings served as food.
*Beauveria brongniartii* is regarded the most promising pathogen in microbial control against *Melolontha hippocastani* F. in Germany. Bioassays for virulence were conducted with 26 strains of different origin against second and third instar larvae (L2, L3) of *Melolontha hippocastani* from an outbreak area North of Karlsruhe, Germany. The virulence was compared to 2 strains of *Beauveria bassiana* and 2 strains of *Metarhizium anisopliae*. 0.1 ml of a suspension of $10^5$, $10^6$ or $10^7$ conidia/ml were applicated topically on each grub. The temperature for application and rearing was 19 °C. The mortality during 90 days and the $LT_{50}$ (lethal time, duration up to 50 % mortality) were determined.

**Results and discussion**
The most common pathogens in the investigated *M. hippocastani* populations are *Rickettsiella melolonthae* and *Beauveria brongniartii* (Fig.1). Especially among the grubs *B. brongniartii* holds 50 % of all detectable infections. Only in areas with simultaneous appearance of these both pathogens a fast breakdown within one cockchafer generation is possible. If the population density does not reach about 80 L1/$m^2$ or 30 L2/$m^2$ the infection pressure seems to remain too low for a fast epizootic. Though these pathogens are present some forest stands suffer from stable cockchafer populations below this density level for the durance of at least 3 generations. Other pathogens such as the Microsporidia *Nosema melolonthae* and *Pleistophora tenua* (Trzebitzky 1992) and Nematodes show little impact on mortality dynamics. A varying amount from 5 to 47 % undeterminate mortality occured in the samples from the field (not shown in Fig.1).

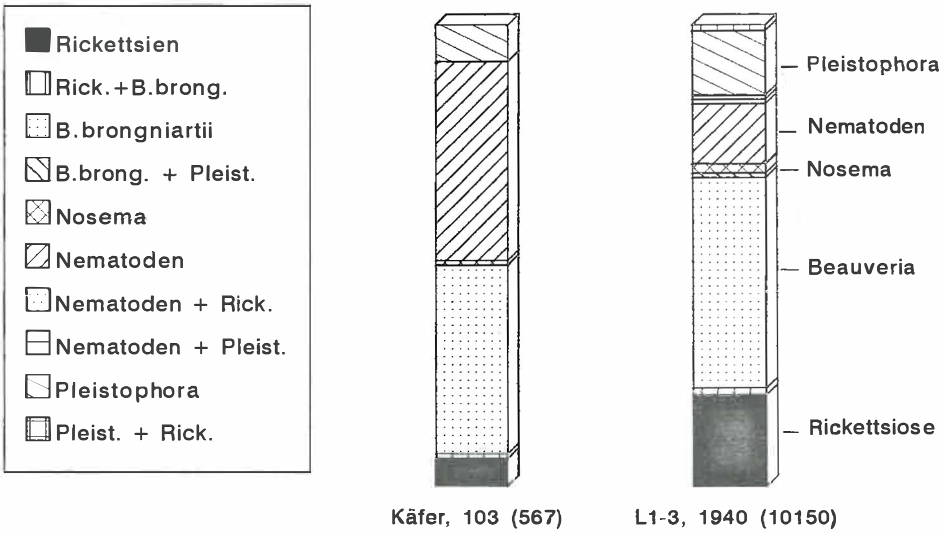


Fig. 1. Distribution of the pathogens within the samples from the outbreak area North of Karlsruhe (Southwest Germany). After digging the grubs and adults were reared in the laboratory and diagnoses were made after death. Double infections are indicated by special signatures.

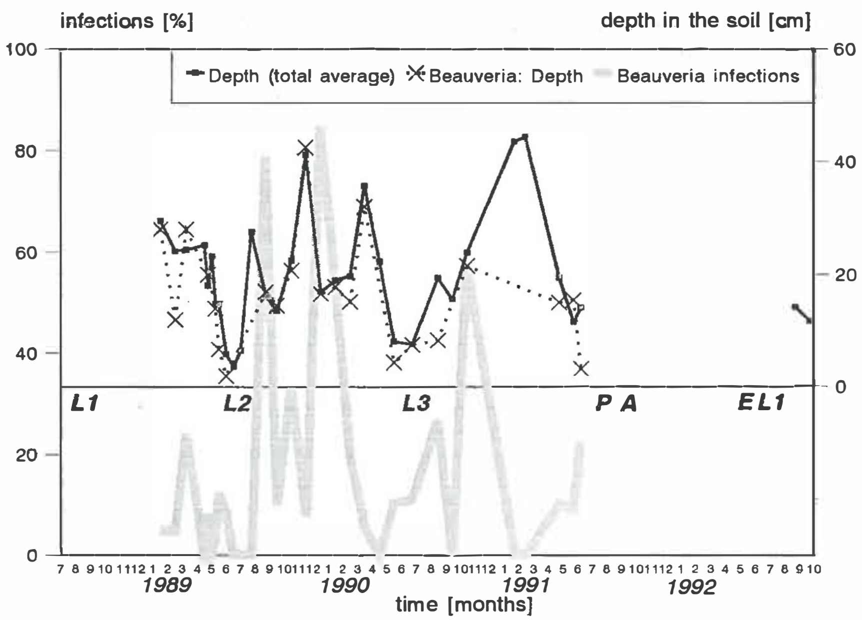


Fig. 2. Vertical migration and *Beauveria* infections from 1989 to 1992 in a population North of Karlsruhe measured by monthly sampling. The presented generation declined from 85 L1, 65 L2, 30 L3 to 10 adults and 70 L1 in the following generation (always individuals/m$^2$). Other pathogens were also present reaching low infection levels. A = Adults, P = Pupae, L = Larvae, E = Eggs

In the soil, the different instars exhibit a wide range of vertical migration (Fig.2). During the cold period in winter some grubs reach a depth down to 1 meter in the sandy soil. According to the weather several vertical migration activities can be observed. *Beauveria brongniartii* appears in all depths and infections occure throughout the year, indicating a high independence of the temperature. The particular depth of the host during the annual migration and the humidity of the soil seem to be of higher importance for infections.

If the winter before the flight period is mild and wet, the adults are susceptible for *Beauveria* infections and might die in the soil to a high amount. Up to 60 % mortality due to *Beauveria* could be stated in the winter 1991/92 among the adults. New infections with *Beauveria brongniartii* occur mainly after upward migrations of the grubs. The highest infection rates can be stated when grubs roam in a depth of about 20 cm. Especially in June during moulting all instars stay in this horizon. Compared with the average depth distribution *Beauveria* infected individuals mostly can be found nearer to the ground surface (Burchard & Trzebitzky 1994).

It is not easy to estimate the duration of *Beauveria* infections under natural conditions. The incubation periods in the samples after the transfer to the laboratory are shown in Fig.3. One half of the infected individuals already died during the first 4 weeks, overall 90 % of the infections lead to death within 90 days. It must be kept in mind, that all animals from the field samples had been stressed in many ways. Aditionally the temperature of 19 °C in the laboratory exceeds the natural level in the soil. It could be observed that infected grubs continue feeding until death.

In the bioassays the virulence of the isolates can be well distinguished by using a concentration of $10^6$ conidia/ml. With higher dosis of $10^7$ conidia/ml the mortality reaches 100 % within 4 weeks for all fungal strains. It is not possible to highten the mortality and the speed of the infection beyond this level. If the spore density on the host surface is too high a concurrence for nutriments and humidity among the germinating spores exists (Trzebitzky 1994).

A low concentration of $10^5$ conidia/ml leeds to a very long incubation period that exeeds the test durance of 90 days (Fig.4). In some fungal strains mycelial growth outside the host is not obligate after death due to *Beauveria* infection. It seems to be necessary to screen for good growth and abundant spore production befor using a strain in field trials.

In accord with the experience of Ferron (1967) second instar larvae (L2) proved to be less susceptible for *Beauveria* infection than L3.

At low temeratures (8 - 12 °C) the incubation period is very long and mortality goes not significantly beyond the control (Fig.5). Germination on a suitable surface still is possible at lower temperatures but takes much longer as could be shown in germination assays on solid medium. After 48 h at 7 °C only 10 % germination occures, at 15 °C between 10 - 50 % can be reached, at 25 °C all spores germinate within 36 h.

Wide differences in virulence within the autochthone isolates from the outbreak area could be detected. Isolates from the spots with highest infection rates proved to be not the most virulent ones. The highest virulence was found in isolates from *M. hippocastani* populations with medium population densities and relatively low infection level. Two strains isolated from *M. melolontha* in Switzerland (kindly provided by S. Keller, Zürich-Reckenholz)) were also very active against the grubs of *M. hippocastani*. The two *B. bassiana* strains, one from *M. hippocastani* and one from *Diprion pini* cocons, proved to be very weak in virulence.

*Metarhizium anisopliae* strains exhibited high virulence in the laboratory but no infections could be obtained in treated field plots (Trzebitzky & Schmid-Vielgut 1990).

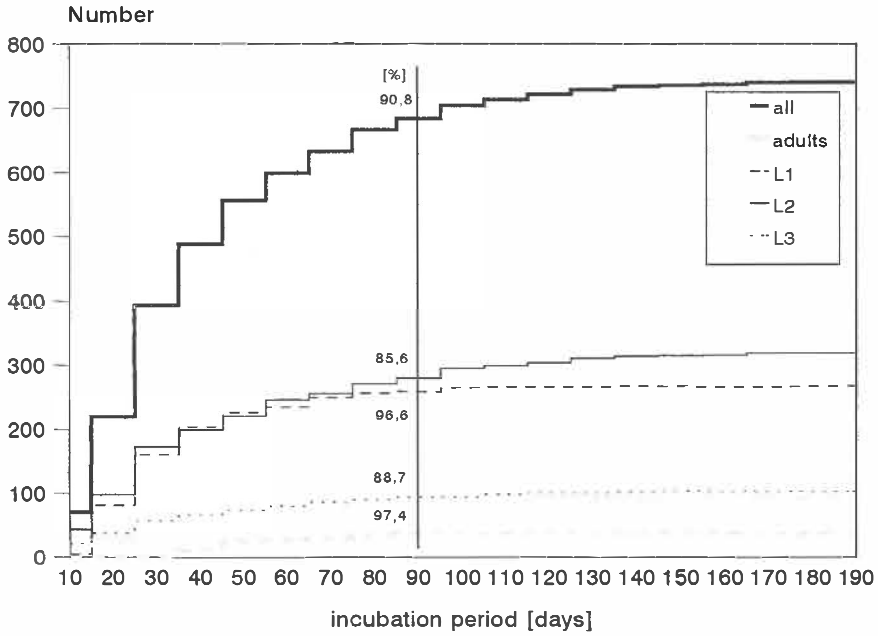


Fig.3. The incubation periods of *Beauveria brongniartii* in the samples after the transfer to the laboratory. Grubs were reared individually at 19 °C in damp peat/sand mixture.

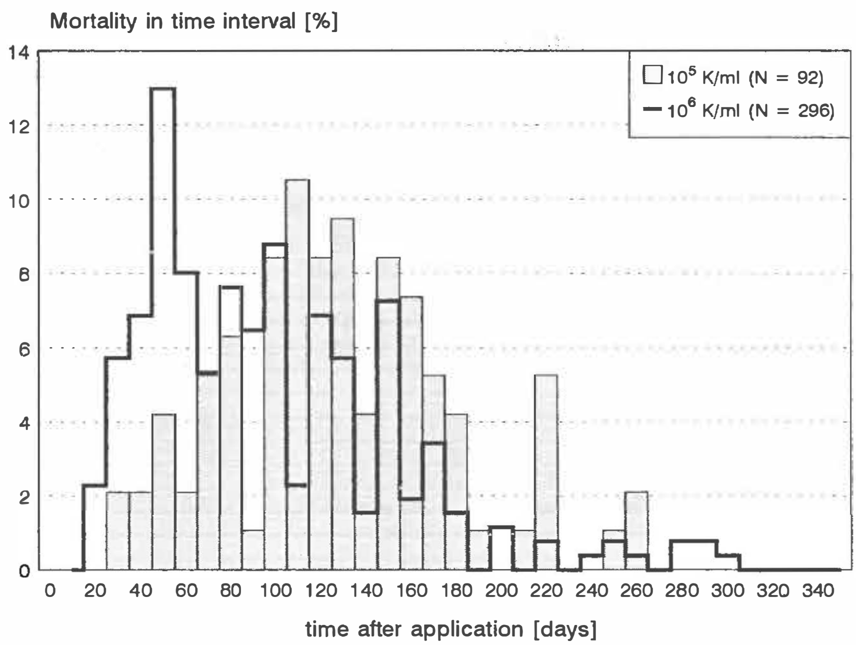


Fig.4. Different incubation period of *B.brongniartii* in bioassays with grubs of *Melolontha hippocastani* treated with $10^5$ and $10^6$ conidia/ml.

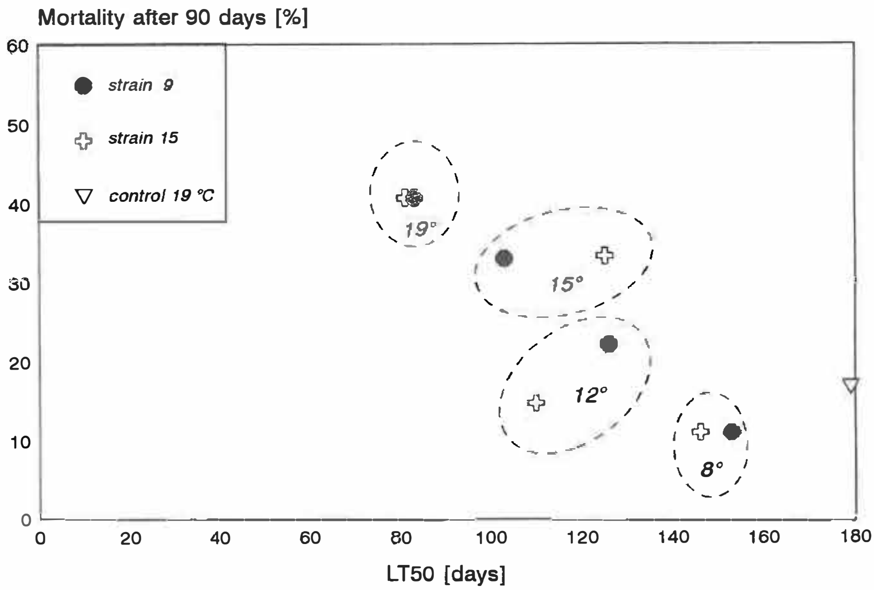


Fig.5. The influence of the temperature on mortality and $LT_{50}$ (lethal time) in a bioassay with L3 of *Melolontha hippocastani* treated with $10^6$ conidia/ml.

In germination assays with coinidia on lipid extracts (solvant: hexane/chloroform) of the cuticula from *M. hippocastani, M. melolontha* and other Coleopteran species a high stimulatory effect of homologous and heterologous cuticle extracts was found, but no correlation between stimulation level on this simulated host surface and virulence was detectable (Trzebitzky 1994).

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# OCCURRENCE OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BRONGNIARTII* IN THE SOIL OF VALLE D'AOSTA AND INFESTATION LEVEL OF *MELOLONTHA MELOLONTHA**

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SUMMARY - The deuteromycete *Beauveria brongniartii* (Sacc.) Petch, one of the most efficient natural control agents of *Melolontha melolontha* L., in Valle d'Aosta, Northwest Italy, is rare within the larval population of the beetle.

In order to verify if the extremely low number of larvae found to be affected by mycosis is due to the poor presence of the fungus in the soil, in spring-summer 1994 the relation between the level of diffusion of *B. brongniartii* in the soil of Valle d'Aosta and the distribution of larvae of *M. melolontha* has been studied.

For each of the 193 sampling points in the European cockchafer infestation area along the Morgex-Montjovet valley-bottom line, the presence of *B. brongniartii* in the soil, the cockchafer larval population density and the frequency of the fungus within the larvae were determined.

In 79.8% of samples *B. brongniartii* was absent. In 58% of the remaining samples the fungal presence was less than $1 \times 10^3$ Colony Forming Units/gram dry soil and in 13% higher than $1 \times 10^4$ CFU/g d.s., with a maximum value of $8.41 \times 10^5$ CFU/ g d.s..

The larval density in the soil of Valle d'Aosta was between 0 and 72 larvae/m$^2$, with a mean value of 9.7 larvae/m$^2$.

Of the 1978 larvae collected only 2 were affected by mycosis.

The analysis of variance and the Duncan test did not show any relation between the infestation level of *M. melolontha* larvae and the diffusion of *B. brongniartii* in the soil of Valle d'Aosta.

The deuteromycete *Beauveria brongniartii* (Sacc.) Petch is one of the most effective natural control agents of *Melolontha melolontha* L., the common European cockchafer, whose larvae cause huge damages to erbaceous and woody crops in Italy (Valle d'Aosta, Trentino Alto Adige) and in center Europe (Switzerland, Germany, Austria).

In contrast with the situation of other zones infested by *M. melolontha* (Keller, 1986; Zelger, 1991), in Valle d'Aosta (Bondaz *et al.*, 1991) the fungus is recovered within the larval population of the insect at a very low frequency; the number of dead adults covered by the micelium of *B. brongniartii* is also very limited.

*Research supported by a grant of Regione Autonoma Valle d'Aosta

Previous researches (Ozino, 1988; Cravanzola *et al.*, 1994b) have excluded the possibility to attribute the phenomenon to a limited virulence of the fungus. In fact the isolates obtained from mycotic larvae of *M. melolontha* from different Valle d'Aosta areas have determined, in laboratory infection essays, a very high mortality on may bug larvae, with values ranging from 70 to 100% in 35-63 days, according to the isolates.

Moreover, the same strains were inoculated in different soils of the Valle d'Aosta territory and showed an entomopathogenic action sufficient for the microbial control of the cockchafer and a good capacity of diffusion and persistence in soil. Virulence was still present 4 years after inoculation (Cravanzola *et al.*, 1994a).

So, it was hypothesized that the modest natural diffusion of the fungus within the larvae of the coleopter in Valle d'Aosta is due to the poor presence of the mycete in the soil.

Aim of the present paper is to estimate the level of diffusion of *B. brongniartii* in relation to the distribution of larvae of *M. melolontha* in the Valle d'Aosta territory verified in the spring-summer 1994.

## MATERIALS AND METHODS

The *M. melolontha* infestation area (8000 hectares) was divided in 18 zones along the valley-bottom line, from Morgex (923 m altitude) to Montjovet (406 m altitude).

In each zone sampling points were established, from a number of 3 as a minimum (Montjovet) to 23 as a maximum (Chevrot-P.Felinaz), accordind to the zone size, for a total of 193.

These sampling points were located in fields belonging to the normal Valle d'Aosta farming cycle (meadow, pasture, orchard).

For each sampling point the presence and the distribution of *B. brongniartii* in the soil, the *M. melolontha* larval density (III instar larvae in 1994) were determined and the frequency of the deuteromycete within the larval population of the coleopter was verified.

For the evaluation of the presence of *B. brongniartii*, for each point the soil of the cultivated layer (0-30 cm depth) was sampled by a hand-drill along the diagonals of the considered lot.

The *B. brongniartii* presence in the soil was evaluated by plating serial dilutions of each sample on Sabouraud dextrose agar with 0.25 g/l of Actidione and of 0.5 g/l of Chloramphenicol for selectiveness (Veen and Ferron, 1966).

The results were expressed as number of Colony Forming Units (CFU)/gram of dry soil.

Each test was carried out in triplicate.

The may-bug infestation level in each monitoring point was determined counting the number of white grubs found in a 0.04 $m^3$ soil sample (0.16 x 1 x 0.25 m) obtained with a Blessinger hoe.

The larval density was expressed as number of larvae/$m^2$.

The frequency of the deuteromycete within the larval population of the beetle was determined counting in the same soil sample the number of larvae affected by *B. brongniartii*.

The significance of all data was examined with the analysis of variance and the Duncan test.

# RESULTS

In 79.8% of samples *B. brongniartii* resulted absent.

In 58% of the remaining samples the fungal presence was less than $1 \times 10^3$ CFU/g d.s. and in the 13% higher than $1 \times 10^4$ CFU/g d.s., with a maximum value of $8.41 \times 10^5$ CFU/g d.s. (table 1).

Table 1 - Diffusion rate of *Beauveria brongniartii* in the 18 zones considered.

| ZONES | | number of samples | % of samples with *Beauveria brongniartii* | CFU/g d.s. (minimum-maximum) |
|---|---|---|---|---|
| A | Villaret-Derby | 6 | - | - |
| B | Lavancher-Morgex | 7 | 14.2 | $2.63 \times 10^2$ - $1.75 \times 10^4$ |
| C | La Ruine-Villaret | 12 | 33.3 | $7.5 \times 10$ - $8.3 \times 10^4$ |
| D | Arvier-Villeneuve-Rumiod | 11 | 9.1 | $2.4 \times 10^2$ |
| E | Sarriod-Sarre | 20 | 45 | $9.1 \times 10$ - $1.16 \times 10^5$ |
| F | Jovençan-Gressan | 15 | 33.3 | $9.1 \times 10$ - $1.87 \times 10^3$ |
| G | Chevrot-P.Felinaz | 22 | 22.7 | $7.8 \times 10$ - $5.5 \times 10^3$ |
| H | Pollein-G.Pollein | 19 | 21.1 | $6.9 \times 10$ - $4.2 \times 10^3$ |
| I | G.Brissogne-Brissogne | 16 | 6.2 | $7.5 \times 10$ |
| J | Brissogne-St.Marcel | 9 | - | - |
| K | Nus | 5 | - | - |
| L | Fenis-Champagne | 6 | 33.3 | $3.8 \times 10^3$ - $8.41 \times 10^5$ |
| M | Fenis | 6 | 33.3 | $7.8 \times 10^2$ - $2.01 \times 10^3$ |
| N | Septumian-Margnier | 12 | 33.3 | $2.42 \times 10^3$ - $2.2 \times 10^4$ |
| O | Prelaz-Torin | 7 | 14.2 | $6.6 \times 10^2$ |
| P | Ussel-Torin-Glereyaz | 11 | - | - |
| Q | Borgo Montjovet | 6 | - | - |
| R | Montjovet | 3 | - | - |

As shown in figure 1, the higher distribution of *B. brongniartii* was detected in the following zones: Sarriod-Sarre (the deuteromycete was present in 45% of samples), La Ruine-Villaret, Jovençan-Gressan, Fenis-Champagne, Fenis, Septumian-Margnier (the fungus was found in 33% of samples).

*B. brongniartii* was never isolated in the following zones: Villaret-Derby, Brissogne-St.Marcel, Nus, Ussel, Torin-Glereyaz, Borgo Montjovet e Montjovet.

The average cockchafer infestation level in 1994 was of 9.7 larvae/$m^2$.

As illustrated in figure 2, remarkable differences in the mean number of grubs per $m^2$ among the different zones were detected: for instance, 0 larvae/$m^2$ in the zone of Montjovet and 20.7 larvae/$m^2$ in the zone of Arvier-Villeneuve.

However the larval may-bug population seems to have a higher diffusion in the territory among Arvier-Villeneuve and G.Brissogne-Brissogne: in fact the mean larval density accounted for 20.7 larvae/$m^2$ in the zone Arvier-Villeneuve, 11.2 in the zone Sarriod-Sarre, 18 in the zone Jovençan-Gressan, 15.9 in the zone Chevrot-P.Felinaz, 9.4 in the zone Pollein-G.Pollein and 10.9 in the zone of Brissogne.

In all the other zones the mean value of larval infestation never exceeded 6.8 larvae/$m^2$.

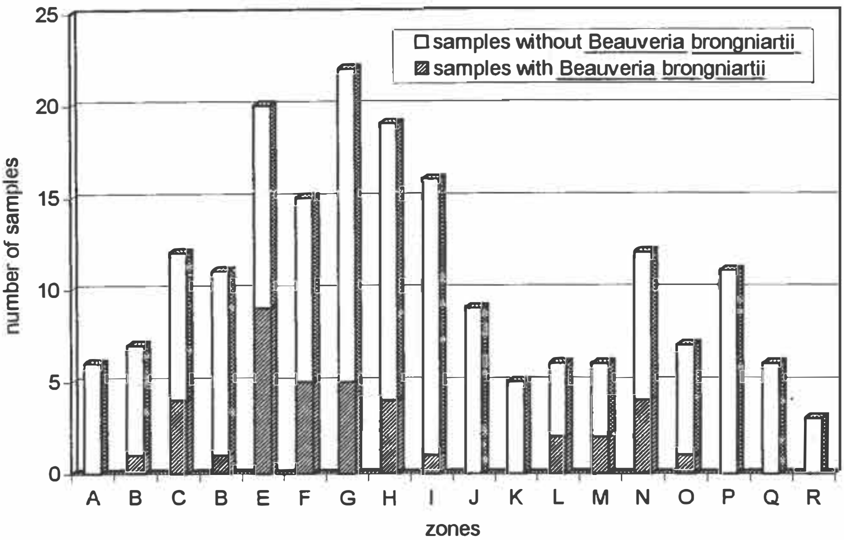


Figure 1 - Diffusion of *Beauveria brongniartii* in the different considered zones.

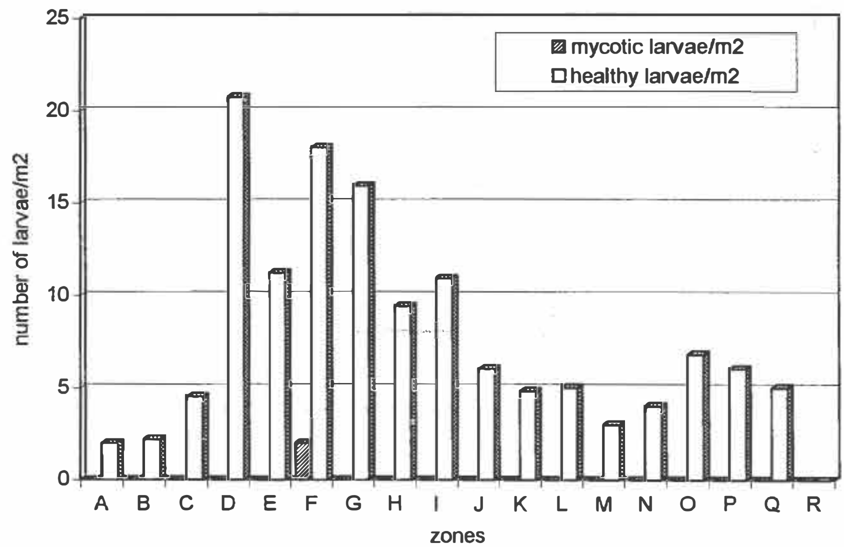


Figure 2 - Degree of infestation by *Melolontha melolontha* in the different zones considered and frequency of mycotic larvae.

Moreover in each zone the larval distribution resulted to be not homogeneous: for example, in the zone Pollein-G.Pollein in a total of 19 samples, in some cases the larval density was 0 larvae/m$^2$ and in some other up to 72 larvae/m$^2$ .

As shown in figure 2, from 1978 larvae collected, only 2 were affected by mycosis, in the zone Jovençan-Gressan.

Variance analysis and Duncan test failed to show any correlation between the diffusion of *B. brongniartii* in the soil and the grade of infestation of may-bug larvae: the larval density appeared in fact high, moderate or scarce but always without any relationship with the level of diffusion of the biological control agent.

## CONCLUSIONS

The above reported results, although restricted to one year only of the experimentation, show a very particular situation, probably due to soil and climate conditions of Valle d'Aosta.

In the main part of samples (79.8%) the entomopathogenous fungus was absent and in the remaining 20.2% the deuteromycete has reached levels often very low (< $1x10^3$ CFU/ g d.s.) only in some cases higher than $1x10^4$ CFU/g d.s..

No relationship between this distribution of *B. brongniartii* in the soil and the infestation level of *M. melolontha* larvae was detected.

Particular concern should be attributed to the extremely low diffusion of the fungus within the larval population in Valle d'Aosta (Bondaz *et al.*, 1991), in contrast with the situation of other European zones heavily infested by *M. melolontha.*

This suggests that the entomopathogenic fungus, due to unfavorable environmental conditions, fails to growth at an optimum level for the production of a sufficient virulence.

On the other hand, the relationship between the presence of the entomopathogenic fungus and the mycosis induced mortality of *M. melolontha* has been surely ascertained (Ferron, 1978; Keller *et al.*, 1986).

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# ESSAI D'EPANDAGE A LA VOLEE DE GRAINES ENROBEES DE *BEAUVERIA BRONGNIARTII.* INSTAURATION ET PENETRATION DANS LE SOL

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## RESUME

L'inoculation et l'établissement dans le sol de *Beauveria brongniartii* (Sacc.) Petch est un problème difficile à résoudre car cette moisissure est particulièrement sensible au rayonnement solaire. La seule technique qui ait donné des résultats satisfaisants est celle de l'enterrement de graines sous le gazon à l'aide d'un semoir conçu par la Station Expérimentale de Laimburg (Bozen); cette technique comporte toutefois de sérieux inconvénients, par exemple la difficulté d'oeuvrer sur les terrains en pente ou l'impossibilité de desservir de très grandes surfaces. Nous avons donc tenté de perfectionner la méthode d'épandage à la volée par la distribution de graines enrobées (avec une charge d'inoculation de 6-10X$10^{10}$ sp/m²) sur la neige fraîche (4 parcelles) ou sur l'herbe haute (6 parcelles). La neige fraîche et l'herbe haute pourraient en effet avoir la capacité de protéger le mycelium du rayonnement solaire pour le temps nécessaire à son instauration dans le sol.

Les résultats obtenus jusqu'à maintenant démontrent une bonne capacité du champignon *Beauveria brongniartii* de s'instaurer et de pénétrer dans le sol, même s'il y a encore des questions que nous espérons résoudre les années prochaines.

## INTRODUCTION

L'inoculation et l'établissement dans le sol de *Beauveria brongniartii* (Sacc.) Petch est un problème difficile à résoudre car cette moisissure est particulièrement sensible au rayonnement solaire. Plusieurs techniques ont été expérimentées: pulvérisation de blastospores par hélicoptère, distribution de suspensions de conidies, épandage à la volée de graines enrobées de mycelium, enterrement des graines sous le gazon à l'aide de semoirs conçus à cet effet. Cette dernière technique a été adoptée dans la province de Bozen car les autres n'ont pas donné de résultat satisfaisant.

L'inoculation à l'aide de semoir conçu par la Station expérimentale de Laimburg (BZ) pose toutefois de gros problèmes dans notre région: difficulté ou impossibilité d'oeuvrer sur les terrains en pente et dans les vergers irréguliers ou avec branches tombantes, impossibilité de desservir de très grandes surfaces.

Nous avons donc envisagé une méthode d'inoculation plus appropriée aux conditions de notre territoire.

Le but était de vérifier la capacité de la neige et de l'herbe haute de protéger du rayonnement solaire le mycelium de *B. brongniartii* répandu à la volée et de vérifier la capacité du mycète de pénétrer dans le sol.

MATERIEL ET METHODES

Les traitements ont été effectués dans un verger (Jovençan) et dans trois prairies permanentes (Sarre, Pollein,Saint-Marcel).

Comme variantes expérimentales nous avons essayé l'épandage à la volée de graines mycosées sur neige fraîche (4 parcelles à Pollein et à Sarre) et sur herbe haute (6 parcelles à Jovençan et à Saint-Marcel).

La dose d'inoculation a été de 200 g de graines enrobées/m² équivalent à 6-10 $X10^{10}$ sp/m².

La surface des parcelles traitées est la suivante:

Sarre-Pollein - 9 m² - (4 parcelles, tot. 36 m²);
Jovençan - 10 m² - (4 parcelles, tot. 40 m²);
Saint-Marcel -100 m² - (2 parcelles, tot. 200 m²).

Les données indiquées dans les tableaux se rapportent à des analyses microbiologiques d'échantillons représentatifs prélevés dans les différentes parcelles; nous avons employé le milieu de culture Sabouraud rendu sélectif avec actidione et chloramphenicol (Veen *et al.*, 1966).

Pour ce qui concerne les deux parcelles de Saint-Marcel, nous avons voulu vérifier si *B. brongniartii* pénétrait en profondeur dans le sol: voilà pourquoi nous avons pensé prélever et analyser des échantillons dans trois différents horizons des parcelles.

RESULTATS

Les premiers contrôles démontrent la présence de *B. brongniartii* 4-5-11 mois après l'inoculation (respectivement à Sarre-Pollein, à Saint-Marcel et à Jovençan) dans 8 des 10 parcelles traitées, avec une charge comprise entre 1,16X10 et 3,01X10³ sp/m² dans les sols où *B. brongniartii* n'était pas présente au moment de l'inoculation (tableaux 1 et 2). A Saint-Marcel nous retrouvons *B. brongniartii* dans tous les trois horizons considérés. Les derniers contrôles, effectués 1,5-2 ans après l'inoculation, sont assez satisfaisants pour ce qui concerne le champ expérimental de Saint-Marcel, mais soulèvent beaucoup d'interrogations dans les autres cas: par exemple, nous ne retrouvons plus *B. brongniartii* dans 4 parcelles où elle était présente lors du premier contrôle (Pollein a,b; Sarre d; Jovençan f).

Par contre, en octobre 1995 nous trouvons pour la première fois *B. brongniartii* dans la parcelle Jovençan g.

CONCLUSION

Les résultats obtenus démontrent une bonne capacité du champignon *B. brongniartii* de s'instaurer et de pénétrer dans le sol, même suite à un seul traitement (Saint-Marcel).

Certes, les derniers contrôles nous posent beaucoup de questions que nous espérons résoudre les années prochaines, en développant cette recherche qui vient de commencer. Il faut, d'abord, comprendre quels sont tous les facteurs qui interagissent avec l'installation et la pénétration du champignon dans le sol de notre Région, qui présente des aspects pédoclimatiques particuliers.

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TABLEAU 1

Contrôles d'instauration de *Beauveria brongniartii* dans le sol.

| LIEU | DATE INOCULATION | CHARGE AVANT INOCULATION sp/g sec | CHARGE INOCULATION sp/m² | CONTROLE JUIN 1994 sp/g sec | CONTROLE OCTOBRE 1995 sp/g sec |
|---|---|---|---|---|---|
| Pollein a | février 1994 | 9,00 x 10 | $6,2 \times 10^{10}$ | $1,71 \times 10^{2}$ | 0 |
| Pollein b | février 1994 | $2,02 \times 10^{2}$ | $6,2 \times 10^{10}$ | $6,47 \times 10^{2}$ | 0 |
| Sarre c | février 1994 | $3,78 \times 10^{2}$ | $6,2 \times 10^{10}$ | $7,54 \times 10^{4}$ | $1,37 \times 10^{3}$ |
| Sarre d | février 1994 | $6,41 \times 10^{3}$ | $6,2 \times 10^{10}$ | $8,73 \times 10^{4}$ | 0 |
| Jovençan e | juillet 1993 | 0 | $10 \times 10^{10}$ | $1,01 \times 10^{3}$ | $5,10 \times 10^{2}$ |
| Jovençan f | juillet 1993 | 0 | $10 \times 10^{10}$ | 1,16 x 10 | 0 |
| Jovençan g | juillet 1993 | 0 | $10 \times 10^{10}$ | 0 | $2,55 \times 10^{3}$ |
| Jovençan h | juillet 1993 | 0 | $10 \times 10^{10}$ | 0 | 0 |

TABLEAU 2

Contrôles d'instauration et de pénétration de *Beauveria brongniartii* dans le sol.

DATE INOCULATION: JUIN 1994

CHARGE AVANT INOCULATION: 0 sp/g sec

CHARGE D'INOCULATION: $10 \times 10^{10}$ sp/m²

| Contrôles (sp/g sec): | 0 - 5 cm | | 5 - 10 cm | | 10 - 20 cm | |
|---|---|---|---|---|---|---|
| | novembre 94 | octobre 95 | novembre 94 | octobre 95 | novembre 94 | octobre 95 |
| Saint Marcel i | $1,52 \times 10^{3}$ | $4,36 \times 10^{4}$ | $1,92 \times 10^{3}$ | $2,62 \times 10^{3}$ | $6,50 \times 10^{2}$ | $6,30 \times 10^{2}$ |
| Saint Marcel l | $3,01 \times 10^{3}$ | $2,85 \times 10^{3}$ | $1,92 \times 10^{3}$ | $1,70 \times 10^{3}$ | $2,70 \times 10^{3}$ | $6,75 \times 10^{2}$ |

# CURRENT STATUS OF *MELOLONTHA MELOLONTHA* CONTROL BY THE FUNGUS *BEAUVERIA BRONGNIARTII* IN AUSTRIA

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## ABSTRACT

A three year field study (experimental area; grassland - 12.5 ha) has been initiated in Kramsach/Tyrol. Since the spring of ´94 *Beauveria brongniartii* - a host specific entomopathogenic fungus - has been applied (25 kilograms per ha of fungus-colonized barely) to preventively treat *Melolontha melolontha* infestation. In order to guarantee a large scale application of the biological control agent in the near future the necessary data with regard to the Austrian plant production legislation will be obtained. Initial results concerning the effectiveness of the biological control agent *B. brongniartii* in the highly *M. melolontha* infested grasslands are herewith presented.

## INTRODUCTION

Over the past ten years *Melolontha melolontha* (cockchafer) has been appearing in large quantities in agricultural and grassland areas in the Provinces Carinthia, Loweraustria, Salzburg, Tyrol and Upperaustria. Although the infestation is usually localized, the level of damage in the infested areas has consistently reached levels between 50 and 100 percent. At present, mainly mechanical techniques for controlling *M. melolontha* are used in Austria. The agricultural experts advise harrowing and/or milling the infested areas, although the degree of effectiveness is inadequate (60 to 90 percent). In addition, these techniques have to be repeated periodically after each outbreak of a new *M. melolontha* population. This results in repeated high costs for the treatment and new seeding of the grasslands. The sanitation costs of the highly damaged areas is approximated at 15,000 ÖS per ha (according to Chamber of Agriculture, 1995). It should be noted that the labour costs (necessary mechanical and manual labour) are not calculated in the above estimate.

As a result the expert opinion is that the promising, preventive plant protection technique *Beauveria brongniartii* should be applied in Austria (Buchgraber, 1994). Accordingly, the goal of the project - in addition to the production of the biological pest control agent, the quality control development, etc. - is the realization of a three-year field study (total life cycle of *M. melolontha* in the test area) in order to register the *B. brongniartii* as a biological control agent with respect to the Austrian plant protection legislation. The final result of these efforts should be large scale production and application of *M. melolontha* biological control agent.

## FIELD STUDY PARAMETERS

Over a period of three years (begin: spring 1994) a field study in Kramsach/Tyrol (experimental area 12.5 ha) is being conducted in which 25 kilograms of fungus-colonized barley is applied with a slit-seeder twice a year. The following parameters are being studied in the sandy, humus and stony soils (3 applied areas and 2 control areas): Standard evaluation of some chemical and physical parameters; determination of the density of *M. melolontha* infestation (all stages); *B. brongniartii* density (cfu per $m^2$, in upper 10 cm); vertical distribution of spores in layers of 0-10 and 10-20 cm; autochthone bacteria and fungus density (cfu per $m^2$, in upper 10 cm). Further, the environmental compatibility - the pathogenic impacts and competitiveness of the beneficial fungus *B. brongniartii* respectively the phytotoxicity of the fungus - must be determined. Additionally, climactic effects (temperature and precipitation level) must also be determined.

Because the field study is in its beginning stages, only a snapshot of the process can be offered at this time, i.e. the data which have been accumulated thus far should not yet be weighed too heavily.

## RESULTS AND DISCUSSION

### Cockchafer infestation in the selected test and control areas

To evaluate the *M. melolontha* infestation and to estimate the success rate of the biological control agent in all of the cockchafer's developmental stages three test areas (TA; *B. brongniartii* treated grassland) and two control areas (CA; untreated grassland) were selected in Kramsach (12.5 ha in total). The selection of the test areas was done according to soil types (sandy - TA1, humus -TA2 and stony-TA3) and field locations (flat grasslands in the Inn valley). Each area (in six divided plots) was evaluated for both the *M. melolontha* infestation and the fungus induced mortality rate of the *M. melolontha* in different development stages (three larvae stages, pupa and adult cockchafer).

At the time of the first sampling (May 30, 1995) only small numbers of *M. melolontha* larvae could be found in the test and control areas (infestation density between 2 and 21 larvae per $m^2$, indication of presence of *M. melolontha* interim population). The cause of the detected low levels was the prior swarming of the *M. melolontha* population (three year developmental cycle), which began approximately on the 20th of April and was still underway due to advantageous weather conditions. The pupa skins of the adult cockchafers could be found at a depth of 40 cm which allowed an estimation of the number of the adult *M. melolontha* per $m^2$ in the test and control areas. The calculated adult *M. melolontha* density exceeded the threshold of 5 *M. melolontha* per $m^2$ (Keller, 1986) so that a targeted *M. melolontha* pest control to reduce damage to grasslands in the future appeared absolutely necessary (figure 1).

The threshold of 5 per $m^2$ represents the density at which the reduction of the pest becomes necessary. A second sampling (late August 1995) confirmed the suspicion that because of the intensive *M. melolontha* swarming in the spring high levels of larvae could be found in the Kramsach fields. Accordingly, an average larva density of over 100 larvae per $m^2$ was recorded (figure 1).

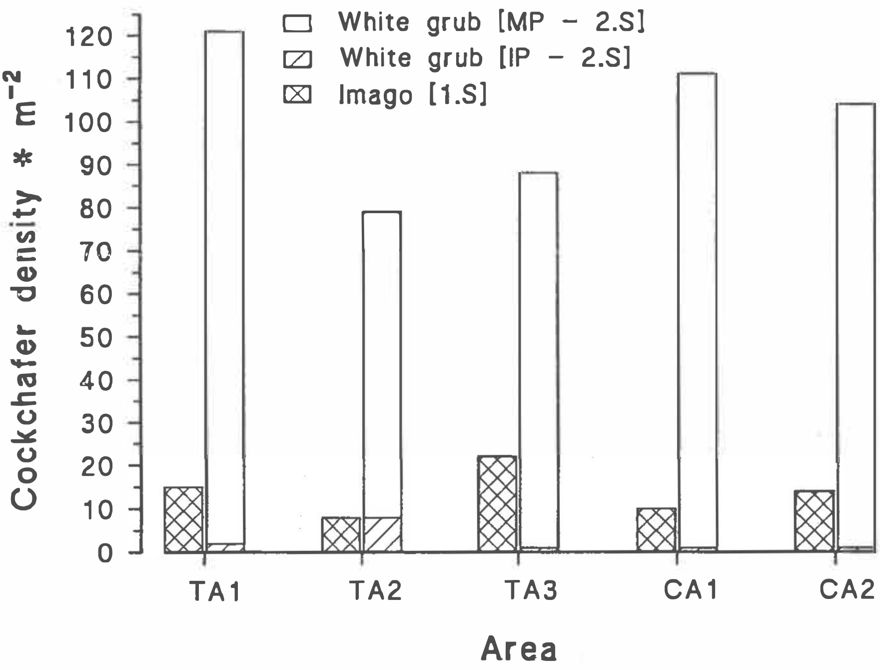


Figure 1 Determination of the *Melolontha melolontha* density (all developmental stages) in the Kramsach, Tyrol area according to samplings in the grassland areas (Test areas..TA 1, 2 and 3; control areas...CA 1 and 2) in May (1.S...1st sampling) and August (2.S...2nd sampling). *Melolontha melolontha* main population (MP; swarming period - 1995), interim population (IP). Explanation see text.

In all of the test areas (with exception of control area two; K2) dead larvae could be found, although it was only in the treated grasslands where *B. brongniartii* infected larvae could be detected (up to 2% of the total population; figure 2). An increased mortality between 0 and 27% was recorded. The causes of *M. melolontha* deaths are varied and must be related to „natural" epizootic (nematodes, mites, bacteria, etc.) as well as mechanical damage (agricultural machinery). The dead larvae were gathered and put on the surface of water-agar (agar without nutrient media) after surface desinfection ($H_2O_2$; 30% w/v). After a few days of incubation at 20°C some of the rotting grubs were detected with symptoms of *B. brongniartii* infection. Also some were determined to have been attacked by nematodes. Still, it could not be ruled out that the larvae deaths were natural and the nematode attacks post mortem.

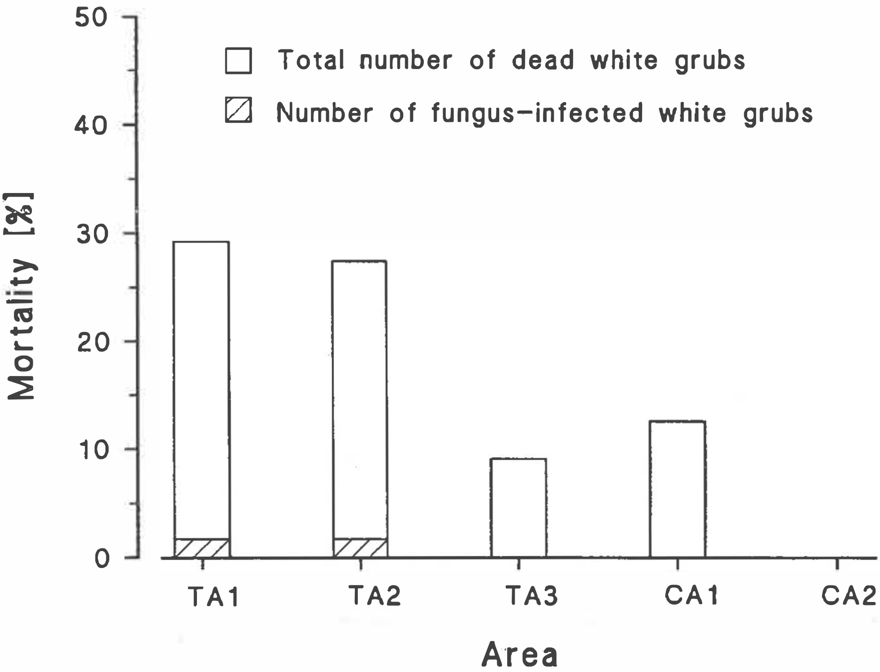


Figure 2 Assessment of *Melolontha melolontha* mortality (data in percent, time of sampling late August 1995) in the three selected test areas (TA1, 2 and 3) and the two control areas (CA 1 and 2) in the grasslands of Kramsach, Tyrol. In addition to the unspecified total mortality, the mortality caused by *Beauveria brongniartii*, fungus-infected larvae, is separately shown.

Enrichment of the entomopathogenic fungus - *Beauveria brongniartii*

*B. brongniartii* could not be reisolated from the soil of the untreated control plots (figure 3). The cfu (colony forming unit) concentrations were all under the detection limit (lower than $10^3$ cfu / g dry weight soil). Conversely, a Beauveria density between $2*10^3$ and $5*10^4$ cfu / g dry weight soil could be detected in the twice treated test areas, so that a highly significant correlation between application with fungus-colonized barley and the presence of *B. brongniartii* in the soil resulted. These results suggest that *B. brongniartii* does not naturally occur in relevant concentrations in the Kramsach region. Additionally, *Metarrhizium anisopliae* and two other possible entomopathogenic fungi (*Penicillium sp. and Paecilomyces sp.*) could be isolated from naturally infected larvae from this region. These fungi do not compete with *B. brongniartii* in terms of virulence and host specificity (Keller, 1991).

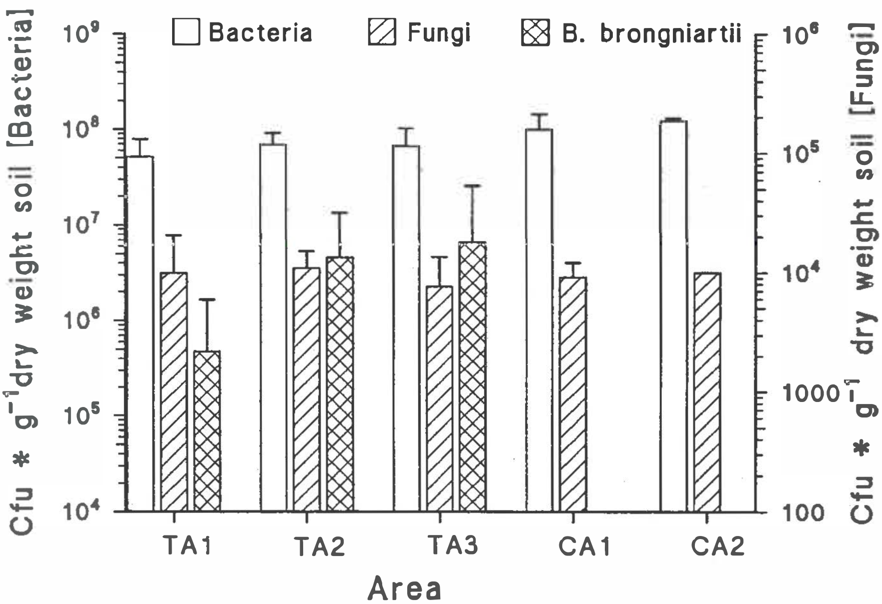


Figure 3 Assessment of the average autochthone bacteria and fungus density of the soil and evaluation of *Beauveria brongniartii* density in the three test areas (TA 1, 2 and 3) and the two control areas (CA 1 and 2). Sampling period May 1995.

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# The influence of soils on the growth of *Beauveria brongniartii*

S. KELLER, B. PÄRLI and C. JACOBER


## Abstract

Field trials to control white grubs with *Beauveria brongniartii* indicated that greater efficacy was achieved in meadows than in orchards. A method was developed to test the hypothesis that orchard soils suppressed fungal growth. Soil samples were taken from orchards and from adjacent perennial meadows at six localities. Native (untreated), humidified and, in one series, pasteurised soils were investigated. In 14 comparisons out of 56 we found statistically significant differences; in 5 cases growth was better in soils from orchards and in 9 cases it was better in soils from meadows. We therefore conclude that orchard soils were not fundamentally different from meadow soils. The observed differences in field trials may have occurred by chance or may reflect farm specific practices.


## Introduction

The fungus *Beauveria brongniartii* (Sacc.) Petch has been successfully used in grassland to control larvae of the cockchafer *Melolontha melolontha* L. In orchards, however, this microbial control method has not established. Results achieved were comparable to those achieved using a chemical control, but both were considered insufficient by farmers. This may have been due to the higher sensitivity of this crop or to the lower damage threshold respecitvely. We also noticed that the fungus induced mortality in orchards tended to be below that in meadows (Keller et al., 1992). The regular application of fungicides in orchards and/or the accumulation of residues like copper were considered as possible causes of this effect. However, neither trials (Keller et al., 1993) nor soil analysis (Matzke, pers. comm.) gave sufficient evidence for such an effect. Therefore we decided to examine soils from orchards for the presence of natural fungistatic or fungitoxic characteristics.

## Materials and Methods

To investigate the growth of *B. brongniartii* in soils we used a commercial preparation. This consisted of peeled barley kernels completely colonised by the fungus. These kernels were referred to as "fungus kernels".
We took 4 soil samples (to a depth of 2 to 10 cm) from each of 6 orchards and their adjacent perennial meadows. The soil from each sample was cleared of stones and other larger particles and placed into plastic boxes measuring 9 x 12 x 5 cm. These boxes were half filled with soil. Sixty fungus kernels were then distributed evenly on the surface and covered with more soil from the same sample. The boxes were closed and incubated at 20°C. After 15 to 17 days the fungus kernels were collected and sieved through 6 and 8 mm meshes. Kernels belonging to the fractions > 6 mm and > 8 mm were counted. For each fraction the number of kernels from orchard soils was compared with that from meadow soils using the Mann Whitney-U-test.
In one experiment using soil samples taken between April and August, we compared fungal growth in untreated soils from all six localities and in soils from four of the localities after an addition of 40 ml water per box. In a second experiment using soil samples taken in September, we compared fungal growth in soils from all 6 localities prepared in three different ways: (1) untreated, (2) water saturated and (3) pasteurised (70°C for 60 minutes repeated the following day). The water content of the untreated soils determined by air-drying was between 19 and 27% weight.

**Results**

In the first experiment the number of fungus kernels with a diamter of >6 mm did not statistically differ between samples from orchards and meadows, either in untreated or in humidified conditions (fig. 1). The fraction of kernels with a diameter > 8 mm resulted in one significant difference in the untreated soil and two significant differences in humidified soils. In all three cases fungus growth was better in soils from orchards (fig. 1) . Also among the non significant values the total number of fungus kernels in fraction >8 mm tended to be higher in soil from orchards.
The results of the second experiment are summarized in table 1. Within the untreated soils three localities had significantly different numbers of fungus kernels in either fraction. In 5 cases the growth was better in soils from meadows and in one case it was better in soil from an orchard. Among the water saturated soils the growth of the fungus was more regular. Only in one case was growth better in the soil from an orchard.
Among the pasteurized soils sites 4 and 6 had statistically significantly different numbers of fungus kernels in either fraction. In all cases more kernels were present in soils from meadows.
In general there were only small differences in the number of fungus kernels between soils from orchards and adjacent meadows as well as between the treatments. An exception was the pasteurized soils which contained in fraction >8 mm on average 1.5 - 2.0 times more kernels than native and water saturated soils.

**Table 1:** Influence of different soil treatments on the growth of *B. brongniartii* on barley kernels in soils from orchards and adjacent meadows. Each number is the average of four replicates. An asterisk means that a statistically significant difference at the 5%-level between orchard and meadow exists.

| site | soil treatment | % fungus kernels > 6 mm orchard | meadow | > 8 mm orchard | meadow |
|---|---|---|---|---|---|
| 1 | native (untreated) | 91.4 | 80.1* | 36.1 | 26.1 |
| 2 | | 83.4 | 88.4 | 32.1 | 32.4 |
| 3 | | 78.9 | 83.6 | 17.0 | 32.8* |
| 4 | | 85.4 | 82.2 | 28.9 | 27.1 |
| 5 | | 77.6 | 90.5* | 20.9 | 52.8* |
| 6 | | 80.4 | 90.1* | 22.6 | 35.4* |
| 1-6 | average | 82.9 | 85.8 | 26.3 | 34.4 |
| 1 | water saturated | 81.2 | 79.7 | 31.7 | 31.4 |
| 2 | | 91.2 | 88.3 | 24.5 | 25.4 |
| 3 | | 88.4 | 90.9 | 18.7 | 26.0 |
| 4 | | 84.5 | 90.2 | 28.0 | 26.5 |
| 5 | | 87.2 | 90.6 | 35.2 | 26.4* |
| 6 | | 87.8 | 92.8 | 20.8 | 19.6 |
| 1-6 | average | 86.7 | 88.8 | 26.5 | 25.9 |
| 1 | pasteurized | 76.8 | 82.8 | 41.5 | 37.0 |
| 2 | | 84.5 | 91.4 | 28.0 | 38.3 |
| 3 | | 91.0 | 92.4 | 44.8 | 56.3 |
| 4 | | 76.7 | 92.9* | 37.5 | 67.0* |
| 5 | | 90.4 | 81.6 | 50.0 | 46.4 |
| 6 | | 89.2 | 96.6* | 48.6 | 63.1* |
| 1-6 | average | 84.8 | 89.6 | 41.7 | 51.4 |

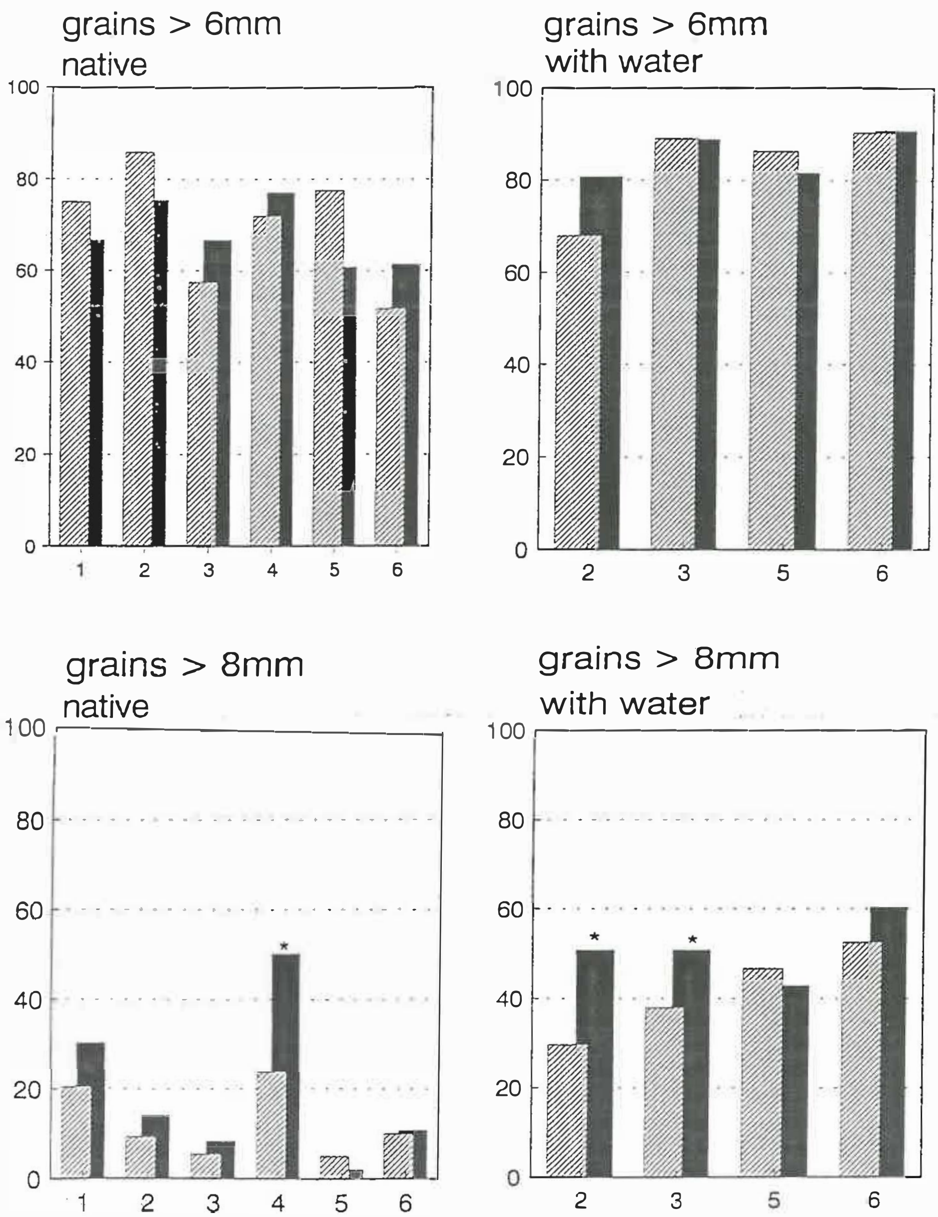


**Fig. 1:** Growth of *B. brongniartii* on barley kernels in native (untreated) and humidified soils from meadows (hatched bars) and from orchards (black bars). The height of the bars is given as percentage of all fungus kernels. The asterisks indicate statistical significances at P ≤ 5%.

**Table 2:** Numbers of statistically significant growth of *B. brongniartii* on barley kernels in soils from orchards and meadows

| Series | Samples | Treatment of soils sample | Dimension of fungus kernels (mm) | Nr investig. soil pairs | Nr of soil samples with significant differences | Growth better in soils from |
|---|---|---|---|---|---|---|
| 1 | 1-6 | native | > 6 | 6 | 0 | |
| | | | > 8 | 6 | 1 | orchard |
| | 2,3,5,6 | humidified | > 6 | 4 | 0 | |
| | | | > 8 | 4 | 2 | orchard |
| 2 | 1-6 | native | > 6 | 6 | 3 | orchard (1)<br>meadow (2) |
| | | | > 8 | 6 | 3 | meadow |
| | 1-6 | humidified | > 6 | 6 | 0 | |
| | | | > 8 | 6 | 1 | orchard |
| | 1-6 | pasteurised | > 6 | 6 | 2 | meadow |
| | | | > 8 | 6 | 2 | meadow |
| 1 + 2 | | | | 56 | 14 | orchard (5)<br>meadow (9) |

## Discussion

In these experiments the growth of *B. brongniartii* in soils from meadows and adjacent orchards was expressed by the number of kernels in two size classes (fractions). The more kernels present the better was the growth. The summary of all statistically significant differences in the number of fungus kernels (table 2) demonstrated that the results were not uniform. In the first experiment the fungus tended to grow better in soils from orchards whereas in the second experiment growth was generally better in soils from meadows. Therefore, we conclude that no systematic differences existed between the soils beneath these two crops. However, we must consider that the two experiments were not completely comparable, since the samples for the first one were taken between April and August while those for the second were taken in September. In addition to this there may also have been differences due to farm specific practices.

The observation that growth of *B. brongniartii* was best in pasteurised soils indicated that soil organisms inhibited this fungus independently of the origin of the soil, which confirms the observations of other authors, e.g. Joussier (1977).

The growth assay presented here can also be used to test side effects of pesticides on insect pathogenic fungi (Keller, 1994).

## Acknowledgements

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# Integrated control

# Protection of orchards from white grubs *(Melolontha melolontha L.)* by placement of nets

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In canton Thurgau, northeastern Swizerland, the damage to agricultural crops by the white grub *(Melolontha melolontha L.)* has steadily increased in the past years. Affected areas include grasslands but most especially orchards.
In Spring of 1994 during the flight period of the cockchafer, 150 ha were covered with fine meshed netting. The goal was to prevent the females from depositing eggs. The netting was placed on the soil and connected between the treelines. In some orchards where there was already hail netting in place, side nets were attached to inclose the area completely.
With the placement of these nets the white grub population was significatly reduced. In the proteced areas, the white grub population was under the tolerance limit.

## Introduction

In canton Thurgau the damage to agricultural crops due to the white grub *(Melolontha melolontha L.)* has steadily increased in the past years. Affected areas are primarily orchards, grasslands are also impacted. Almost half of canton Thurgau's 1500 hectares of orchards are within the breeding range of the cockchafer (adult form of the white grub). Apple trees are especially sensitive to root damage caused by the white grub, depending on the age and rootstock of the tree. A single white grub can ruin a young tree. Practical experience has also shown that neither chemical nor biological *(Beauveria brogniartii)* insecticides can adequately protect young orchards from middle to heavy infestation by the *Melolontha* larvae. That means that new methods have to be developed.

As a result of independent small-site experiments in the flight year 1991, and because of positive results in South Tirol (Varner, 1992; Zelger & Wolf, 1993) nets have been tested in canton Thurgau.

## Material and methods

In 1994 150 hectares of orchard were covered with netting, to prevent the female cockchafers from depositing eggs. The netting was placed on the soil in wide lanes of 4, 4.5 or 5m. They were connected between tree lines with nails or clips. About 12'000 square meter of netting were used per hectare. In a few orchards, where hail nets were already in place, side nets were attached to inclose the area completely. Two different types of black netting (Novatex Company, Sirtori Italy; Sistex Company, Verdellino Italy) and one green netting (Tegum Company, Zürich Switzerland) were used. The mesh size was 4 x 8 mm.

The nets were placed in Spring 1994, shortly before or during the feeding flight of the cockchafer, with the last nets being placed one week after the flight started. While a few

cockchafers could leave the experimental sites, most of them were held back by the netting. The netting was kept in place from May 3rd to June 10th, when the field flight ended.
Placing and connecting the nets took an average of 40 hours per hectare. Treatment of the adult cockchafers with the insecticide Phosalone (3 l per hectare), in cases of high density under the net, was also recommended. Experiments of Zelger & Wolf (1993) have shown that females were nevertheless able to deposit fertile eggs without their traditional feeding flying pattern.

In October 1994 four orchards and in 1995 ten orchards were controlled against infestation by the white grub. Counts to measure grub infestation were made by taking soil samples at conclusion of the experiments. In total 532 soil diggings, each 50 x 50 cm wide and 40 - 50 cm deep, were done.

## Results and discussion

Table 1 and 2 show that the white grub population rate was effictivly reduced by placing nets on the soil. Six of eleven controlled sites were not infested at all. In three orchards the density was under one white grub per square meter. In another two orchards between 1.2 and 1.3 larvae per square meter could be found. The average number of white grubs in the unprotected control sites was nine per square meter. There was no significant difference between the three types of netting nor were the nets damaged by tractors driving over them during chemical treatments. The cockchafers did not succeed in getting through the mesh. The nets also had a repellent effect on the females flying from the woodland borders to the fields. They flew over the orchards covered by netting to deposit their eggs in a unprotected site. Occasional temporary landings by the female cockchafer did not result in infestation, e.g. the cockchafers did not slip through openings in the netting near the tree trunks.

**Table 1: Density of white grub (L2 per square meter) in orchards with netting protected sites and unprotected control sites.**

| Area/Site | | Type of netting | Insecticide | Number of samples | Density per sample | Density per square meter |
|---|---|---|---|---|---|---|
| Guggenbühl | 1 | Sistex | with | 32 | 0 - 1 | 0.3 |
| | 2 | unprotected | --- | 16 | 0 - 6 | 4 |
| Güttingen | 1 | Novatex | without | 16 | 0 | 0 |
| | 2 | unprotected | --- | 16 | 0 - 1 | 1 |
| Donzhausen | 1 | Tegum | without | 16 | 0 | 0 |
| | 2 | unprotected | --- | 16 | 0 - 15 | 22 |
| Biessenhofen | 1 | hail net | with | 16 | 0 - 1 | 0.5 |
| | 2 | unprotected | --- | 16 | 0 - 3 | 2 |

Table 2: **Density of white grub (L3 per square meter) in orchards with netting protected sites and unprotected control sites.**

| Area/Site | | Type of netting | Insecticide | Number of samples | Density per per sample | Density per square meter |
|---|---|---|---|---|---|---|
| Guggenbühl | 3 | Sistex | with | 14 | 0 | 0 |
| | 4 | unprotected | --- | 30 | 0 - 5 | 7 |
| Buchackern | 1 | Sistex | with | 16 | 0 - 1 | 0.8 |
| | 2 | unprotected | --- | 28 | 0 - 8 | 8 |
| Heldswil | 1 | Sistex | with | 16 | 0 - 2 | 1.2 |
| | 2 | unprotected | --- | 36 | 0 - 11 | 13 |
| Hagenwil | 1 | Sistex | with | 18 | 0 - 2 | 1.3 |
| | 2 | unprotected | --- | 18 | 0 - 6 | 10 |
| Güttingen | 3 | Novatex | without | 16 | 0 | 0 |
| | 4 | unprotected | --- | 16 | 0 - 6 | 10 |
| Kesswil | 1 | Novatex | without | 16 | 0 - 1 | 0.5 |
| | 2 | unprotected | --- | 20 | 0 - 4 | 4 |
| Donzhausen | 3 | Tegum | without | 16 | 0 | 0 |
| | 4 | unprotected | --- | 16 | 0 - 5 | 6 |
| Götighofen | 1 | Tegum | with | 16 | 0 | 0 |
| | 2 | unprotected | --- | 16 | 0 - 5 | 9 |
| Biessenhofen | 3 | hail net | with | 28 | 0 - 2 | 1.1 |
| | 4 | unprotected | --- | 16 | 0 - 1 | 1.0 |
| Kesswil | 2 | hail net | with | 16 | 0 | 0 |
| | 2 | unprotected | --- | 20 | 0 - 4 | 4 |

White grubs that were found in some protected areas did in all probability not descend from females involved in the normal feeding period, but rather the grubs originated from cockchafers already trapped under the net. The experiments of Zelger & Wolf (1993), and Keller et al. (1996), showed that a few females could deposit a small number of fertile eggs without the flying feeding pattern.
The attempt to fight the trapped cockchafers under the net with an insecticide did not always lead to a white grub-free soil. Treatment at the right time seems to be critical for success.

The white grub population under hail netting, inclusive side nets, is as low as the white grub population under soil-placed netting. In Biessenhofen and Kesswil 0.5, respectively 1.1 white grubs per square meter were found in the protected test sites (Table 1 and 2). However the density in the unprotected control field also was very low (2.3 per square meter). Once again the treatment of the adults with an insecticide did not lead to a complete extermination.

## Conclusions

Protected orchards - with hail netting or soil placed netting - are not always free of white grubs. However, compared to unprotected areas, the larvae population is much lower and it is below the tolerance limit for young orchards. Experimental results with soil placed netting are very similar to those of Varner (1992) and Zelger & Wolf (1993).

It is estimated that nets can prevent damage in orchards up to a density of about 30 cockchafers per square meter (Keller et al. 1996). At the moment placing nets is the only effictive method to protect crops susceptible due to *Melolontha* larvae.

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# EXPERIENCES ABOUT THE CONTROL OF THE COMMON COCKCHAFER (*Melolontha melolontha* L.) IN TRENTINO BY MEANS OF PLASTIC NETS.

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## SUMMARY

None of the known methods of control allows a satisfactory solution to the great problem of the common cockchafer (*Melolontha melolontha* L.) in orchards. In fact some methods present severe limits in their effectiveness and others are very slow in their activity. A very interesting experience was carried out in Trentino in 1991, in order to search for a method able to interfere on the population of this Coleoptera species. The chosen areas were Mezzocorona and San Michele a/A and the trial was performed on a 12 ha area of apple orchards and vineyards. We covered these 12 ha with nets made of plastic material: we used both anti-hail and shading types. In 1994 we covered 25 ha with plastic nets.
The aim was to prevent the exit of adults from the covered areas as well as a new infestation from oviposing females.

## INTRODUCTION

In the region of San Michele a/A and Mezzocorona in Trentino Province an increasing area near the river Adige has been affected over the past few years by the presence of larvae of the common cockchafer (*Melolontha melolontha* L.). The affected area amounted to 100 hectares before the 1991 flight, to 150 hectares after that flight and to 250 hectares after the 1994 flight. The affected area consists of apple orchards and vineyards. The vines are not susceptible to damages once they have reached 2-3 years of age, while apple trees are extremely sensitive to the damages inflicted on the root system by the larvae of this beetle. In particular dwarf rootstocks (M9-M26) are sensitive to even low population densities (5-10 larvae/$m^2$), while seedlings are affected by the presence of the cockchafer only at higher densities (15-20 larvae/$m^2$).
In some cases the infestation reached such an extent that plants perished and had to be substituted, sometimes leading to the replacement of the whole orchard. The farmers have always been well acquainted with the cockchafer, but in our region there has always been more concern about the adults than about the larvae. During these last years however we have learned about the hazard represented by the larvae.
The first measures taken for the control of this pest were by chemical, and where possible, by agronomic means (substitution of apple orchards by vineyards, ploughing....). Because of the poor results obtained it became necessary to find new methods of control. So we resorted to the entomoparassitic fungus *Beauveria brongniartii*. The fungus, which was supplied by Dr. Zelger from the Agricultural Research Centre Laimburg, was applied to the soil on small areas in 1989 and 1990, and on ca. 80 hectares in 1991. As the flight of 1991 came closer, the fear of a further expansion of the cockchafer rose to such a level that it became necessary to find methods capable of faster reducing the population.
In trials undertaken in Alto Adige in 1989 on small orchards it was observed that the flight of the cockchafer was obstructed by covering the soil completely with hail nets. Encouraged by these results (Zelger R., personal communication) it was decided to adopt this system in Trentino on a

larger area. 12 hectares were covered with plastic nets in 1991 and 25 hectares in 1994, in order to prevent the adult cockchafers from leaving and at the same time to avoid a new infestation by ovipositing females.

## YEAR 1991

## MATERIALS AND METHODS

The area of 12 hectares was divided into 21 plots which were covered with different types of nets: anti-hail nets with mesh size 3x10 mm and 4x7 mm; shading nets grade 30% (mesh size 1.8 x 1.8 mm) and grade 50% (mesh size 1x1 mm) of different colours (green, black/green, black, black/white, white). The width of the net was chosen according to the distance between the rows, with an increment of about 15% in order to achieve a better covering of the soil and a superior adaptation of the nets to the traffic of farm machinery. The nets were put in place by the farmers within the first week of April.

Different systems of fixing the nets along the tree rows were used (u-shaped pieces of wire of 40-50cm length, clipping together with a stapler,...).

5 days (40 hours) of working time per hectare were needed for the placement of the nets. The cost of the nets ranged from 470 Lire/$m^2$ to 600 Lire/$m^2$ and were wholly covered by the *Servizio Vigilanza e Promozione dell'attivitá agricola della Provincia Autonoma di Trento*.

The plots to be covered were chosen in the foci of infestation preferring apple orchards or newly planted vineyards which were afflicted by damages during the previous years.

During the period of flight females of the cockchafer were captured at regular intervals in the woods and under the nets. Part of them were kept separately in cages of plexy-glass in the laboratory feeding them with their typical substrates of the woods (beech and oak) and of the meadow (Rumex, Lamium, Taraxacum and Graminaceae). The other part was preserved in alcohol and examined at a later stage.

On these females the development and maturity of the eggs or the occurred oviposition were evaluated.

The controls during October were carried out by taking samples consisting of 4-8 holes per hectare, the dimension of the holes being 50x50 cm with a depth of 50-60 cm; the soil was sieved in the field and the resulting individuals were counted. The surveys were performed on plots covered with nets and on adjacent control plots where no practice of containment had been put in place.

## DISCUSSION

### Laboratory results

During the observation of the flight we noticed that the adults under the nets mated and were feeding on the different plant species of the meadow. For this reason a laboratory trial was set up. The separate sampling allowed us to compare females originating from plots covered by nets at two different feeding regimes (meadow plants, plants of the woods) and females originating from the woods.

| FEEDING TRIAL - YEAR 1991 | | | |
|---|---|---|---|
| Females collected in the woods and under the nets and fed in the laboratory on the respective plant species for 10 days | | | |
| | % mature fem. | % fem. begin. mat. | % non mat.fem. |
| Females - woods | 93 | 7 | 0 |
| Females - nets | 0 | 50 | 50 |

From the observation of the females fed in the laboratory it emerged that those fed on plants from the woods had a higher food intake, lived longer and moreover completed the development of the eggs. Those fed on meadow species, on the other hand, had a lower food intake, a shorter life, and were not able to complete the development of the eggs.

| FEEDING TRIAL - YEAR 1991 | | | |
|---|---|---|---|
| Females collected in the woods and under the nets on 30/4 and on 7/5 | | | |
| | % mature fem. | % fem.begin.mat. | % non mat.fem. |
| Females -woods | 80 | 20 | 0 |
| Females nets+meadow | 0 | 90 | 10 |
| Females nets+soil | 0 | 70 | 30 |

The further investigation carried out by periodical sampling of females from under the nets gave a confirmation of the impossibility of reaching the stage of complete egg maturity.

**Field results**

| Year 1991: cockchafer larvae /m$^2$ | | |
|---|---|---|
| | plots with nets | control plots |
| examined plots | 12 | 17 |
| minimum value | 0 | 5 |
| maximum value | 8 | 99 |
| mean value larvae/m$^2$ | 3 | 29 |

In October 12 evaluations were carried out in plots with nets and 17 in adjacent non-covered plots. The situation can be summarized as follows: under the nets the average presence was 3 cockchafer-larvae/m$^2$, whereas it was 29 larvae/m$^2$ in the non-covered plots.

| Year 1991: cockchafer-larvae/m$^2$ | | |
|---|---|---|
| Evaluations between and along tree rows in plots covered by nets | | |
| | between rows | along rows |
| nr. of holes 50x50x50 cm | 32 | 32 |
| holes without larvae | 29 | 18 |
| mean presence larvae/m$^2$ | 0.5 | 5.4 |

A further investigation was performed on an area covered with nets, evaluating the population density of the cockchafer between the rows of trees (where the effect of the covering was superior) and along the rows (joint-area of the nets). Between the rows the mean population was 0.5 larvae/m$^2$ and along the rows 5.4 larvae/m$^2$. The reason for this difference was probably the

presence of entry holes for the females, caused by the imperfect closure of the adjacent nets, and not by a different distribution of the cockchafer larvae within the plot. In fact no significant difference in the presence of larvae emerged between areas along or between tree rows during the different investigations in orchards and vineyards without net covering. It is very difficult to realize a perfect closure of the adjacent nets, particularly around the stems of the trees. A small opening may be sufficient for the insect to enter or to leave. It was exactly in those areas that during the flight period the highest accumulation of adults was observed.
From the samplings carried out under the diverse types of nets no significant differences emerged.

## YEAR 1994

## MATERIALS AND METHODS

25 hectares were covered with a black net of plastic material having a weight of 42 $g/m^2$ and a mesh size of 3x7cm.
The dimensions of the nets were chosen according to the following parameters:
- width: distance between tree rows augmented by 15-20%
- length: length of the tree rows augmented by 5%

in order to achieve a better covering of the soil and a superior adaptation of the nets to the traffic of farm machinery.
The nets were put in place by the farmers within the first week of April.
For the placement of the nets about 50 hours per hectare of working time were needed in the adult orchards, while in those between 1-3 years of age the time needed was slightly inferior (35-40 hours). For the recollecting of the nets 25-30 hours/hectare were required.
The cost of the nets was 300 Lire/$m^2$ and was covered by 70% by the *Servizio Vigilanza e Promozione dell'attivitá agricola della Provincia Autonoma di Trento.*
During the period of flight females of the cockchafer were collected periodically under the nets and the development and maturity of the eggs or the occurred oviposition were evaluated.
The controls during October 1995 (larvae of 2$^{nd}$ year) were carried out by taking samples of 4-8 holes per hectare, the dimension of the holes being 50x50 cm with a depth of 50-60 cm; the soil was sieved in the field and the resulting individuals were counted. The surveys were performed on plots covered with nets and on adjacent control plots where no practice of containment had been put in place.

## DISCUSSION

### Field results

During the flight females from under the nets were collected at different periods and compared with those from the woods.

| YEAR 1994: evaluation maturity eggs females | | |
|---|---|---|
| | females woods | females nets |
| 21 April | half developed | beginning development |
| 27 April | mature | beginning development |
| 6 May | mature | beginning development |
| The return flight started on 25 April and ended around 20 May. | | |

On 27 April as well as on 6 May the females originating from the woods presented mature eggs while those from under the nets were just at the stage of beginning development. Nevertheless, to be on the safe side, a treatment with Phosalone on the nets was recommended towards the end of the flight (8-12 May '94) in order to eliminate the females which perhaps might have completed the maturation of the eggs.

| Year 1995: cockchafer larvae/m$^2$ | | |
|---|---|---|
| | plots with nets | control plots |
| controlled plots | 8 | 14 |
| minimum value | 0 | 0 |
| maximum value | 3 | 38 |
| mean larvae/m$^2$ | 0.4 | 7.5 |

In October 1995 8 controls on plots with nets and 14 on adjacent non-covered plots were carried out. The situation can be summarized as follows: under the nets there was a mean presence of 0.4 larvae/m$^2$, whereas in the non covered plots the mean presence was 7.5 larvae/m$^2$.

## CONSIDERATIONS

The obtained results show a very interesting efficiency of the method of covering the soil with nets, revealing a promising way of interference on the cockchafer population. It is very important to place the nets with accuracy, avoiding as far as possible the presence of entry holes for females coming from outside. The laboratory as well as the field observations confirm in fact that the females which remain trapped under the nets and feed on herbaceous plants are not able to complete the maturation of the eggs. With such a diet they are in fact able only to survive their feeding substrate not being qualitatively sufficient for reaching oviposition.

After 15 - 20 May the nets were removed and it could be ascertained that they had not been damaged by the passage of farm machinery except in a very few cases where the nets had been placed on soil without grass cover. Moreover, the grasses never grew through the meshes of the nets but simply lifted them during growth.

Among the different tested systems of fastening the nets the best one was inserting a 5-6 cm long nail through the edges of two adjacent nets. Two nails per tree (one on each side) were needed to close the nets completely around the trunks.

For what concerns the colour the least visual impact was given by black coloured nets.

## CONCLUSIONS

The search for alternatives to chemical pest control is one of the most important commitments of integrated crop production. In some instances, as in the case of the cockchafer, it becomes a necessity because of the ever more evident limitations of the chemical methods of control. A further point in favour of this system, which also is perfectly in line with the principles of integrated production, is the potential of the cockchafers as organic manure. Already in 1886 (Bollettino Agrario) it was recommended to collect the cockchafers by percussing the trees an to re-utilize them as manure. Even in this period it was already known that 100 kg of cockchafers contain 46.8 kg of water, 53.2 kg of dry weight, 1.5 kg of ashes, 0.27 kg of phosphoric acid and 4.29 kg of nitrogen. Since the far 1886 times have changed and the method "by percussion" is no longer realistic, but by covering the soil with nets it might still be possible to take advantage from a "natural fertilization with cockchafers".

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# EXPERIMENTS TO REDUCE *MELOLONTHA HIPPOCASTANI* F. DAMAGES IN THE HESSIAN RHEIN-MAIN-PLAIN

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## 1. Abstract

During the flight period of *Melolontha hippocastani* F. in 1994 the following methods to reduce the damages of this species were tested in field and in combined field and laboratory studies: a) insecticides on the basis of natural substances: „Neem Azal“ agents (= seed extracts from the neem tree, *Azadirachta indica*), „ENVI-Repel“ (= extracts from garlic plants), b) biological insecticides: *Beauveria brongniartii* (= blastospore suspension of the insect-pathogenous fungi), c) chemical insecticides: „Rubitox“ (active substance: phosalone), „Decis“ (active substance: deltamethrine), d) mechanical methods: soil treatment with a rotary hoe.

Under field conditions neem agents had no immediate letal or repellent effect on the beetles but resulted in changes of behaviour: defoliation decreased, the intake of food was subsided completely after two to three days, the flight activity and the egg production was almost perfectly reduced and the beetles did not look for any protection against rainy weather. Beetles forced to feeding on neem treated leaves had no egg production under laboratory as under controlled field conditions. The „ENVI-Repel“ agent did not show any repellent or disturbing effect. There were also no effects of *Beauveria brongniartii* because of the lost of spores ability to germinate. „Rubitox“ and „Decis“ showed an immediate letal effect within 3 - 4 hours which continued about 10 days. Thereby defoliation was reduced successfully.

Negative side effects on other arthropode species were only recognised the day after the treatment with „Decis“. One week and four weeks after the application of the insecticides there were no significant differences on other species between treated and untreated areas.

## 2. Introduction

There is a natural occurence of *Melolontha hippocastani* F. (NIKLAS, 1974) in the Hessian Rhein-Main-Plain. Flight strains with a four year developing cycle are in the hessian forest districts of Lampertheim, Bensheim and Darmstadt (DUBBEL, 1991).

During the last outbreak in the years 1949 to 1956 there was a heavy use of chemical insecticides. The following 30 years there was no damage caused by the cockchafer or its larvae. 1986 damage of 20 to 30 ha young stands was recognized. Up to 1990 the infestation area increased to about 200 ha. In both years chemical control was prohibited, so the infestation area in 1994 enlarged to about 10.000 to 15.000 ha. Under normal conditions the defoliation caused by the adult beetles every four years is not dangerous for the existance of the stands and the loss of biomass production is tolerated by forestry. Compared with that the damage of the roots during the development of the larvae is much more dangerous for the existance of the young stands. But under the special conditions of the Rhein-Main-Plain (population and industry centre, air pollution, subsided ground water level, overlapping of social and protection functions of the forest) both, damage of the roots and defoliation can lead to death of the stressed stands on great areas. Therefore control of *Melolontha hippocastani* must be directed at prevention of larvae feeding <u>and</u> defoliation.

At this time in forestry only the mechanical soil treatment with a rotary hoe is to be used for a few years before and after planting. Other methods either are too expensive or ineffective (e. g. collecting of the beetles), not developed far enough for use in practice (biological methods), or there are no licensed pesticides for use in forests. In addition there are many problems because of ecological disadvantages following an pesticid application.
In field and in combined field and laboratory experiments it should be tested wether several control methods could reduce the damage caused by *Melolontha hippocastani* combined with a well tolerance towards the environment.

## 3. Methods

The following control methods were tested:

a) insecticides on the basis of natural substances:
- „Neem Azal" agents
- „ENVI-Repel"

b) biological insecticides:
- *Beauveria brongniartii*

c) chemical insecticides:
- „Rubitox"
- „Decis"

d) mechanical methods:
- soil treatment with a rotary hoe

The experiment areas were located in the forest districts of Bensheim and Lampertheim in the Hessian Rhein-Main-Plain. They are characterized by an altitude of 90 - 100 m above zero, a mean temperature of 9,5° C and an average rainfall of 650 mm p. a.. The soil is a sandy deposition from Rhein and Neckar with low or middle nutrient and water contents.
The flight period in spring 1994 started at 17.04. and continued to 08.06., strongly influenced by the weather conditions. At 08.05. female percentage reached 49,5% so that the application took place at 09./10.05.94 in young oak (*Quercus robur*) and red oak (*Quercus rubra*) stands in the infestation centre.

## 4. Results

### 4.1 Direct effects on the target organisms

Within 3-4 hours after application of „Rubitox" the first cockchafers fell down from the trees and died a few hours later. Until the next morning about 50% of the beetles lay on the ground, some still living but with reduced activity. The next three days the rest of the beetles died. About one week after the application successful recolinization with cockchafers from untreated areas began.
The insecticid „Decis" also showed immediate letal contact effects. In addition to this a strong repellent effect was recognized. For about two weeks no cockchafers were seen on this area. Successful recolonization started about two and a half week after application.
On the „Neem" treated areas no immediate letal or repellent effects were seen. Instead of this changes of behaviour occured: the beetles had a highly decreased activity, they did not fly any more and did not look for protection when the weather became cool and rainy.
On the areas treated with *Beauveria* respectively „ENVI-Repel" wether immediate nor delayed direct effects on the cockchafers were recognized.

**4.2 Loss of leaf mass**

The loss of leaf mass was determinated at three dates: one day before as well as four weeks and twelve weeks after the application. The results are shown in figure 1.

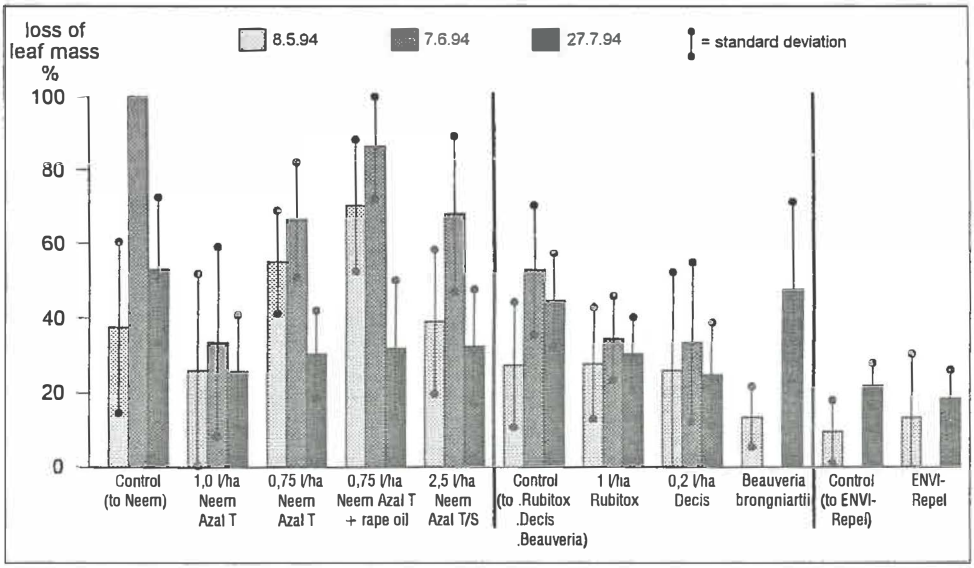


Fig. 1: Loss of leaf mass during the flight period of *Melolontha hippocastani* in spring 1994 (N = 20).

Within one month there was 100% defoliation on the control area to the „Neem" variants. On the other hand the „Neem" treated areas showed a defoliation percentage from 35% to 85%. After finishing of the lammas shoots the defoliation percentage decreased to 53% on the control area and about 30% on the „Neem" treated areas. On the „Rubitox" and „Decis" treated areas the loss of leaf mass only increased for a few percent and on the *Beauveria* and „ENVI-Repel" areas there were no differences seen between treated and untreated areas.

**4.3 Egg production and larvae development**

In figure 2 the number of larvae found in one m² in August 1994 on each area is shown.
On the „Neem" treated areas larvae density was much higher than on the control area to this treatment. Only the area treated with a rotary hoe showed strongly reduced amounts. Opposit to that larvae density on the control area to the „Rubitox", „Decis" and Beauveria traetments was the highest of all experiments whereas the treated areas had reduced densities in the range of the „Neem" variants.

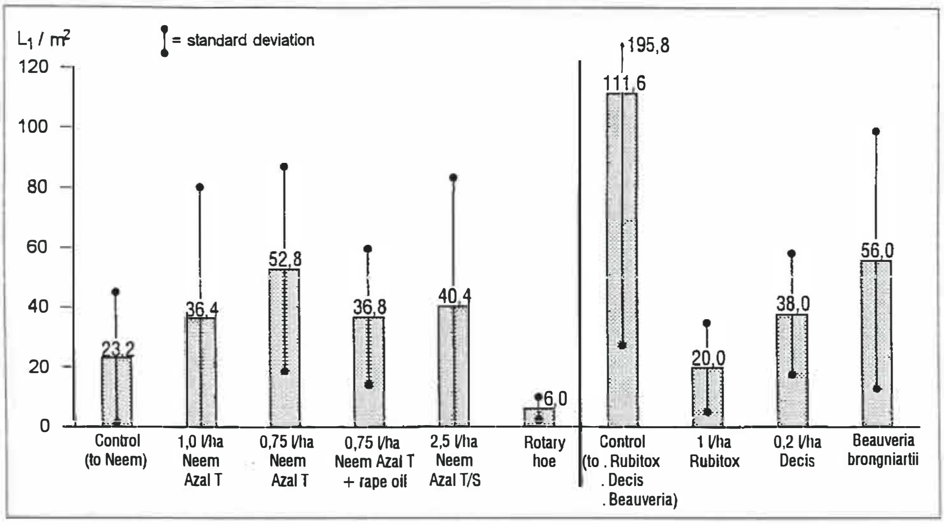


Fig. 2: Larvae density of *Melolontha hippocastani* in one m² in August 1994 (N = 10).

Two and a half week after the application cockchafers from several treated and untreated areas were collected ant brought into laboratory. Beetles coming from the „Neem“ and „Rubitox“ treated areas had strongly reduced egg production and larvae hatching whereas beetles from the „Decis“ area produced even more eggs and larvae than those from the untreated control area (table 1).

| Variant | Ø number of clutches /10 females | Ø number of eggs / 10 females | Ø number of eggs / clutch | Ø number of eggs / female | proportion of damaged eggs (%) | Ø number of larvae / 10 females | hatching - rate (%) |
|---|---|---|---|---|---|---|---|
| **Control** | 5 | 149 | 29,8 | 14,9 | 0,7 | 79 | 53 |
| **Neem 1,0 l/ha** | 3 | 39 | 13,0 | 3,9 | 5,1 | 6 | 15 |
| **Neem+rape oil** | 2 | 22 | 11,0 | 2,2 | 27,3 | 7 | 32 |
| **Rubitox** | 3 | 47 | 15,7 | 4,7 | 6,4 | 19 | 40 |
| **Decis** | 7 | 157 | 22,4 | 15,7 | 0,0 | 88 | 56 |

Tab. 1: Egg production and larvae development of Melolontha hippocastani collected two and a half week after application from the experiment areas.

In another experiment the crowns of 6 trees on every area were wrapped into a net the day after the application. 50 female and 20 male cockchafers were set inside the net so that the beetles were forced to feeding on the treated leafs. Two weeks later the cockchafers were collected and brought into laboratory. Egg production and larvae development is shown in table 2.

| Variant | ∅ number of clutches /10 females | ∅ number of eggs / 10 females | ∅ number of eggs / clutch | ∅ number of eggs / female | proportion of damaged eggs (%) | ∅ number of larvae / 10 females | hatching - rate (%) |
|---|---|---|---|---|---|---|---|
| **Control** | 7,0 | 155 | 22,1 | 15,5 | 1,9 | 90,7 | 58 |
| **Neem 1,0 l/ha** | 0 | 0 | 0 | 0 | -- | 0 | 0 |
| **Neem 2,5 l/ha** | 0 | 0 | 0 | 0 | -- | 0 | 0 |
| ***Beauveria*** | 8,3 | 190,7 | 22,9 | 19,1 | 7,9 | 80,3 | 42 |

Tab. 2: Egg production and larvae development of *Melolontha hippocastani* forced to feeding on different treated crowns under field conditions for two weeks.

Egg production and larvae development of the beetles coming from the „Neem" treated areas was reduced to zero whereas the cockchafers coming from the *Beauveria* treated area produced more eggs than the control beetles. But in this case the propotion of damaged eggs was higher than in control so that the number of larvae decreased.

### 4.4 Side effects

Side effects on non target organisms were seen especially the day after the application on the „Rubitox" and „Decis" treated areas. Mainly great numbers of dead larvae of the gypsy moth (*Lymantria dispar*) and weevils (*Curculionidae*) as well as lower numbers of other butterfly larvae, beetles, flys and spiders were found on plastic canopys in the stands. Within one week dead fall decreased rapidly, only some dead larvae of the gypsy moth were found on the „Rubitox", „Decis" and „Neem" treated areas.
In addition several flight traps and ground traps were brought out on treated and untreated areas to examine more side effects on non target arthropod organisms. The traps were emptied five times: one day before as well as one day, one week, one month and one year after the application. In the first two cases the traps were active for 24 hours and in the next three cases they were active for 3 - 6 days. Negative side effects were only seen on the „Decis" treated area one day after the application when the number of individuals, species and arthropod families was strongly reduced. On all other areas and all other dates the changes in composition of the arthropod fauna on treated areas were quite similar to those on the untreated control areas.

## 5. Discussion

Under field conditions the aim of protection of the endangered young oak stands by reducing larvae production was only reached with the well known method of soil treatment with a rotary hoe. But this method can only be used in a short time before and after planting and the problem is transferred to the neighbour areas. The application of the several insecticides was

only partly successful under field conditions: the loss of leaf mass was reduced but larvae density did not decrease effectively. The reason for that was not an ineffectiveness of the means in principle but the small experiment areas in comparisation to the whole infestation area so that flights from untreated to treated areas were possible in a wide range.
The effectiveness of the several means in principle was seen in the combined field and laboratory studies. Intake of leaf mass, egg production and larvae developement were strongly reduced when the trees were treated with „Neem". The „Neem" preparates only act as a feeding poison and do not show any contact or repellent effects (SCHMUTTERER and KAETHNER, 1988; ROHDE and BRESSEM, 1995). So they are more selective than the other insecticides and would be to prefere until other biological control methods with an even higher selectiveness would be developed for use in practice.

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# Control of the Cockchafer *Melolontha melolontha* in the Kraichgau with NeemAzal-T/S

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## ABSTRACT

NeemAzal-T/S was sprayed against the European cockchafer *Melolontha melolontha* (Scarabaeidae, Coleoptera) in the Kraichgau (Baden-Württemberg) in 1995. This azadirachtin preparation causes a long lasting feeding inhibition after intake with food. NeemAzal-T/S reduces egg production to less than 3%. It is as efficient as the synthetic insecticide Rubitox (Phosalone) in the control of the cockchafer population. The side effects against nontarget organisms are clearly lower for NeemAzal-T/S than for Rubitox.

## INTRODUCTION

The larvae of the field European cockchafer *Melolontha melolontha* cause damage in orchards in the Kraichgau in the south of Heidelberg for more than ten years. Trials to control the white grubs with the fungus *Beauveria brongniartii* demonstrate clearly that this pathogen alone cannot efficiently reduce the *Melolontha* population. Until now, no other entomopathogens are available for control of the grubs (Glare et al. 1992, Schnetter 1988, Trebitzky 1994). *T*wo insecticides were applied by the Landesanstalt für Pflanzenschutz Baden-Württemberg in 1995: Rubitox(Phosalone) and NeemAzal-T/S against the adults of *Melolontha melolontha.*
The aim of the investigations in Heidelberg was to analyse the influence of the neem preparation on the egg production of the European cockchafer .
The side effects of the neem application and the influence on the grub population in orchards was investigated by the Landesanstalt.
The subtropical neem tree Azadirachta indica produces numerous substances which are biologically active. Azadirachtin possesses insecticidal activity. It is a mixture of triterpenoids, which causes after uptake with the food a feeding stop and disturbes metabolism, metamorphosis, and egg production (Rembold 1995, Schmutterer & Rembold 1995). The preparation *NeemAzal-T/S* was succesfully applied against adults of *Melolontha hippocastani* and caterpillars of the Gypsy moth *Lymantria dispar* in Hessen in 1994 (Schmutterer & Nicol. 1995).
Because of this convincing results cockchafer control was partly conducted with *NeemAzal-T/S* in the Kraichgau in 1995.

## MATERIAL AND METHODS

*NeemAzal-T/S* is an extract from neem kernels, contains 10 g Azadirachtin per litre and is formulated in vegetable oil with detergents.
After leaving the soil the adults of *Melolontha melolontha* are concentrated at a small edge strip of the forest for some days. During this time, as soon as the male to female ratio has reached 1:1, a strip, 100 m broad, was treated with NeemAzal-T/S by helicopter: in Horrenberg 11 ha and in Bruchsal 4 ha.

The *neem* preparation was sprayed in 75 l water per hectar in Horrenberg and 50 l in Bruchsal. To test efficacy under field conditions, after spraying, 4 x25 couples of beetles were exposed to treated oak branches in gauze bags in the field for 5 days in each area. Control groups (2 x 25 couples) were kept on untreated branches. Additionally, a further 25 couples from a treated and an untreated area were collected in the forest 7 days after neem application. Subsequently, each group was kept with untreated oak leaves in cages with soil under open-air conditions for four weeks.
The side effects of NeemAzal-T/S and Rubitox were controlled after spraying by the Landesanstalt für Pflanzenschutz during 3 and 4 days respectivily. Dead insects, spiders and mites were collected in the test areas with plastic cases and foils. During September 1995 the grub density of the new *Melolontha* generation was determined in the orchards near the edges of forest, where the insecticides were applied.

## RESULTS AND DISCUSSION

### Inhibition of feeding and egg production

In the groups of chockchafers, that fed on treated oak leaves, there was a clear reduction of feeding. In comparison with the control groups the food intake was reduced to less than 10%. This feeding inhibition was not reversible on untreated leaves. Nevertheless, the mortality of the cockchafers was more or less similar in all the groups. At the end of the trials after 4 weeks the soil in the cages were controlled for eggs. Table 1 shows the results. In the groups from Horrenberg the egg production was reduced by neem ingestion to 3% or less. In the groups from Bruchsal the egg production reached 45%. An explanation for this worse result is that the beetles were fed in bags on oak branches 2-3m above ground. It can be assumed that in the Bruchsal trial these branches were sprayed incompletely by the helicopter. Thus not all the females ingested enough Azadirachtin. Additionally it is possible that several females had already finished food intake for completing the oogenesis. The better result in the Horrenberg trial could also have been caused by the higher volume of water used for neem application.

### Reduction of grub population

In both areas where neem was applied, numerous females were registered flying back from the forest to the orchards for deposition of eggs. Unfortunately, it could not be examined whether these females produced normal eggs. In addition, it was not possible to decide whether these females originated from treated or untreated areas of the forest.
The inhibitory effect of neem extracts on the egg production should have an influence on the grub frequency in breeding sites. Although the area of neem application was rather small, the grub density in the neighbouring orchards was clearly reduced in comparison with the number of beetles which was counted in the soil in the early spring of the same year, as Table 2 shows. The number of grubs of the new *Melolontha* generation was much less than would have been expected in the absence of insecticide application. The reduction rate in Table 2 is a theoretical calculation, because the role of the loss of adults and L1 instar larvae and the influence of the dispersion behaviour of the beetles are unknown. But the data demonstrate that the synthetic insecticide cannot control the *Melolontha* population more efficiently. In order to get a more efficient reduction of the grubs in the orchards , the areas for neem application against the cockchafers would have to be larger.

Table 1: Influence of Neem application on the egg production of the uropean cockchafer (*Melolontha melolontha*)

Field trials 1995. NeemAzal T/S 3 l/ha

| Test area | Number of eggs per female | |
|---|---|---|
| | Control | Neem / % of control |
| Horrenberg a) | 8,28 c) | 0,19 / 2,3 % d) |
| Horrenberg b) | 4,04 ) | 0,12 / 3,0 % ) |
| Bruchsal a) | 6,42 c) | 2,93 / 45,6 % d) |

a) exposed 5 days in gauze sacs

b) exposed 7 days free-living

c) 2 x 25 couples

d) 4 x 25 couples

e) 1 x 25 females

Table 2: Control of the European cockchafer (*Melolontha melolontha*) in the Kraichgau in 1995 with NeemAzall T/S and Rubitox

Reduction of grub number after insecticide application against the beetles

| | number of beetles in soil per $m^2$ ( april 1995 ) | number of grubs per $m^2$ ( sept. 1995 ) | theoretical grub number per $m^2$ * (no treatment) | theoretical reduction rate |
|---|---|---|---|---|
| **NeemAzal T/S** | | | | |
| Horrenberg | 40 | 10 | 200 | 95 % |
| Bruchsal | 12 | 12 | 60 | 80 % |
| **Rubitox®** | | | | |
| Jöhlingen | 32 | 14 | 160 | 91,5 % |
| Bruchsal | 5 | 20 | 25 | 20 % |

* Assumption: 50 % females, 50 % loss, 20 eggs per female

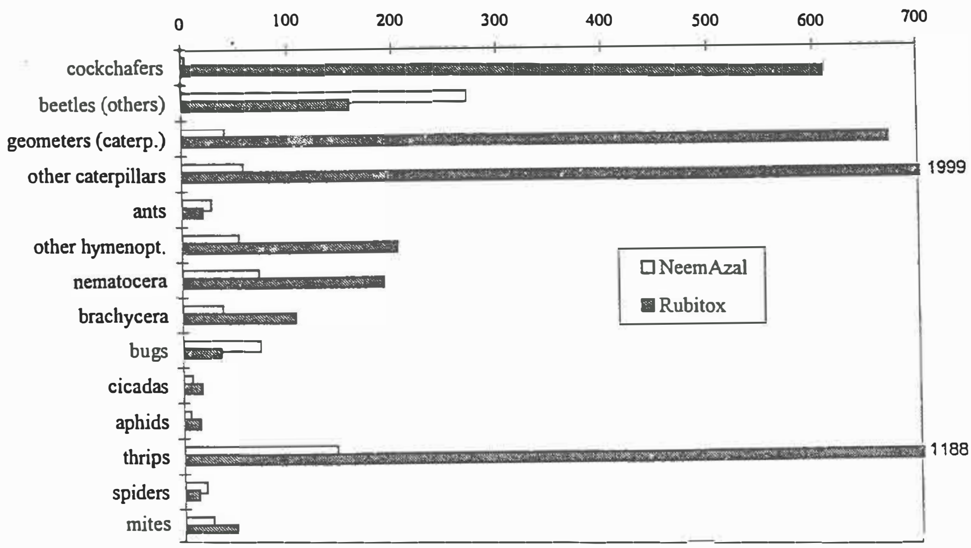
**Side effects against nontarget organisms**

Because azadirachtin must be taken during feeding, only leaf-feeding insects should be damaged by NeemAzal-T/S. To investigate the side effects of this neem preparation insects, spiders, and mites were collected under the trees after insecticide application. The results of the neem and rubitox treated areas are compared in figure 1.

The side effects of NeemAzal-T/S were clearly lower than after rubitox application. Only small species of beetles and bugs were damaged somewhat more. Because NeemAzal-T/S does not possesse an acute toxicity and causes only a feeding stop as first reaction after intake, it can be assumed that formulation ingredients are responsible for the lethal effects (Schmutterer 1995). Investigations in orchards (Viñuela et al. 1996) demonstrate that the predator *Chrysoperla carnea* (Neuroptera) is not damaged by NeemAzal-T/S. Further side effects against nontarget organisms might be possible through an influence on the fecundity. The reduction of the egg production of cockchafers demonstrates, that such an effect may be found in other insects too, if they take in azadirachtin during oogenesis.

An important advantage of neem products has to be emphasized. No toxic effects are known against vertebrates. Thus there are not any preharvest conditions for use on foodcrops by the EPA registration in the United States.

Figure 1: Mortality of European cockchafer and nontarget organisms after application of NeemAzal - TS (3l/ha in 75 l water) and Rubitox (2 kg/ha in 50 l water)



**Aknowlements**
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# Trials to prevent and control the White Grubs of the Common Cockchafer (*Melolonta melolontha* L.) in the State of Baden-Württemberg/Germany

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SUMMARY:

A number of field experiments has been initiated in 1986 aiming at preventing or controlling the White Grubs of M. melolontha in Baden-Württemberg. While excluding experimental details, a brief report on the experiences made are presented in this contribution.

Based on the fact that the Common Cockchafer females avoid both bare and completely covered soils for oviposition, effects of soil cover on the infestation levels were studied. Plantation of perennial crops, (e.g. vineyards and apples, was postponed into the year of flight and the soils kept bare until the end of the flight period. In addition some of the bare soils have been cultivated once or twice in the following summer before sowing the plant cover of the alleys. Accordingly the grub density was reduced and remained on a low level for more than one cockchafer generation. During the 1994-flight some alleys of a vineyard were not mulched, whilst others were cultivated by a rotary digger twice a week to remove soil cover. The grass cover could not be removed sufficiently in the actual fertile soil, because in spring time the manpower required to operate more often is not available. In the autumn 1994 the grub density was highest in the control site and lowest in the non-mulched alleys.
Beginning in 1989 different types of polyethylene nets and fleeces were used to cover the soils to prevent Common Cockchafer females from reaching their oviposition locations. The results showed that these physical method was successful in limiting the infestation. Beetles emerging under the cover were able to lay eggs without any previous feeding. Insecticide treatments may be essential under such conditions when males and females are able to meet each other. Therefore the use of fleeces is not applicable in practice.

Young White Grubs are reported to be quite susceptible to soil cultivation (HORBER und WÜST 1958 and LÜDERS 1958). Rotary digger, disc harrow, tine cultivator and tine harrow are appropriate implements. A considerable reduction of the larvae lasting for several years has been achieved by cultivating twice or more often in opposite direction. But the mechanical methods find their limits on sloping fields (erosion risk), under wet soil conditions, in case of water protection and in integrated farming systems.

Biological control trials were also initiated 1986. Blastospores of *Beauveria brongniartti* (Sacch.) petch. were sprayed at the edges of an infested forestry strip. However, the flight was toor weak to justify any interpretation of the results. Negative side-effects on non-target-organisms were not observed in this experiment. A further experiment using the same pathogen was carried out. Considering the essential conditions for the fungus infestation (temperature min. 18 C, windless and darkness), which hardly will coincide in this country no further experiments are planned.

Experiments with conidiospores of the same fungus started in 1990 in the Kraichgau region including about 17 hectares. 25 kg/ha of infected whole wheat grains (produced in our institute) have been applied. In the 1991 autumn assessment about 30% of the White Grubs were infected. In addition 40 kg/ha (product of Samen-Schweizer, Thun/Switzerland) were applied both in autumn 1994 and spring 1995. In the 1995 assessment the infection rate has been very low.

Since the 1994-flight a new series of experiments were set up at the Kaiserstuhl site in vineyards and in an apple orchard. Pathogen spores were applied in both autumn 1994 and spring 1995 at a rate of 40 kg/ha. White Grubs were sorted out of soil samples (0,25 $m^2$) and the diseased individuals were counted in the field. This year 8% of the larval population collected from the vineyards were found to be diseased, whilst almost none of the larvae in the apple orchard have shown infection symptoms. The rate of larval infection by the pathogen did not really differ from those occurring naturally there. The most probable cause seems to be the unsuitable farm implements available concerning the given soil types. Further experiments have to be carried out using specific machinery.

At the lab levels the efficacy of different parasitic nematode species was verified, indicating that field experiments would not be promising. GLAS (Regierungspräsidium Freiburg) performed a trial in a meadow with few sweet cherry trees using *Heterorhabditis bacteriophora* (POINAR 1975), On a rainy day. Hardly one infected grub could be sorted out of the soil.

Contrary to other countries the state government is accepting to legalize the use of insecticides against the adults at forestry edges. This is despite the strong rejection of forest managers to the idea of using pesticides. First trials to evaluate Phosalone and Azadirachtin were set up this year. The preliminary results have been presented by SCHNETTER.

Soil insecticides to control White Grubs don't affect the population density at all, but sometimes can prevent grubs from feeding on the roots. Since they are, applied in full rates, rather expensive, three trials were carried out applying various insecticide as well as conidiospores of *Beauveria brongniartii* (Sacch.) Petch. (washed off the grains), placed close to the roots of apple trees by a soil injector. The results obtained, however, were not promising. The use of soil insecticides cannot be recommended

Table 1: Valuation of the results

| Measures | Value | Estimation |
|---|---|---|
| **preventive** | | |
| postponing the planting dates | ++ | appropriate for permanent crops to be planted |
| keeping the soil bare over the whole flight period | ++ | not appropriate on slopes, during a wet summer, in water conservation areas and within integrated plant production programmes |
| cockchafer nets | + | appropriate for existing fruit gardens |
| + insecticide | ++ | in case of high cockchafer density beneath the nets |
| **controlling** | | |
| soil cultivation | ++ | not appropriate on slopes, during a wet summer, in water conservation areas and within integrated plant production programmes |
| *Beauveria brongniartii* | | |
| blastospores | - | conditions cannot be guaranteed |
| conidiospores | +- | not yet developped for use in practice |
| Nematodes | - | not yet developped for use in practice, far too expensive |
| Insecticides | | |
| adults (feeding on leaves) | ? | appropriate |
| grubs | - | in case of need only |

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Z.angew.Entomologie **42** (1) 1-88

# Methods to prevent and to control infestation of Common Cockchafer (*Melolontha melolontha* L.)

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## Summary

Farmers remember well the effective cockchafer control by helicopter at the edges of forests obtained in the years after World War II. Especially they did not forget that the costs both of insecticides and application had been payed by the government of Baden-Württemberg. But as long as treatments of forestry edges are not feasible they are waiting for a scheme of simple, effective, cheap methods that are applicable under all conditions. The more I come to the details the more it becomes obvious that this challenge can only be accomplished inadequately.

The recommendations are adapted to the situations in the state of Baden-Württemberg and are based on the work of HORBER (1958), HORBER UND WÜST 1958) and LÜDERS (1958), whose results can be relied on even today. Within the following scheme (table 1) conidiospores of *Beauveria brongniartii* (Sacch.) Petch. deliberately aren't mentioned because until yet we got no positive results from our experiments. In addition as far as we know such a product will never been registered in Germany. Recommendations concerning the control of White Grubs by insecticides also are not included because for this purpose insecticides are not registered and in the future presumably will never be.

The scheme is based on the principle of protection of the threatened crops and not to control adults feeding outside the fields. For that purpose farm husbandry has to be orgenized in such a manner that all the preventive measures and control methods can be carried out.

## Prevention measures

Common Cockchafer females avoid bare soils and turn to other sites for ovipositing. In the year of flight the farmers can make use of this behaviour by keeping the soils bare during the whole flight period with

a) late crops (e.g. late potatoes, beets) plantletmay not emerge before the end of flight

b) the alleys of permanent crops (e.g. fruit trees, grapevines, hops, nurseries) established in spring season.

The females also avoid densely and high grown crops. So early crops (e.g. winter grains, early potatoes), grassland and growth of set aside fields should cover the soils as completely as possible. In an area, however, where the females cannot turn to a more suitable site for ovipositing this prevention measure doesn't work.

In all those fields where the soil cannot or is not allowed to be bare during the flight period oviposition can effectively be prevented by covering the ground with polyethelene nets. But since cockchafers emerging under the nets are able to mate and deposite fertile eggs without previous feeding an insecticide has to be sprayed additionally over the nets when the density of beetles is high enough that males and females can meet each other.

Control measures

The effectivity of soil cultivation by a rotary digger, (less appropriate: disc harrow, tine cultivator or tine harrow) against young White Grubs (1st and 2nd larval stages) has been elaborated by HORBER UND WÜST (1958) and LÜDERS (1958). Therefore cultivation is most effective when carried out in the year of flight when hatching of the grubs has finished. The most suitable period of time is july until september, except in the case of severe drought when the White Grubs are living deeper in the soil. Soil cultivation for a second time for a second time in the opposite direction will improve the efficacy. The range of application includes early crops (after harvesting) of agriculture, most crops of horticulture, alleys of nurseries, alleys of fruit gardens or grapevineyards, hops (between the rows). Common Cockchafers feeding on the leaves of stone fruit or grapevines may be controlled by insecticides. However, plants which are not grown commercially because of special official regulations in Baden-Württemberg are allowed to be treated with an suitable insecticide.

When farmers comply with these recommendations as far as possible in the following year crops normally may seldom be damaged.

As easily can be seen the scheme does not include all the ranges of application e.g. dense plantations of nurseries or in public green spaces and others. In addition to this political demands to farmers creating conflicts concerning the aims to comply with as well as given conditions limiting the applicability of the methods recommended in the scheme may impede or even make impossible to carry out the methods (tables 2 and 3).

Preventive measures on the other side are the less appropriate to avoid Common Cockchafer damages the higher the population density is. This is also true concerning the geographical spread and the actual phase of the cycle. Hence preventive measures have to be initiated at the end of the latent period. It is the task of the advisory service to establish a monitoring system so that farmers can initiate preventive methods as early as possible. But none of the accountings available include reliable hints concerning the actual phase of the cycle. We also don't know which phase the actual Common Cockchafer populations in the state belong to. Since the state institute has not got either records of past cycles nor the capacities required to perform a monitoring system, it is very important to have the possibility of treating forestry edges to control the adults of Common Cockchafer.

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**Table 1**: Recommended methods to prevent infestation by white grub and to control

| range of application | methods in the year of flight | |
|---|---|---|
| | from pupal stage until the end of the flight | from hatching until september |
| agriculture<br>early crop<br>late crop | <br>high growth, maximum density,<br>sowing/planting late into bare soil | <br>rotavating after harvest |
| legumes, ornamental plants | | rotavating |
| asparagus yielding | cockchafer net (+ insecticide)[1] | |
| hops | late cutting, bare soil | rotavating after hilling up and after harvest |
| strawberries | cockchafer net | rotavating before planting |
| fruit<br>yielding<br>stone fruit — beetles feeding on leaves<br><br>young plantation — autumn before year of flight<br>**or** spring of year of flight<br>autumn of the year of flight | <br>cockchafer net (+ insecticide)[1]<br>insecticide<br><br>bare soil **or**<br>green cover + cockchafer net (+ insecticide)[1]<br>early crop | <br><br><br><br><br><br>rotavating before planting |
| nurseries, public green spaces<br>individual plants | <br>bare soil and/or cockchafer net (+ insecticide)[1]<br>plantation after the end of oviposition | |
| grapevines — young plantations | bare soil and/or cockchafer net (+ insecticide)[1]<br>plantation after the end of oviposition | rotavating before planting |
| set aside fields | high growth, maximum density,<br>no mowing before the end of oviposition | rotavating in case of need |
| grassland | complementary seeding at the end of may in the year before flight, in the year of flight first mowing after flight period | |

[1] in case of need only

Table 2: Conflicting aims

| political demands to agriculture | demands for prevention and control whithout insecticides |
|---|---|
| extensification | intensification (manpower, energy, time) |
| reducing tillage | intensive tillage |
| integrated farming | bare soil not allowed |
| green cover | bare soil at times not allowed |
| protection against erosion | intensive tillage |
| waters protection | intensive tillage |
| official regulations in the state | no insecticide allowed in crops not grown commercially |

Table 3: Limiting factors

| factor | effects on Common Cockchafer control |
|---|---|
| consciousness of the problem | infestation is perceived too late |
| wet summer | husbandry impeded or impossible |
| very stony soil | dense and high growth can hardly be achieved |
| dense plantings | tillage impossible |
| sandy soils | frequent tilling ruins soil structure |
| erosion risk | tilling not feasible |
| waters protection | tilling not allowed at times |
| no insecticides registe-red | chemical control impossible |

# The population dynamics of the cockchafer in South Tyrol since 1980 and the measures applied for control

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Abstract:

This paper refers mainly to the infested area of the "Unterland"-region south of Bozen (Bolzano).
The present massive increase of the cockchafer population in South Tyrol has had its beginning in 1980. After a culmination of the damages to the orchards in the years 1986-89 there was a continuous decline of the larvae density as a result of the taken measures of control.
Covering the soil in areas of high larvae-density during the flight period in order to prevent oviposition proved successful. For areas of low population density the application of the fungus *Beauveria brongniartii (Sacc.) Petch.* is recommended as a strategy for the long term regulation of the cockchafer population.

## Introduction

The present massive increase of the cockchafer population in South Tyrol has had its beginning in 1980. The infested areas were initially very localized, but have continuously been expanding ever since. At present around 6000 hectares of cultivated area, mainly orchards, are affected (Fig. 1).

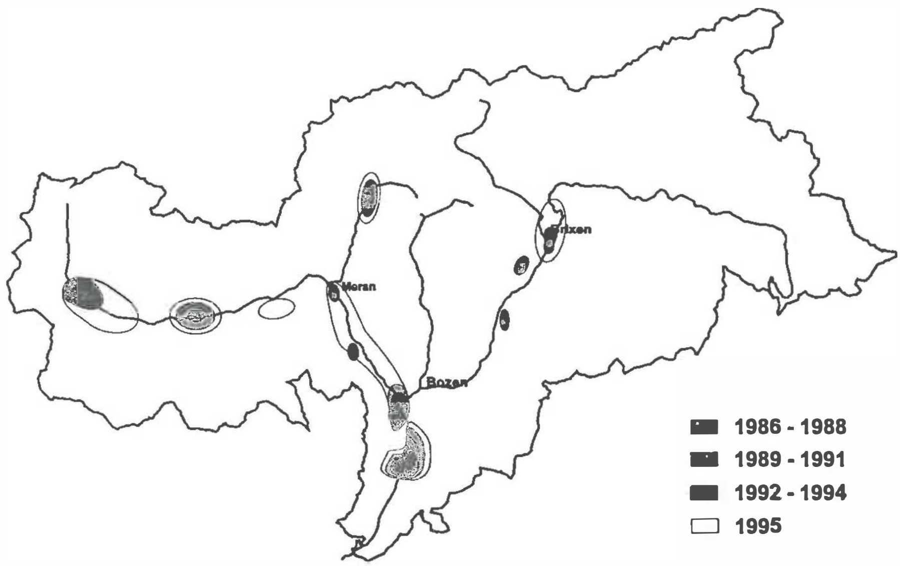


Fig. 1: Development of the cockchafer infestation in South Tyrol

In most of the affected areas prevails the common cockchafer *(Melolontha melolontha L.)* with a 3-years-cycle; only in the upper Vinschgau-valley and in the Völs-area of the Eisack-valley predominates the chestnut chafer *(Melolontha hippocastani F.)* with a 4-years-cycle.
In 1986 a project was started aimed at finding methods of control which should ensure a limitation of the damages as well as a long term regulation of the pest population. As this latter aspect in particular may only be achieved for a whole flight area, the different methods of control were tested not only on small-scale plots, but in the whole affected area.
This present paper deals with the oldest and at same time largest infested area in South Tyrol, which is located in the "Unterland"-region south of Bozen (Bolzano) and covered an area of around 800 hectares at the beginning of the project.

## The development of the cockchafer infestation since 1980

Since the beginning of the monitoring in 1986 evaluations of the larvae population have been carried out each year in autumn. The evaluation of the larvae-density always occurs at the same 10 selected locations within the affected area, with 8 holes being dug per location; the holes have a size of 50x50 cm with a depth of 20-80 cm, according to the depth of hibernation of the cockchafers. The relative results since 1986 are summarized in table 1.

Tab. 1: Mean larvae density per m² in the area of infestation "Unterland". (Alluvial plains of Adige-river; autumn surveys).

| Year (stage) | Generations | | | | |
|---|---|---|---|---|---|
| | 1980 - 82 | 1983 - 85 | 1986 - 88 | 1989 - 91 | 1992 - 94 |
| 1st year (L2) | ? | ? | 90 (50 - 320) | 35 (20 - 90) | 7 (0 - 52) |
| 2nd year (L3) | ? | ? | 50 (30 - 120) | 18 (10 - 60) | 4 (0 - 40) |
| 3rd year (adults) | ? | ? | 25 (15 - 45) | 5 (2 - 30) | |
| damages | none | locally limited | massive; eradictions | slight - heavy | none - slight |

There are no exact figures available for the generations from 1980-85. According to witness from farmers, larvae were frequently spotted during soil tillage; there were no accounts of damage from the generation 1980-1982, the 1983-85 generation however was reported to have caused occasional damages. Massive damages were caused thereafter particularly by the following generation (1986-88). Several orchards had to be eradicated as a consequence of the damages caused by the larvae. The mean densities of larvae in the different years of development were 90, 50 and 25 per m$^2$ respectively.
The larvae density of the generation 1989-91 was substantially reduced, not at least due to the the various measures applied for control. The density dropped from 35 larvae/m$^2$ in the first year of the development cycle to 5/m$^2$ in the third year. This generation was still causing damages, although not to the same extent as the previous generation. For the generation 1992-94 a further reduction of the larvae-density was observed. The mean values were 7, 5 and 4

larvae/m$^2$ in the first, second and third year of development respectively. Also the damages to the fruit-trees were clearly reduced; they were limited to those orchards where no adequate control had been carried out. By 1995 the flight in this affected area had suffered such a reduction that there remains no risk of damages from the present generation.
Despite the substantial decline of the larvae-density over these last years the extension of the flight area has increased, both towards north and south. This may be explained with the flight behaviour of the swarming adults. It has been observed that the cockchafers fly more or less straight towards the feeding trees at the valley border; the departure towards the areas of oviposition however takes place also at differing angles, thus determining a colonization of new cultivated areas after each flight, independently of the population density of the initial focus of infestation.

Type and success of control measures

The different measures of control, which were adopted in South Tyrol for minimizing the damages, may be distinguished into various phases characterized each by a certain principle of control.
1980-85: hardly any control. This is the phase of the onset of the cockchafer-gradation. The importance of the infestation, by that time still being low and not causing any damage, was not recognized, and thus no measures of control were taken. Only as the first damages appeared in 1985 in localized areas, soil treatments with insecticides were carried out, which however had little prospect of success due to their late timing.
1986-88: chemical phase. After the massive flight in spring 1986 trials were started with several soil insecticides applied either as liquids around the trees or as granules incorporated into the soil (Zelger, 1987). These trials already revealed that chemical control of the larvae is labour-intensive, expensive and of limited success. Nevertheless, the farmers made massive use of insecticides, as there was no other option available. At the end of this generation of larvae it became clear that despite the repeated and large scale applications of chemicals the cockchafer population could not substantially be reduced, although in some instances a massive use of insecticides could limit the damages to the trees. The population density of the cockchafer in the autumn preceding the year of the flight had remained at such high levels that heavy damages to the orchards by the subsequent generation had to be expected.

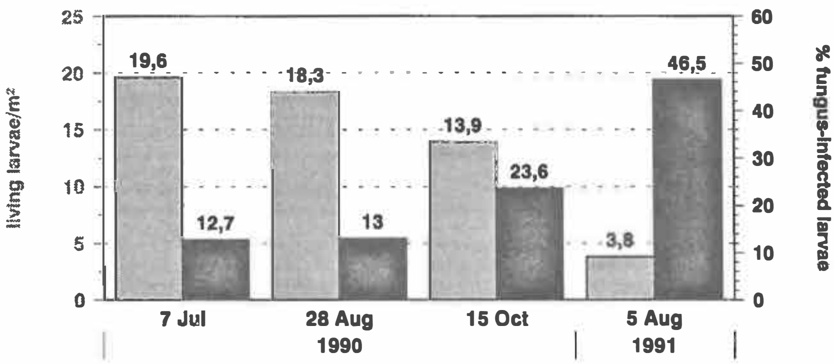


Fig. 2: Density-variations and infection rate of larvae after application of fungus-inoculated barley. (Orchard "Psenner" - Treatments: autumn 89, 12.7. and 4.9.1990; flight year 1989)

1989-91: biological phase. Following the positive results obtained in Switzerland (Keller 1983, Keller, 1986) in 1989, blastospores of *Beauveria brongniartii (Sacc.) Petch.* were sprayed also in the "Unterland"-region onto the swarming cockchafers at the margins of the woods. Despite the high rates of infection the effectiveness of this control measure was not sufficient (Zelger, 1993). At the same time a mass propagation on barley grains of the cockchafer-pathogen was started. In 1989 and 1990 the antagonist was applied in a broadly organized action to about 80% of the affected area. With purposely built seeding machines about 30 kg/ha of the fungus-inoculated barley was sown into the soil. Since the success of this action became not immediately visible, the initially very high acceptance among the farmers for this method of biological control faded again very quickly. Only in a few orchards the treatments were repeated during the following years. Fig. 2 shows the results of density assessments in a commercial orchard (43 ha), where the fungus was applied three times. It emerges that there was very little initial effect, but that the density could be substantially lowered towards the end of the generation. This was observed also in other orchards were the fungus had been applied several times. These experiences have shown that although an application rate of 30 kg/ha cannot safeguard the integrity of an orchard in the short term, a substantial reduction of the larvae density over a period of a few years may well be possible. These positive results of the field application of *Beauveria brongniartii* have subsequently brought about a renewed strong increase in the acceptance of this biological method of cockchafer-control.

1992- : mechanical phase. Already during the flight of spring 1989 it was attempted to prevent the females from ovipositing by covering the soil. Among the various tested materials nets proved to be the most suited for the purpose. In spring 1992 65% of the cultivated area in the flight area of the "Unterland"-region was covered in a large scale action. The success was impressive (Zelger 1995). Subsequently no further damages were detected in the covered orchards, and also the density of larvae had been very strongly reduced in the whole flight area. In spring 1995 the nets were posed again despite the low flight intensity. The situation at present is that much of the cultivated area has been nearly cleared of the infestation.

## Discussion

During the recent cockchafer gradation in South Tyrol various methods of damage containment and/or regulation of the population were tested in trials. Those showing promising results were subsequently applied on a large scale in the flight area of the "Unterland"-region.

The use of insecticides against the beetles during the maturation feeding in the woods has never been seriously taken into consideration for reasons of water protection (nearby river) and because of the experiences from the fifties and sixties. Also the use of soil-applied insecticides did by far not bring the expected and necessary results. As a consequence, such treatments are recommended, if at all, only in situations where preventive measures have not been taken in time and damage is imminent because of the advanced degree of infestation.

In areas of high infestation pressure soil-covering as a prevention against oviposition is the most appropriate method. This procedure is however very labour-intensive and costly (material and labour: 5.000.000 Lire/ha), but in the case of high value crops such as orchards the costs pay off at an expected level of damage of 10-20%.

Such a method, however, acts only against that particular generation, whose oviposition is prevented. Flying populations, which lie in between, are not affected. Such populations may build up again, if no action is taken against them. This was observed at least in South Tyrol. The covering-method is moreover too expensive and therefore not recommended for the marginal zones of an infested area, where the population density lies below the damage threshold.

A valuable approach is offered by the encouraging results obtained from the fungus-applications. The fungal antagonist can infect the cockchafer at all stages of development. Its application should therefore be directed against populations of low density where a covering of the soil is not remunerative. In such cases a timely and repeated application may prevent a stronger build-up of the population. Moreover, also in heavily infested areas the population density can be reduced after repeated soil-coverings to such levels that a further application becomes uneconomical. This is at present the case in the "Unterland"-region. In such circumstances it should be aimed to prevent a renewed build-up of the population through the application of the fungus.

Based on the acquired experiences and on the above considerations the following guidelines for the control of the cockchafer population are presently in force in South Tyrol:

- In areas with a population density above the damage threshold (and with highly priced crops) the soil should be covered with nets in order to prevent oviposition.
- In all other areas with a low degree of infestation (e.g. on the margins of infested areas) repeated soil applications of the fungus *Beauveria brongniartii* have to be carried out. The same applies for areas where the level of infestation was lowered under the damage threshold by the use of nets.
- The use of soil-applied insecticides should be discouraged because of the environmental impact and the low efficiency.

These guidelines, which have been put into practice by most of the fruit growers in the affected areas of the "Unterland"-region, although not completely eradicating the infestation, have successfully prevented further damages during the last years.

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## Summary of the discussion on standardisation

S. Keller

The discussion revealed a need for standardisation mainly of field methods. The statements of the participants are summarized as follows:

Sample unit: Most workers use sample units measuring 50 cm x 50 cm with a depth depending on soil characteristics and the position of Melolontha or the season respectively. This is in contrast to French colleagues who proposed sample units measuring 17 cm x 100 cm mainly by statistical reasons. However, such a sample unit has two main disadvantages: it is not practicable if the hole has to be deep and it has a longer border line. To sample a square meter the borderline is more than 14 m compared to 8 m for the 50 cm x 50 cm unit. The probability for unprecise countings and killing Melolontha is therefore nearly doubled.

Number of sample units: Usually 4 sample units (50 cm x 50 cm)/ha or area unit are used for population surveys over large areas and 16-20 sample units/plot are used for control trials.

Infections rates: Infection rates may be determined in the field based on sampling dates. But better results are attained by rearing the undamaged individuals collected during sampling. The rearing period should be limited for larvae, e.g. 3 months or until pupation depending on the age.

Egg depositing experiments in the laboratory: They should be done with the same soil type and include a standard e.g. females cought on the egg depositing flight.

Complete standardisation cannot be achieved but authors should precisely state the methods used.