CHAPTER 12

BIOLOGICAL CONTROL OF SCARABS AND WEEVILS IN CHRISTMAS TREES AND GREENERY PLANTATIONS

Jørgen Eilenberg, Charlotte Nielsen,
Susanne Harding and Susanne Vestergaard

1. Introduction

In Danish forestry the economically most important insect problems and consequently the most intensive use of chemical insecticides occur in the production of Christmas trees and decoration green (Kirkeby-Thomsen and Ravn, 1997; Ravn, 2000). Nordmann fir (*Abies nordmanniana*) and noble fir (*Abies procera*) are the dominant tree species in this production today. *Abies nordmanniana* and *A. procera* cover approximately 22,600 ha and 9,200 ha, respectively. *A. nordmanniana* is mostly used for Christmas trees, while *A. procera* is mostly used for decoration green. Other conifer species to be used for the same purposes are only grown on a negligible scale.

Christmas trees and decoration green are both important for the home market and export. In 2003, more than 9 mio Christmas trees and 30,000 tonnes decoration green were exported to several countries in Central and Northern Europe with Germany as the main recipient. The prices obtained by the producers vary depending on quality, but can typically be around EUR 10 per tree of 200 cm height and EUR 1 per kg decoration greenery. The total export value for Denmark was thus above EUR 150 mio in 2003. The market demands a very high product quality, and no damage from feeding of insect pests is accepted. Only the highest quality with the required shiny, dark green needle colour of Christmas trees and decoration greenery without any signs of insect feeding on the needles are saleable at reasonable prices. It is therefore a prerequisite for the producers to ensure pest control.

Control of insect pests in the Danish production of Christmas trees and decoration green has historically been based on the use of chemical pesticides. However no chemical pest control have been permitted in state forestry since 2003 (The Danish Environmental Protection Agency, 1998), and this applies to the 4,600 ha production area for Christmas trees and decoration greenery. For privately owned forests there is a political wish from the state authorities to phase out chemical pesticides (Ravn, 2000). Further, there is an increasing desire from consumers to buy organically grown Christmas trees produced without chemical pesticides. The products are associated with strong emotions among consumers: Christmas trees and decoration green are linked to family traditions during the Christmas period, which in Denmark lasts about one month.

Biological control using natural enemies of the pest populations may thus provide an attractive alternative to conventional chemical treatment. This chapter provides a short review

J. Eilenberg and H.M.T. Hokkanen (eds.), An Ecological and Societal Approach to Biological Control, 247–255.

of the first major European experiments to implement biological control in the production of Christmas trees and decoration green.

2. *Melolontha melolontha* in Christmas tree plantations

The European cockchafer, *Melolontha melolontha* (Coleoptera: Scarabeidae) is a serious pest in the production of Christmas trees in Denmark (Harding, 1994). The larvae feed on the roots of especially young trees causing extensive and lethal damage in the plantations (Harding, 1994). The cockchafer has a four-year-life cycle and the larvae dwell in the soil for 3 growth seasons. Feeding by the small larvae has no major impact on the vitality of the trees, but the large third instar larvae may totally eradicate the root system. A single individual is capable of damaging several trees. The damage results in discoloration of the needles and in case of extensive feeding on the root system the trees eventually die. Plantations on land which were recently converted from agricultural fields into Christmas tree plantations are particularly subjected to damage with the result that intensive re-plantation is required in patches throughout the plantations after a few years. Chemical control of the soil-dwelling larvae of *M. melolontha* is not allowed in Danish forestry and mechanical control is not possible in these perennial crops. The growers have therefore no current options to control the scarab larvae and thus prevent attack.

The fungus *Beauveria brongniartii* is considered to be the most important natural enemy of *M. melolontha* and promising results of biological control have been obtained in orchards and pastures in Central Europe using barley kernels colonised by this fungus (Keller, 1992; Zelger, 1996; Keller *et al*., 1997; Enkerli *et al*., 2004). It was therefore sensible to test the efficacy and applicability of this control method in the Christmas tree plantations. The studies in Denmark were therefore based on European strains of *B. brongniartii* (BIPESCO 1 or 2). The first field experiments were carried out in the spring 2000, which happened to be a flight year of *M. melolontha* (Vestergaard *et al*., 2002). Our experiments were based on inoculation biocontrol strategy that relies on establishment of the fungus for at least one season after application (Eilenberg *et al*., 2001). In a Christmas tree plantation at Vallø, (Zealand) kernels with *B. brongniartii* were inoculated in the following two ways: 1) kernels were placed in holes of 10 cm depth around existing small trees, or 2) kernels were thoroughly mixed with the soil from the planting hole and placed around the new tree during re-plantation.

In order to assess the damage, we developed a score index of the health status of the trees, based on needle colour. Category 5 meant that the trees had no damage and that the needles were shiny and dark green. Trees in category 4 had a slight discoloration of their needles, indicating decreasing vitality. Trees in category 4 were in risk of ending in a lower category before harvest. Category 1-3 showed substantial discolouration and needle loss, category 1 referring to trees verging on death. Category 0 was used to characterize dead trees. Health scores were performed in autumn 2001 and 2002, more than one and two years, respectively, after treatments. In addition to assessing the health score we also surveyed the persistence of the fungus over time and the effect of fungal treatment on non-target insects.

The application of fungus via inoculation kernels in the soil during re-plantation resulted in statistically significant effects of the treatment. It is especially noteworthy that in the treated plots there were 30 % more trees in category 5 compared with untreated plots, which clearly indicates the advantage of the treatment. The density of *B. brongniartii* colony forming units (cfu) in the soil was approximately the same one year after application, as the density immediately after application. The lowest density found was 10^3– 10^4colony forming units (cfu's) per g soil while the highest density was 10^5– 10^6 cfu's per g soil (Vestergaard *et al*., 2002). In spite of intensive sampling of insects from several orders we did not find any infected non-target insects in the plots. Thus, long-term control of cockchafers can be achieved using simple methods.

Based on these data a second experiment was initiated in Northern Jutland in spring 2002 in order to: 1) test the dosage of fungus needed for successful control of scarab larvae in the plantations and 2) apply the fungus by a simple and practically feasible method. The experiments were carried out in an area where severe and lethal damages on newly planted Christmas trees had occurred on several occasions at four-year intervals. In connection with an extensive replanting resulting from a major attack by *Melolontha* larvae two different dosages were tested: 1) 10 g barley kernels per tree and 2) 30 g barley kernels per tree. The colonised barley kernels were simply thrown directly into the planting hole before the small tree was inserted into the hole, thus avoiding the time consuming thorough mixing of kernels into the soil as done in the previous experiment.

After 6 months an effect of the biocontrol treatments was apparent: In the *B. brongniartii* treated plots 98% (low dosage) and 100 % (high dosage) of the trees were scored as category 4 and 5 compared to about 93% of the control trees. This effect increased significantly after 1 ½ year when almost 13% of the untreated trees had been killed and another 25% showed decreasing health (category 1-3). None of the treated trees died and only single trees showed discolouration. The benefits of the biocontrol agent were apparent more than two years after the application. There was no difference in effect between the low and the high dose of fungus applied.

Based on our finding we suggest an easily applicable system for long lasting control of *M. melolontha* in *A. nordmanniana* plantations: during plantation or re-plantation, kernels with the fungus *B. brongniartii* are simply placed in the planting hole.

3. *Strophosoma* spp. in decoration green plantations

In the Danish production of decoration green, weevils (Coleoptera: Curculionidae) are frequently occurring insect pests. In particular, two species from the genus *Strophosoma,* the nut leaf weevil *S. melanogrammum* and *S. capitatum* are economically important pests (Harding, 1993; Kirkeby-Thomsen and Ravn, 1997; Thorbek and Ravn, 1999; Ravn, 2000). The damage is caused by the adult weevils feeding on the needles. The weevils feed on current-year needles as well as older needles. Although weevil damage is observed in the whole canopy, the damage is most pronounced in the top of the crown, where also the needles of the leader are frequently heavily grazed upon. The damage may result in economically significant losses for the growers, since branches harvested and sold as decoration green must be completely without damage.

The two weevil species exhibit a fascinating life cycle, which was studied as part of the experiments by means of sticky traps, funnel traps, emergence cages, and soil samples. In spring, overwintering adults emerge from the soil and start feeding on the needles before oviposition, which takes place on the shoots in the tree canopy. In order to get to the top of the trees the weevils need to climb the stems. After egg hatch the first instar larvae drop from the canopy to the ground in early summer. The 'shower' of small larvae can be as high as almost 3000 larvae per m^2. The larvae enter the soil and spend the rest of their time as larvae in the upper soil layers feeding on small roots. The following year the larvae pupate and emerge into the new adult generation in August-September. The new generation of adults start feeding on the needles again before they hibernate in the soil. Thus, the complete life cycle is approximately 15-18 months for both species.

Only very little is known about the naturally occurring enemies of *Strophosoma* spp. However, although no records existed of insect pathogens on the weevils, initial bioassay in the laboratory documented that fungal biocontrol agents could infect both adults and larvae of this pest insect. Under field conditions the adult weevils could potentially be targeted by a soil application in late spring upon emergence from the soil and before oviposition in the canopy and the larvae could be targeted by a soil application in mid summer. An application in mid summer would possibly also persist to infect the new generation of adult weevils emerging from the soil later in the season. We therefore decided to test if a biocontrol agent could successfully be applied in spring or summer.

As a biocontrol agent the fungus *Metarhizium anisopliae* (BIPESCO 5) was used . The fungus is not a natural occurring enemy of *Strophosoma* species in Denmark, and the fungus did no toccur naturally in the soil in the selected *A. procera* plots. *M. anisopliae* was chosen because of existing knowledge about the growth conditions *in vitro* in medium-large scale and thus its potential for industrial production. The fungus is available in several countries, including some European, as a product for insect control, including weevil species from other genera, for example the genus *Otiorhynchus,* but mostly in rather controlled environments: glasshouses or strawberry crops. It had, however, never been tested before against *Strophosoma* spp. in perennial cropping systems, and the application in a stand of *A. procera* gave additional challenges: grass, shrubs, wilted twigs and branches from earlier harvests covered parts of the area. Conidia of *M. anisopliae* were suspended in an aqueous suspension before application as an inundation biocontrol agent using a personal back-pack spray device.

The evaluation of the effect of the fungal treatment was based on estimations of population densities of the two *Strophosoma* species. This was measured by weekly counts of adult weevils emerging from the soil. In addition, we assessed the prevalence of *M. anisopliae* infections in adult weevils collected after the spring application. Finally the persistence of the fungus over time as well as infection of non-target arthropods was assessed.

Immediately after fungal application we detected up to 90 % *M. anisopliae* infection in the adult weevils in the treated plots, compared to only a single infected weevil found in the untreated control plots during the entire experimental period (Nielsen *et al*., 2004).

The summary results on population effects are shown in table 1. Both spring and summer applications resulted in a lower density of the target, but the effect was not apparent until one year after application. The highest reduction was 60 % compared with untreated plots measured in autumn 2001. In none of the cases, however, did we document a long-term effect, despite the presence of significant amounts of inoculum in the treated plots up to 419 days after treatment. Inoculum was still detectable in autumn 2004, more than 3 years after application. Among non-target invertebrates collected in treated plots we documented *M. anisopliae* on ticks, on coleopterans and on hemipterans, thus some non-target effects were present.

Table 1: Effects of Metarhizium anisopliae *applied as a biological control agent against weevils from the genus* Strophosoma *in a Danish stand of* A. procera*. The plots, which were treated in summer 2000, were treated again in summer 2001. A 'YES' means that a reduction in target weevils was obtained, a 'NO' means that this was not the case*

Treatment	Autumn 2000	Spring 2001	Autumn 2001	Spring 2002	Autumn 2002	Spring 2003	Autumn 2003
Summer 2000	NO	NO	**YES**	**YES**	**YES**	NO	NO
Spring 2001			NO	NO	**YES**	NO	**YES**

Based on these data we conclude that it is feasible to obtain a significant level of control by applying *M. anisopliae* on a regular basis. A range of questions arises, however, concerning biocontrol:

- What is the optimal time of application and is control solely possible by spraying using personal equipment?
- Is it ecologically sound to use a fungus, which was not a naturally occurring enemy of the target, did not occur naturally in the soil in the forest plots, and may infect certain non-target invertebrate species?
- Should other methods be tested, for example strips baited with fungus on tree stems to infect beetles climbing to the top?
- Is the public concerned about inundating high amounts of a fungus into a forest eco-system, which is regarded as natural (although the tree itself is an exotic species)?

4. Grower's attitude

We assume that growers will adopt to biological control options, provided that the methods work and that standard technology can be used or, that some cheap and easy new technology is available. The Christmas tree and greenery growers' attitude to new technologies like biocontrol is that they are willing to pay an additional cost of 10 – 25 % compared to the conventional control methods (K. Østergaard, pers. comm.). We therefore assume that a biocontrol product can be accepted even if it may cost more than a conventional product.

Our main method for application of *B. brongniartii* as a biocontrol agent to the soil of Christmas trees was based on low technology: applying kernels with fungus into the planting hole during plantation or re-plantation. The method is based on an existing *B. brongniartii* product, and an application of biocontrol will provide protection of a high value crop at a very low cost. The main obstacle will probably be convincing the growers that many of them would have to use the fungus as prophylaxis, despite the fact that only some areas will suffer from serious damage. We feel, however, that the implementation of an easy to use guideline will assist the growers in benefiting from the biocontrol.

The method for application of *M. anisopliae* as a biocontrol agent of weevils needs optimization before we can recommend it to the growers and prepare easy to use guidelines.

5. Public perception

It is normally assumed that many consumers prefer crops produced without chemical agents such as conventional pesticides. The present situation in Denmark with many organic products sold at a higher price than conventionally grown food products documents that consumers are in general willing to pay a higher price in order to avoid chemicals. The sales of organically grown Christmas trees and decoration green have increased in Denmark over the years and may point towards a future demand for such products. The question is how the public will accept biocontrol in Christmas trees and greenery plantations and how much more they are willing to pay for such products. Our studies were not accompanied by socio-economic studies, which could document the public attitude. It is, however, our impression that Christmas trees and decoration green provide an excellent example of crops, for which biocontrol programmes will immediately receive public acceptance for a number of reasons:

1) Christmas trees and decoration green are products with strong emotional aspects. They are used in the month of December as part of Christmas celebrations and, for many people, a range of important family events. In Denmark, Christmas time is the most significant family event during the year. The trees and branches of conifers to be used should reflect the importance of this emotional significance by being environmentally sound.
2) The trees are grown in forests and woods and especially around major cities, the public are frequent visitors using the production areas. The plantations and the pest control attempts are thus highly exposed to the public, and our studies received much public attention. The biocontrol studies of *M. melolontha* were subjected to a presentation in prime time news on the major national television channel, the first of our biocontrol experiments ever to receive such exposure. The story was presented very positively as an example of environmentally sound plant protection and research directed to public benefit. As part of our experiments to control weevils in the greenery plantation, we placed a poster in the forest plot with a short explanation of the purpose of the studies, our names and how to contact us. The plot was situated near a small road used by people biking or walking in the forest. Responses by people passing by and watching our sampling and other activities during the season were very positive and we regard the poster as an important element in the communication with the public. The local newspapers reported on the experiments and local people, who were curious to learn about the biocontrol experiments, frequently approached the people employed in the forest. We received *no* negative comments to our application of a fungus by inundation in the plots. Based on these personal experiences with communication with the public we conclude therefore that implementation of biocontrol in both Christmas trees and in decoration green would be very well received.

6. Perspectives

Concerning the development from an initial potentially good idea onto a marketable product and application methods for commercial use, an obvious question is, however: who pays? The growers' economic situation is often very sensitive to small changes in the market prices, and in some years the growers face an economically difficult situation. The grower's attitude is thus that they support biocontrol experiments on their sites without compensation, while they do not have funding for the development of biocontrol products. For companies involved in biocontrol products, the Christmas tree and decoration green markets are inferior, due to the overall small (yet increasing) number of hectares in Europe. Therefore, it is solely to be expected that companies will only contribute by using existing products with very few modifications for a new market. Public funding has so far financed the major parts of the studies presented here. Potentially additional funding for testing can be achieved but it is not to be expected that public funding can in itself guarantee product development.

We believe that biocontrol of pest insects in Christmas trees and decoration green has great potential (table 2).

Table 2: Overview of important parameters on our evaluation of the potential for biocontrol in the two crop-pest-fungus systems

1: Products based on Metarhizium anisopliae *exist but so far for other targets and/or other cropping systems*
2: Based on the assumption that inundation by simple spraying equipment will be tested further

Parameter	*A. nordmanniana* *M. melolontha* *B. brongniartii*	*A. procera* *Strophosoma* spp. *M. anisopliae*
High value of crop	YES	YES
Economic losses due to pest insect	YES	YES
Chemical control feasible	NO	NO
Biocontrol agent a natural enemy of target insect	YES	NO
Biocontrol agent commercially available	YES	NO[1]
Application system simple and cheap	YES	YES[2]
Biocontrol based on inoculation	YES	NO
Biocontrol based on inundation	NO	YES
Immediate effects on target	NO	NO
Effect of biocontrol after approx-imately one year	YES	YES
Lasting effects on target expected	YES	NO
Non-target effects in treated plots	NO	YES
Grower's acceptance	YES	YES
Public acceptance	YES	YES

Biocontrol in these crops will meet the consumer's demands and a successful biocontrol in crops with emotional significance has further value as models for teaching the public about biocontrol in general. It is therefore our hope that the obvious advantages of biocontrol combined with easily applicable technologies will allow the development towards practical biocontrol in Christmas trees and decoration green in Europe and elsewhere.

Acknowledgements

Christina Wolsted, Jan Martin and Rasmus Eliasen performed major parts of the technical work. Bent Christensen provided information about production economy and export. *B. brongniartii* was produced by Agrifutur (Italy) and *M. anisopliae* was produced by Prophyta GmbH (Germany). Helen Roy gave valuable comments to the manuscript. The studies were in parts financed by EU (BIPESCO, FAIR6-CT98-4105) and The Danish Environmental Protection Agency (No 7041-0317 and 7041-0081).

References

The Danish Environmental Protection Agency (1998). Agreement on phasing out the use of plant protection products in public areas. Copenhagen, 3 November, 1998, http://www.mst.dk/homepage/

Eilenberg, J.; Hajek, A.E.; Lomer, C. (*2001).* Suggestions for unifying the terminology in biological control. Biocontrol 46: 387-400.

Eilenberg, J., Nielsen, C., Vestergaard, S., Harding, S., Frølander A., and Augustyniuk, A. (2003). Biological control of weevils (*Strophosoma* spp.) in Danish greenery plantations. IOBC/WPRS Bulletin 26: 55-58.

Enkerli, J.; Widmer, F.; Keller, S. (2004). Long-term field persistence of *Beauveria brongniartii* strains applied as biocontrol agents against European cockchafer larvae in Switzerland. Biological Control, 29: 115-123.

Harding, S. (1993). Gråsnuder – et aktuelt skadedyr [*Strophosoma* – an actual pest insects] (In Danish). Skoven No 8:330-331.

Harding, S. (1994). Oldenborren. [The Cockchafer] (In Danish). Skoven No 6-7: 270-271.

Keller, S.; Schweizer, C.; Keller, E.; Brenner, H. (1997). Control of white grubs (*Melolontha melolontha* L) by treating adults with the fungus *Beauveria brongniartii.* Biocontrol Science and Technology, 7: 105-116

Kirkeby-Thomsen, A., and Ravn, H.P. 1997. Skadedyr. [Pest insects] (In Danish). In Lundqvist, H. (ed.). Miljøvenlig Juletræsproduktion. En statusopgørelse. Pyntegrøntserien No. 2-1997. Danish Forest and Landscape Research Institute, Hørsholm. 157 pp.

Nielsen, C.; Eilenberg, J.; Harding, S.; Vestergaard, S. (2004): Biological control of weevils (*Strophosoma melanogrammum* and *S. capitatum*) in greenery plantations in Denmark. Danish Environmental Protection Agency, Danish Ministry of the Environment, Copenhagen, Denmark, Pesticides Research No 71, 84 pp.

Ravn, H.P. (2000). Status for de vigtigste Skadevoldere - ind i det ny årtusind med og uden pesticider [Status for the most important pests] (in Danish). Beretning, Skov- og Landskabs-konferencen 2000, 98-104.

Thorbek, P. and Ravn, H.P. 1999. Strophosoma spp. Videnblad Pyntegrønt no. 5.5-3. Danish Forest and Landscape Research Institute, Hørsholm. 2 pp.

Vestergaard, S.; Nielsen, C.; Harding, S.; Eilenberg, J.(2002): First field trials to control *Melolontha melolontha* with *Beauveria brongniartii* in Christmas trees in Denmark. IOBC/WPRS Bulletin, 25: 51-58.

Vestergaard, S.; Nielsen, C.; Eilenberg, J.; Harding, S. (2002): Nye bekæmpelsesmetoder overfor gråsnuder og oldenborrer [New control methods against curculionids and cockchafers] (In Danish). PS Nåledrys No 40, 32-35.

Zelger, R. (1996): The population dynamics of the cockchafer in South Tyrol since 1980 and the measures applied for control. IOBC/WPRS Bulletin, 19: 109-113.