Population Management of Cone and Seed Insects in Spruce Seed Orchards

Olle Rosenberg
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2011

Acta Universitatis agriculturae Sueciae
2011:38

Cover: *Cydia strobilella* searching for a suitable site for ovipositing on a *Picea abies* (L.) Karst. flower.
(photo: O. Rosenberg)

ISSN 1652-6880
ISBN 978-91-576-7583-5

Print: SLU Service/Repro, Uppsala 2011

Population Management of Cone and Seed Insects in Spruce Seed Orchards

Abstract

Seed orchards have been established in order to produce high quality seeds for reforestation and forestation. However, seed production in spruce (*Picea abies* (L.) Karst.) seed orchards is severely hampered by cone- and seed-feeding insects. Therefore it is of great importance to find methods to reduce damages from insects. This thesis summarizes and discusses results presented in four papers concerning various methods and chemicals (insecticides and a pheromone) for damage reductions in spruce seed orchards.

Area-wide application of the biological insecticide Turex 50 WP was shown to reduce damage by two of the four most serious pest species. Concerns were then raised that feeding by insects that are not affected by this insecticide may increase following its application, in response to the consequent increases in the availability of food and space, resulting in little no or difference in overall damage.

A follow up study indicated that there would probably not be any problem with increased feeding by the larvae survived and that spraying of an insecticide not affecting all species would probably be cost effective. However, various species-related and abiotic factors (e.g. rain and temperature) affect the efficacy of insecticide treatments, both among and within years, and thus should be taken into account.

A system that would be less sensitive to weather and also may affect all pest species and at the same time avoid affecting the surrounding environment is injectable systemic insecticides. In order to increase the cost efficiency a study was performed where insecticide was combined with the flower stimulating hormone gibberellin and successfully reduced damages and increased number of flowers.

In order to know if and when an insecticide application should be carried out, pheromone for trapping insects is a useful tool. But in order to do so there must be a pheromone available. During the spring of 2009 a pheromone for *C. strobilella* was identified and synthesized. The study showed that the amount of pheromone released from the female was extremely low, 1 pg, so the male antenna is supersensitive in order to find females. This implies also that this species can be a good candidate for mating disruption.

Keywords: Seed orchard, pest management, cone insects, seed insects, insecticide, *Dioryctria abietella*, *Eupithecia abietaria*, *Cydia strobilella*, *Strobilomyia anthracina*

Author's address: Olle Rosenberg, Skogforsk,
Uppsala Science Park, 751 83 Uppsala, Sweden
E-mail: olle.rosenberg@skogforsk.se

The quality of seed, as well as seed production, are both directly and indirectly of importance for the existence of mankind and also of a considerable number of animal organisms

Enar Andersson, *Studia Forestalia Suecica* No. 23, 1965

Contents

List of Publications

This thesis is based on the work described in the following papers, which are referred to by the corresponding Roman numerals in the text:

I Rosenberg, O. & Weslien, J. 2005. Assessment of cone-damaging insects in a Swedish spruce seed orchard and the efficacy of large-scale application of *Bacillus thuringiensis* variety *aizawai* x *kurstaki* against lepidopterans. *Journal of Economic Entomology* 98: 402-408.

II Rosenberg, O., Weslien, J. & Nordlander, G. Effects of different insect species assemblages on Norway spruce seed quality after spraying with insecticides. (Manuscript)

III Rosenberg, O., Weslien, J. & Almqvist, C. Stem injections with insecticide and gibberellin reduce cone damage and increase flowering in a spruce seed orchard. (Manuscript)

IV Wang, H.-L., Svensson, G.P., Rosenberg, O., Bengtsson, M., Jirle, E.V. & Löfstedt, C. 2010. Identification of the sex pheromone of the spruce seed moth, *Cydia strobilella* L. *Journal of Chemical Ecology* 36: 305-313.

Papers I and IV is reproduced with the kind permission of journal publishers.

The contribution of Olle Rosenberg to the papers included in this thesis was as follows:

I In collaboration with Jan Weslien, I designed the field work and was responsible for most of the field work, analysis and writing.

II Together with my supervisors, Jan Weslien and Göran Nordlander, I planned the study. I did all the field work and most of the analyses and writing.

III I planned the study in collaboration with my co-authors, Curt Almqvist and Jan Weslien. I did the majority of the field work, analyses and writing.

IV The study was planned together with Christer Löfstedt. My contribution to the fieldwork was about 30 % and writing 10 %.

1 Introduction

1.1 Seed orchards

Seed orchards are the primary sources of material used in forestation and reforestation in Sweden as well as many other countries. According to a thorough review of tree breeding history in Sweden by Werner (2010), the first inventory of suitable trees for use in breeding started during the 1930´s and the main criteria for selecting trees for seed orchards were established between the years 1944-1951. These criteria included superior growth and several quality-related traits (e.g. branch characteristics and stem shape), these trees were called plus trees. In order to produce large numbers of copies (ramets) of plus trees (clones), branches are cut and used directly as cuttings (from young trees) or grafted on root stocks (Almqvist *et al.*, 2007).

Clones from plus trees were used to establish the first set of *Picea abies* (L.) Karst. seed orchards between 1955 and 1973 (Hannerz *et al.*, 2000). Trees from these seeds have about 10 % increased growth compared to trees from forest stand seeds (Rosvall *et al.*, 2001). The second set of orchards, established during 1982-2002, was mainly based on tested progenies from plus trees in existing orchards and new plus trees from forest stands. The new plus trees were selected to increase the genetic base of seed orchards and to expand the base population for the breeding program (Karlsson & Rosvall, 1995). Seeds from these orchards provide trees with about 10-20 % higher growth rates than unselected trees (Rosvall *et al.*, 2001). Currently, the third set of orchards is being established, using tested progenies from plus trees, resulting in seeds giving trees with further growth enhancements; up to 25 % compared to trees from unimproved seeds (Rosvall *et al.*, 2001). The increases in growth and quality obtained by using seedlings from improved seeds enable higher sustainable harvests of high quality timber and considerable increases in both land values and incomes for forest owners (Li *et al.*, 1999; Mangini *et al.*, 2003; Almqvist *et al.*, 2008).

It takes between 10-20 years from establishment for a *P. abies* seed orchard to start producing cones, and the orchard needs management during this period (Almqvist *et al.*, 2007). Therefore, a seed orchard is costly to establish and maintain. Further, *P. abies* seed orchards only produce cones abundantly in "mast" years every 5-10 years. Today there is a deficiency of improved *P. abies* seeds in Sweden and important factors limiting the cone crops are cone- and seed-feeding insects (Almqvist *et al.*, 2008). Hence, effective insect control in *P. abies* seed orchards can substantially increase the seed crop, and thus reduce the seed deficit (Almqvist *et al.*, 2010).

1.2 Cone- and seed-feeding insects

Pest insects that attack seeds and cones are currently a global problem, but both the plant and insect species that raise concerns vary somewhat among countries (Turgeon, 1994). In Europe the same insect species infest cones and seeds of *P. abies* (cf. Trägårdh, 1917; Holste, 1922; Stadnitskiy, 1971; Annila, 1973; Roques, 1983; Seifert *et al.*, 2000). In Asia several of the pest species are the same, although the hosts may differ (Kobayashi, 1981). In North America, congeneric species cause similar damage to cones and seeds, but affect different tree species (Ebel, 1963; Hedlin, 1973).

Strong interest in cone- and seed-infesting insects first appeared in the beginning of the 20th century (Bakke, 1963 and references therein). Early investigations mainly focused on their biology, distribution and associated parasites (e.g. Trägårdh, 1917; Holste, 1922; Bakke, 1955), although Spessivtseff (1924) was also concerned about the serious cone and seed damage they caused. When seed orchards were established to produce superior seeds and thus increase tree growth, the focus shifted towards pest control (Wiersma, 1978).

In Sweden the most serious damage occurs in *P. abies* seed orchards, although it also occurs in orchards of other species, e.g. *Pinus sylvestris* and *P. contorta* (Almqvist *et al.*, 2007). Both the orders and (to some degree) feeding strategies of the insect species feeding on *P. abies* cones and seeds vary. Four lepidopteran species infest cones: *Dioryctria abietella* Denis & Schiffermüller (Pyralidae), *Eupithecia abietaria* Goetze and *E. analoga* Djakonov (Geometridae) and *Cydia strobilella* L. (Tortricidae). The first three of these species feed mainly on the scales of the cones and the fourth mainly on seeds. The first of these moth species to emerge during the spring is *C.*

strobilella, which emerges in early May and oviposits between the scales during flowering (Annila, 1981). The larvae eat into scales towards seeds directly after hatching, then remain in the cone during the following larval and pupal stages (Annila, 1981). *Cydia strobilella* is dependent on cones for its survival. In a strategy that reduces the risk of all larvae emerging in a year when few cones are produced, approximately half of them emerge after one year and the other half after two years (Annila, 1981).The geometrids *E. abietaria* and *E. strobilella* emerge somewhat earlier than *D. abietella* (Roques, 1983), which emerges during late June to late July (Annila, 1979). The larvae of all three species feed on cone scales and leave excrement that is visible on the outside of the cone. The geometrids leave the cones before or during September (Spessivtseff, 1924), and *D. abietella* leaves the cones by the end of September (Annila, 1979). Spessivtseff (1924) notes that the geometrids can cause severe damages to cones, but they have probably been overlooked since the damage they cause is similar to that caused by *D. abietella* and they leave the cones earlier. The geometrids are not mentioned in a more recent Swedish textbook of forest pests (Eidmann & Klingström, 1990) or in Swedish field studies (Weslien, 1999; Glynn & Weslien, 2004), so they may also have been overlooked in recent years. Larvae of these three species feed on large parts of the scales and occasionally move from cone to cone. Therefore, they are easier to control than species that live exclusively within the cones and seeds (cf. Annila, 1973). The two geometrids and *D. abietella* are not dependent on cones for their survival, and thus are less vulnerable in years when cones are scarce (Spessivtseff, 1924, Annila, 1979).

Three dipteran pest species infest cones. One is the fly (*Strobilomyia anthracina* Czerny, Anthomyiidae), whose larva feed on both seeds and cone tissue. It oviposits on open flowers in May, the larvae leave the cones in June-early July, and prolonged diapause only occasionally occurs (Annila, 1981; Brockerhoff & Kenis, 1997). The two other dipteran species are gallmidges (Cecidomyiidae); larvae of one (*Plemeliella abietina* Seitner) feeds on seeds and the other (*Kaltenbachiola strobi* Winnertz) on the base of the cone scales. Larvae of both species overwinter in their feeding sites, they can remain in prolonged diapause for a year or more and oviposition occurs during May in open flowers (Bakke, 1963; Annila, 1966, 1981).

There is also a hymenopteran pest species, *Megastigmus strobilobius* (Ratzeburg) (Torymidae), which feeds solely on seeds and spends its entire larval stage in them (Annila, 1966, 1981; Skrzypczynska & Roques, 1987). According to Annila (1981), this species emerges from cones formed in the preceding years a few weeks after the end of flowering (late June to early July). According to a study by Skrzypczynska & Roques (1987) this coincides with the time when the cones are almost fully grown. *Megastigmus strobilobius* can remain in prolonged diapause for several years. The phenology and feeding behavior of the mentioned insects are summarized in table 1.

Table 1. *The phenology and feeding behavior of cone infesting insect species* on Picea abies (L.) Karst.

Species	Order [9] Family	Oviposition place	Feeding guild[8]	Larvae phenology
Kaltenbachiola strobi (Winn.) [2]	Dipt. Cecidomyiidae	flower	cone	Overwinters in cone
Plemeliella abietina (Seitn.) [3,5]	Dipt. Cecidomyiidae	flower	seed	Overwinters in cone
Megastigmus strobilobius (Ratz.) [3,5,6]	Hym. Torymidae	cone	seed	Overwinters in cone
Strobilomyia anthracina (Czerny) [5,7]	Dipt, Anthomyiidae	flower	cone (seed)	Leaves cone in late June
Eupithecia abietaria (Goetze) [1]	Lep. Geometridae	cone	cone	Leaves cone in August
Eupithecia analoga (Djakonov) [1]	Lep. Geometridae	cone	cone	Leaves cone in August
Dioryctria abietella (Den. et. Schiff.) [4]	Lep. Pyralidae	cone	cone	Leaves cone in Aug-Sep
Cydia strobilella (L.) [2]	Lep. Tortricidae	flower	seed (cone)	Overwinters in cone

[1]Spessivtseff 1924, [2]Bakke 1963, [3]Annila 1966, [4]Annila 1979, [5]Annila 1981, [6]Skrzypczynska & Roques 1987, [7]Brockerhoff & Kenis 1997, [8]Turgeon *et al.*, 1994. [9]Dipt., Hym. and Lep. refer to Diptera, Hymnoptera and Lepidoptera, respectively.

1.3 Pest management

1.3.1 Chemical insecticides

The possibility of using chemicals to control cone pest insects has been studied since the early 1960s, and several chemicals have been used in practice for this purpose, mainly broad-spectrum organophosphorous-based insecticides, but also dichlorodiphenyltrichloroethane (DDT), Lindane and some pyrethroids. The organophosphates have systemic properties and their application in the following decades (via trunk injections, implanting or conventional spraying) reduced the damage caused by cone-feeding insects (see, for instance, Merkel, 1964, 1969; Nord *et al.*, 1985; Roques *et al.*, 1996; Stein *et al.*, 1993; Mangini *et al.*, 1998) or both cone- and seed-feeding insects (Johnson & Rediske, 1965; Annila, 1973; Hedlin, 1973). In

North America organophosphates and several other insecticides for conventional spraying are still used. The total use of organophosphates in USA has however declined with more than 60 % since 1990 (Grube *et al.*, 2011). There is a concern that pesticides, in order to keep a low risk profile in food production, will be registered for use at too low rates needed for effectively controlling pest insects in seed orchards (Mangini *et al.*, 2003). Restrictions on the use of pyrethroids are also strengthening, at least in Sweden (Swedish Chemicals Agency, www.kemi.se). The only insecticide registered for use in e.g. Swedish and French seed orchards is a variety of *Bacillus thuringiensis*.

Recently, a "new generation" of systemic insecticides has shown promising results for treating trees of various taxa, e.g. pine (Helson *et al.*, 2001; Grosman *et al.*, 2002), elm (Sclar & Cranshaw, 1996; Lawson & Dahlsten, 2003) and hawthorn (Gill *et al.*, 1999). These systemic insecticides can be injected directly into the stems, thereby minimizing potentially harmful effects on the surrounding environment.compared to if they were conventionally applied with e.g. air-blast sprayers. Furthermore, preparations containing emamectin benzoate, abamectin or imidacloprid have been shown to remain active for a year or more after injection (Grosman *et al.*, 2002; Sclar & Cranshaw, 1996).

1.3.2 Biological insecticides

In a laboratory assay, Timonin *et al.* (1980) found that larvae of *C. strobilella* and *S. anthracina* were susceptible to the fungus *Beauveria bassiana*, but it had no detected effect when sprayed on young cones (reference in Brockerhoff & Kenis, 1997b). However, another biological insecticide has proved to be effective against lepidopterous cone-feeding pest insects in field studies. This is the soil bacterium *Bacillus thuringiensis* (e.g. McLeod & Yearian 1981; Cameron *et al.* 1987; Weslien, 1999). In recent trials the biological insecticide *Bacillus thuringiensis* var. *kurstaki /aizawai* (Btk) (Turex® 50 WP) showed no effect on *C. strobilella*. However, it reduced damage caused by *D. abietella* and spraying during flowering did not alter the quality of the resulting seeds (Weslien 1999, Glynn & Weslien 2004).

1.3.3 Pheromones

Sex pheromones can be useful for trapping male moths to assess their numbers, thus aiding decisions in Integrated Pest Management (IPM) strategies regarding if and when insecticides should be sprayed. Trap catches of adult males have been shown to correlate well with damage caused by their offspring in both a field study (Suckling *et al.*, 2005) and simulations

(Robertson *et al.*, 2005). However, the only European cone- and seed-infesting insect for which a pheromone is currently available is *D. abietella* (Löfstedt *et al.*, 2011). Monitoring in northern Europe during three years has revealed that this species may have an extended flight period, from late May to late September (Rosenberg *et al.*, 2010). Neither of two pheromones used for monitoring *C. strobilella* — Z8-12:OH in Poland (Skrzypczynska *et al.* 1998) and E8-12:Ac in Canada (Grant *et al.* 1989) — did not attract any *C. strobilella* in a Swedish trial (unpublished data). Witzgall *et al.* (1996) proposed that a blend of E8,E10-12Ac and E8,Z10-12Ac could be a possible attractant for *C. strobilella*, and more recently Witzgall *et al.* (2010b) found that a mixture of these compounds and the alcohol derivatives E8,E10-12OH and E8,Z10-12OH attracted *C. strobilella* males.

In addition to monitoring applications of pheromones have been used in trials of attract and kill strategies, i.e. to attract male and female pest insects to a smaller area that is then sprayed with insecticide, to protect fruit orchards (Evenden & McLaughlin, 2004; Mansour, M. 2009) and stored products (Nansen & Phillips, 2004). Studies have shown that insecticides do not affect the attractiveness of the pheromone (Evenden & McLaughlin, 2004; Nansen & Phillips, 2004).

Another control strategy is mating disruption, in which immense amounts of sex pheromones are released to disorientate male moths and thus prevent them from finding conspecific female moths that are ready to mate. In North America the efficacy of this technique for disrupting the mating of two cone-feeding *Dioryctria species* (DeBarr *et al.*, 2000) and the seed-feeding *C. strobilella* (Trudel *et al.*, 2006) has been studied. Trap catches in pheromone-treated areas were reduced by 97 % or more for *Dioryctria* species (DeBarr *et al.*, 2000) and by 98 % for *C. strobilella* (Trudel *et al.*, 2006). The latter study also found that pheromone treatment reduced the proportion of infested cones from 56 % to 17 %. According to a review by Witzgall *et al.* (2010a) pheromones are currently used to protect resources covering more than 1 million ha globally.

2 Aims

The aims of the studies this thesis is based upon were to identify suitable methods to reduce damage caused by insects to *P. abies* cones and seeds, using techniques and insecticides that should, ideally, have as little affect on non-target species as possible. In addition, possible approaches to improve the cost efficiency of some of the methods were evaluated.

2.1 Specific questions addressed

I This study evaluated the feasibility of using large-scale spraying of the biological insecticide *Bacillus thuringiensis* var. *kurstaki/aizawai* (Btk) to decrease damage by the lepidopteran pest species. Cones were also dissected throughout the season to assess temporal patterns of insect density.

II Since Btk does not affect all pest species, not even all the moth species, it was hypothesized that reductions in numbers of some species resulting from Btk applications may increase damage by some other species. To test this hypothesis, the effects of several insect species alone, and in all possible permutations, on the number and quality of extracted seeds were examined.

III The objective of this study was to assess the ability of injectable insecticides (which affect the surrounding environment less than sprayed insecticides) to decrease damage by all pest species. In addition, the possibility of increasing the cost efficiency of their use by combining applications with injections of the flower-stimulating hormone gibberellin was investigated.

IV The availability of a pheromone for monitoring of *Cydia strobilella* would be valuable for assessing whether there is a need to spray insecticides and (if so) improve the precision of timing of spraying insecticides, thereby minimizing costs and environmental burdens.

3 Summary of papers

3.1 Experimental sites

The studies were completely (**Papers I**, **II** and **III**) or partly (**Paper IV**) conducted in a Norway spruce (*P. abies*) seed orchard (FP-504 Ålbrunna) in the province of Uppland (59°30´N, 17°32´E). The 25 ha orchard which includes 132 different clones, was established between 1982 and 1987 on abandoned farmland. The trees were approximately 6-m tall at the beginning of the study in 2002, and about 8-m tall in 2006, when the trees were pruned to about 5-m. In the final study in 2008, the trees were about 6-m tall again. A field study was also performed (**Paper IV**) in a *P. abies* clone archive (Maltesholm) in the province of Skåne (55°54´N, 13°59´E).

3.2 Large-scale spraying (Paper I)

The aim of this study was to assess the efficacy of large-scale spraying of Turex 50 WP, (*Bacillus thuringiensis* var. *kurstaki/aizawai* or Btk) against lepidopteran larvae. An air-blast sprayer was used to spray Btk on the 6-m tall trees, with nozzles, pressure and speed optimized to deliver appropriate densities of droplets, of appropriate size for minimizing drift and controlling insects, at the height of the flowers. The spraying was performed during late afternoon to minimize adverse effects of ultra-violet light, high wind speeds and high temperatures. In 2002 sprayings were conducted at times coinciding with the following phenological stages of spruce flower/cone development: early flowering (erected scales, P1), late flowering (scales pointing downwards, P2), cone initiation (scales pointing upwards again, P3) and cone development (when the young cones had just turned downward, P4). These, sprayings were carried out at three different rates, 38, 76 and 114 litres of spray liquid per 100-m tree row. In 2003 the sprayings were carried out at flowering phase P2, P3 or both P2 and P3, at a rate of 38 liters per 100-m tree row. The concentration for both years was 4 g Turex 50 WP per litre water.

Cones were sampled close to untreated control areas throughout the season on five and six occasions during 2002 and 2003, respectively, and then sliced into four pieces to find larvae, which were identified and counted. This procedure provided data on the occurrence and density of insects in the cones, including species that had left the cones at the time of final cone picking in September.

Cones that had been sprayed to assess the efficacy of Btk application (and untreated controls) were externally examined for excrement piles from *D. abietella* and the two geometrids, *E. abietaria* and *E. analoga*. For finding *C. strobilella* also these cones were sliced into four pieces.

To assess the effects of the treatments applied in 2002 two-way ANOVA was used, with rate and phase of Btk application as independent variables, the proportion of infested cones as the dependent variable, and Tukey adjustment for multiple comparisons. The same model was used to assess effects of the treatments applied in 2003, but with block and phase as independent variables. The results showed that the most appropriate time for area-wide application of Btk was during late flowering (Fig. 1).

Figure 1. Late flowering (P2), a phase that was shown to be an appropriate time for application of Btk in order to reduce damage on *P. abies* cones by *D. abietella* and *E. abietaria*.

Spraying on two occasions improved the results in 2002, but not in 2003. Damage by *D. abietella* and *Eupithecia spp*. (mostly *E. abietaria* since only three specimens of *E. analoga* were found during the season), in terms of the proportion of cones affected, was reduced from about 65 % to 25 % in 2002 and from about 90 % to 65 % in 2003. Spraying rates exceeding 38 l per 100-m tree row did not improve the efficacy of the Btk treatment.

Cydia strobilella was not affected by Btk application in terms of the proportion of infested cones.

Cone sampling during the vegetation period showed that the proportion of infested cones was higher in 2003 than in 2002 (Table 1).

Table 1. *Proportions of cones (%) infested by the indicated species during 2002 and 2003 as revealed by sampling between June and September.*

	D. abietella	**Eupithecia spp.*	*C. strobilella*	*S. anthracina*
2002	23	67	80	43
2003	77	77	90	83

*Mainly *E. abietaria*, since only three larvae in total of *E. analoga* were found.

3.3 Impact of insects on seed quality (Paper II)

Since Btk is not effective against all of the pest species there was a concern that feeding by unaffected species might increase following its application, resulting in similar levels of seed damage in treated and untreated cones. Therefore, the objective of this study was to investigate how different insect species, alone or together with other insect species, affect seed quality.

Species assemblages were modified by using two insecticides (separately): Btk (see previous section) active against lepidopteran larvae, and Fastac (see **Paper II**), a pyrethroid active against all insects, plus an untreated control. Insecticides were applied when the scales of most of the flowers were folded back (most in flowering phase P2, but some in P3), which had already been shown to be an appropriate time for applying Btk (**Paper I**). Cone analysis during the summer showed that the number of *Eupithecia* spp. was very low, so nearly all cones with excrement piles were infested by *D. abietella*. Treated and control cones were examined for external signs of *D. abietella* (excrement piles) and *S. anthracina* (bent cones with excrement-filled resin balls). The cones were then sent to a seed laboratory at Skogforsk in Sävar for seed extraction (by the commercially used extraction method), x-ray analysis of seed quality and to find larvae of the seed-feeding insects *M. strobilobius* and *P. abietina*. Total numbers of extracted seeds and extracted filled seeds were counted. The number of seeds with seed insects found was too low for meaningful statistical evaluation. Thereafter the cones were sent back to Uppsala for dissection to find larvae of *C. strobilella*.

Treatment (untreated control, Btk and Fastac) effects on the occurrence/absence of each insect species (dichotomously recorded as 1 and 0, respectively) were analysed by logistic regression with treatment as the independent variable, insect species as dependent variables and Tukey-Kramer adjustment for multiple comparisons. The results showed that Fastac significantly reduced the occurrence of *D. abietella*, relative to the control and Btk treatments, and that of *S. anthracina* relative to the control treatment. No significant effects of Btk on the occurrence of any of the species, relative to controls, were detected and no treatment had significant effects on *C. strobilella* occurrence.

To assess treatment effects on the seed variables, a general linear model (GLM) was used with treatment as independent variable and number of seeds extracted (total and filled) as dependent variables. Fastac treatment resulted in greater numbers of extracted seeds (total and filled) compared to both the control and Btk treatments. Btk had no significant effects, relative to the control treatment, on either of these variables.

The effects of these clusters were evaluated using a GLM with cluster as the independent variable, numbers of seeds extracted (total and filled) as dependent variables, and Dunnett adjustment for the multiple comparisons of numbers extracted from cones with no insects versus cones infested by the other permutations of insects. Insect cluster had strong effects on the results. Numbers of both extracted and filled seeds were significantly lower from cones with no insects than from cones infested with all clusters, except *S. anthracina* alone. Cones infested by more than one species generally yielded fewer seeds than cones infested by a single species, and those infested with *D. abietella* tended to yielded lower numbers of seeds than those not infested by this species (Fig. 2).

Since *C. strobilella* larvae usually overwinter in cones it was possible to estimate their numbers in dissected cones. Therefore, the effects of the treatments on their numbers were evaluated, firstly using a GLM with number of larvae per cone as independent variable and insect clusters (containing *C. strobilella*) as dependent variables. The aim of this analysis was to test the hypothesis that absence of other species resulted in more *C. strobilella* larvae per cone due to the consequent reduction in interspecific competition. The results showed that the number of *C. strobilella* larvae in the cones was not significantly affected by the presence of the other insects in the clusters. The mean number of larvae found was between 2-2.4.

In addition, simple linear regression was applied, with number of *C. strobilella* per cone as independent variable and numbers of seeds extracted (total and filled) as dependent variables. Only data from cones without insects and those infested by *C. strobilella* were included in this analysis. The aim was to investigate if the number of larvae present affected the number and quality of extracted seeds. The results showed that the presence of *C. strobilella* larvae reduced the numbers of extracted seeds and filled extracted seeds by about 18 and 12 per larva.

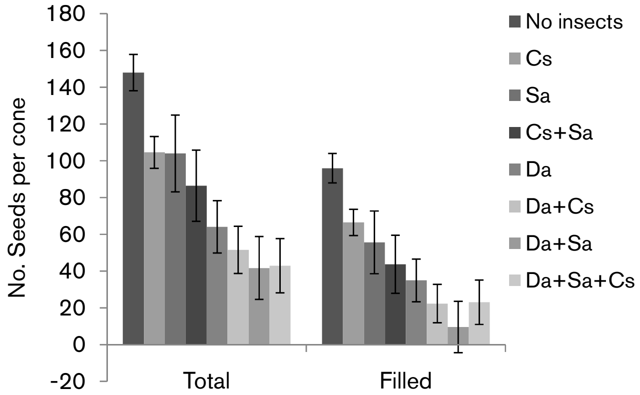

Figure 2. Mean numbers of 'Total' seeds and 'Filled' seeds (±SE) extracted from cones without insects and with insect species alone or in clusters. Abbreviations of species (Da = *D. abietella*, Sa = *S. anthracina* and Cs = *C. strobilella*).

3.4 Stem injections of insecticide and gibberellin (Paper III)

The aim of this study was to assess the efficacy of several injectable insecticides (applied one year before or in the same year as flowering; 2005 and 2006, respectively) for reducing damage by pest species of cones and seeds. Further, in order to increase cost efficiency, injections for flower stimulation was tested. These injections were carried out in 2005. Since the insecticides used had not been tested for this purpose before, it was important to examine their possible effects on seed number and seed quality. The insecticides were injected through the bark into the phloem using a Wedgle™ Direct-Inject™ injection unit. The insecticides used were Greyhound™ [a.i. abamectin, 2 %], Pointer™ [a.i. imidacloprid, 5 %] and two pyrethroids: bifenthrin [7.9 % a.i.] and deltamethrin [4.75 % a.i.]. When referring to these insecticides the name of the active ingredient will be used hereafter. All four insecticides were used for the 2006 injections, whereas only abamectin and imidacloprid were used for injections in 2005.

The flower-stimulating hormone gibberellin $A_{4/7}$ ($GA_{4/7}$) was applied, in ethanol solution, using two different methods. In the first method (which has been used in many earlier experiments) $GA_{4/7}$ is applied using a micropipette through a hole drilled into the stem to the xylem. The second method involves use of the same equipment as used for injections of insecticides. The dose and number of holes or injection sites depended on the trees' diameter at breast height, which varied between 7 and 25 cm.

Cones were examined for external signs of damage by *D. abietella* and *E. abietaria*. Dissections of cones throughout the summer showed that numbers of *D. abietella* and *E. abietaria* were very similar. Seeds were then extracted from the cones and the total number of seeds, number of filled seeds (determined by x-ray anlysis), seed mass per 1000 seeds (excluding empty seeds), and number of seeds infested by *M. strobilobius* were determined in a seed laboratory at Skogforsk, Sävar. No *E. analoga* was found, and the number of *S. anthracina*, *P. abietina* and *C. strobilella* were too low for meaningful statistical evaluation.

To assess the 2006 treatment effects, one-way ANOVA was used with treatment as independent variable and severe damage (> 10 % of cone surface damaged) and damage (< 10 % of cone surface damaged) as dependent variables. All of the insecticides injected in 2006 reduced the proportion of cones with severe damage compared to water injection, but only abamectin decreased the proportion of cones with damage compared to water injection. None of the insecticides had adverse effects on any of the evaluated seed variables.

For evaluation of the 2005 injections, two-way ANOVA (clone and treatment as independent variables) showed that there were significant treatment effects for both damage and severe damage. Pre-planned contrasts were then used to evaluate the effects of the two insecticides alone and in combination with the two gibberellin injection methods (see Paper III for further information). The only treatments that caused significant reductions in proportions of damaged (and severely damaged) cones were injections of abamectin alone and in combination with $GA_{4/7}$. There was no treatment effect on seed variables (Fig. 3).

Clone and infestation by the seed-feeding insect *M. strobilobius* affected the number of filled seeds and seed mass per 1000 seeds. Further, linear regression of the data acquired for the two clones with the greatest numbers of infested seeds showed that for each infested seed there was a loss of one other filled seed.

GA$_{4/7}$ applications, by both methods (drilling and injection), resulted in increases in numbers of flowers, relative to controls (Fig. 4).

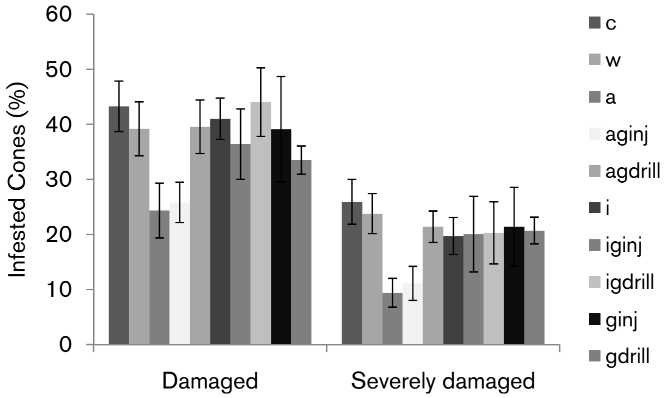

Figure 3. The amount of insect damage in 2006 following injections given in 2005. Mean percentage ± SE of cones classified as 'Damaged' and 'Severely damaged'. Untreated control (c), injections of: water (w), abamectin (a), abamectin + GA$_{4/7}$ injected (aginj), abamectin + GA$_{4/7}$ drilled (agdrill), imidacloprid (i) and imidacloprid + GA$_{4/7}$ injected (iginj), imidacloprid + GA$_{4/7}$ drilled, (igdrill), GA$_{4/7}$ injected (ginj), and GA$_{4/7}$ drilled (gdrill).

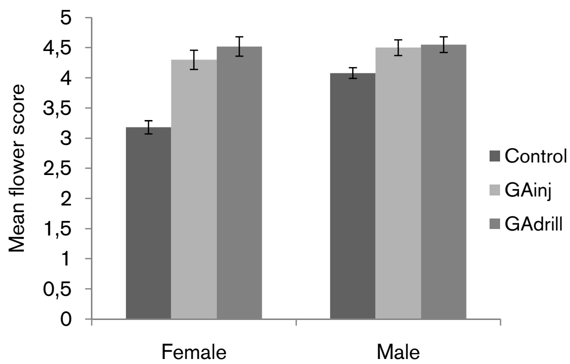

Figure 4. Mean flower score ± SE in 2006 following application of GA$_{4/7}$ given in 2005. Two different methods were used, injection using the same equipment as used for insecticide application (GAinj) or applied using a micropipette through a hole drilled into the trees xylem. The score of the trees varied between 0 (no flowers) to 9 (corresponding to the most abundant flowering in the orchard, > 2000 flowers).

3.5 Sex pheromone for *Cydia strobilella* (Paper IV)

Since earlier studies had shown that *C. strobilella* is difficult to control, a pheromone could be a useful tool for monitoring its flight in order to optimize the timing of insecticide applications or disrupt its mating. However, prior to the study reported in Paper IV, no sex pheromone that was produced by European populations of *C. strobilella* had been properly identified. Therefore, pheromone glands of *C. strobilella* females were dissected, as soon as calling behavior was observed, extracted to identify possible pheromones and male antennae were used to identify active compound(s) in the extracts. The first practical test of candidate pheromones involved use of a Y-glass maze to examine male responses to them. Finally, field experiments were conducted at two locations using Delta traps baited with a rubber septum (pheromone dispenser) at heights of 1.3 m to 4 m, 7-10 m apart, which were checked every second day. Traps were baited with specific components of the extracts in various ratios. Differences in trap catches were then compared by applying ANOVA to log (x+1)-transformed catch data with Bonferroni correction for multiple comparisons. Components, such as E8-12:OH and E8-12:OAc, did not attract *C. strobilella* males in this trial, but a mixture of two components (the isomers E8,E10-12:OAc and E8,Z10-12:OAc) did. It was found that females emitted extremely low amount of pheromone (1 pg for both isomers together).

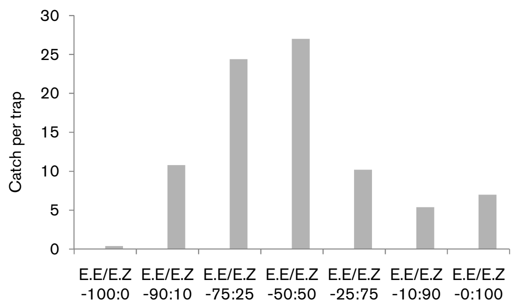

Figure 5. Catches per trap, in Ålbrunna, baited with the isomeric components of extracts from pheromone glands of female *C. strobilella* moths, that proved to be attractive to conspecific males (E8,E10-12:OAc and E8,Z10-12:OAc: EE and EZ, respectively) in indicated ratios.

A blend of E8,E10-12:OAc and E8,Z10-12:OAc proved to be most effective at a ratio of 4:3 and a dosage of 0.3 μg per septum (Fig. 5). Interestingly, ten times higher dosages (3 μg) or more significantly reduced catches.

4 Discussion

4.1 Insect species and their seasonal patterns

To improve knowledge of the phenology and seasonal density patterns of species infesting cones in Swedish seed orchards, cones were sampled between May and September during two consecutive years (**Paper I**). The phenology of *S. anthracina*, *C. strobilella*, *D. abietella* and *E. abietaria* found in this study, was consistent to earlier findings (Spessivtseff, 1924; Annila, 1979; Annila, 1981; Roques, 1983).

One of the most striking findings obtained from the sampled cones was that *E. abietaria* can be a serious pest, as described nearly a century ago by Spessivtseff (1924). However, the species has not been mentioned as a pest in recent Swedish studies (Weslien, 1999; Glynn & Weslien, 2004), and may have been overlooked for two reasons. Damage by *E. abietaria* is difficult to distinguish from damage caused by *D. abietella* (Spessivtseff, 1924; Annila, 1973), and as shown in **Paper I** larvae of *E. abietaria* have already left the cones by early August, before damage is generally evaluated (in late September when seeds have matured). Therefore, it is difficult to assess the relative amounts of damage caused by the two species. Since *E. abietaria* is not present every year, as shown in **Paper II**, *D. abietella* is likely the more important pest species of the two.

In addition, the number of *C. strobilella* larvae decreased substantially from early July to early September, somewhat surprisingly since this species normally overwinters within the cones (Bakke, 1963; Hedlin, 1973). To some degree the reduction can be explained by parasitoids (Brockerhoff & Kenis, 1996), but competition within and/or between species (cf. Mattson, 1986; Denno *et al.*, 1995) might have been a contributory factor, especially since the drop in numbers occurred in the year when there were greater numbers of larvae per cone. According to Tripp (1954), it is not unusual with cannibalism when more than one *C. strobilella* (formerly *Laspeyresia youngana* Kft.) larva feeds in a cone. In large cones however, two or more larvae can survive. This fits very well with the number of *C. strobilella* larvae per cone found during the autumn in **Paper I** and **Paper II** and the greater number found in July, suggesting competition within the species.

Although the seed insects *M. strobilobius* and *P. abietina* cannot be considered as serious pest species according to the studies included in this thesis (**Paper III** and **IV**), it cannot be ruled out that they can be serious some years and at other locations.

4.2 Conventionally applied insecticides

Paper I with area-wide spraying (Fig. 6) confirmed previous reports (using hand and backpack sprayers) (Weslien, 1999; Glynn & Weslien, 2004) that Btk reduces damage by *D. abietella* but has little (if any) effect on *C. strobilella*, at least in terms of the proportion of infested cones. Since no seed analysis was performed in the study it was not possible to evaluate possible differences in seed number and quality between Btk-treated and control cones. However, no effect of Btk-treatment on seeds was found in **Paper II**. Thus, it seems reasonable to assume that Btk had no effect on *C. strobilella* larvae.

Figure 6. Area-wide spraying of *Bacillus thuringiensis* var. *kurstaki/aizawai* on 6-m tall trees in Ålbrunna spruce seed orchard.

In **Paper I** it was found that the most appropriate time for area-wide application of Btk was during late flowering. Possibly because it increases the likelihood that Btk will reach the interior of the cones, where the larvae feed, but also by protection from the closing scales that may prolong the insecticidal activity (cf. Reardon *et al.* 1994), which may otherwise largely be lost within 14 days due to factors such as UV-light (McLeod *et al.* 1983). In contrast, spraying of the pyrethroid permethrin and organophosphate dimethoate is reportedly more effective against *D. abietella* when cones have turned down (Annila & Heliövaara, 1991). In **Paper II**, treatment with the pyrethroid Fastac resulted in much lower proportions of cones infested by *D. abietella* than the control treatment, although it was sprayed during flowering. No effect of Btk on *D. abietella* was detected this year, possibly because the flight period of *D. abietella* was prolonged — as previously recorded in some years (Rosenberg *et al.*, 2010) — and (hence) Btk did not remain active during the whole ovipositing period. According to Cameron *et al.* (1987) this kind of insecticide might be more effective for species appearing for only a short period.

The cones were finally sampled after most larvae had left them. Therefore, it was not possible to discriminate between damage caused by *E. abietaria* and *D. abietella* from external signs, and hence not possible to evaluate the degree to which each species was affected by insecticides in **Paper I**. However, Weslien (1999) showed that the number of *D. abietella* larvae per cone was reduced after Btk treatment, and *B. thuringiensis* reduces damage by other *Dioryctra* species (McLeod & Yearian 1981; Cameron *et al.* 1987), implying that Btk is likely to be effective against *D. abietella*. Since about 80 % of the cones examined in **Paper I** were infested by *E. abietaria* and 23 % by *D. abietella*, and the damage was reduced by 60 %, Btk was also probably effective against *E. abietaria*.

Since Btk does not reduce damage by all species, there was a concern that reduced competition following its application could increase feeding by the remaining species (e.g. Annila, 1973; Roques *et al.*, 1996), especially since a cone is a discrete resource (Mattson, 1986; Denno *et al.*, 1995). However, findings reported in **Paper II**, that numbers of extracted seeds (total and filled) tended to increase with decreasing numbers of species, and that numbers of *C. strobilella* larvae were similar in all species clusters does not support that concern. The within species competition of *C. strobilella* that likely exists, has no practical implication for spraying of e.g. Btk. Instead, the results indicate that it would probably be economically viable to spray Btk as long as the cones are infested by at least *D. abietella* or *E. abietaria*. A drawback is that spraying of Btk has been shown to be most effective during flowering (**Paper I**), which occurs before the flight periods of these two species, why unnecessary sprayings might be difficult to avoid.

Although Fastac did not reduce proportion of *C. strobilella* infested cones in **Paper II**, previous studies have shown that pyrethroids also can decrease damage by *Cydia* species (e.g. Nord *et al.*, 1985). A proper timing of Fastac application might reduce damage also by *C. strobilella*.

4.3 Injectable insecticides

In order reduce damage caused by all pest insects in seed orchards some insecticide(s) other than Btk must be used. Injectable systemic insecticides would be advantageous for this purpose, since their use would minimize possible effects on the surrounding environment. Therefore, in **Paper III** the efficacy of several such insecticides was tested, and the most promising was found to be abamectin, which also reduced damage one year after injection. Although some authors, e.g. Grosman *et al.* (2002), found that injecting insecticide into pre-drilled holes could greatly reduce insect damage, another method was used here, involving inserting a syringe directly into the stem, in order to minimize wounds and to increase the speed of the injections. The resulting reductions in damage were not as great as those reported by Grosman *et al.* (2002) and Gill *et al.* (1999). Since injection is very time consuming, compared to conventional spraying, it can probably only be considered for treating especially valuable trees during flowering seasons. However, in the study by Grosman *et al.* (2002) emamectin benzoate was injected into pine trees, in which cone maturation takes two years, inspiring us to test (and confirm) the possibility that abamectin may still have protective effects a year after injection in *P. abies* trees, although their cones mature within one year. Part of the rationale for this part of the experiment was that flowering can be increased by injecting the hormone gibberellin (e.g. Eriksson *et al.*, 1998), and the results (**Paper III**) show that it might be possible to combine insecticide and gibberellin injections, and thus increase the cost efficiency of the treatments.

4.4 Pheromones

Neither of the formulations for *C. strobilella* used in Polish (Skrzypczynska *et al.*, 1998) and Canadian (Grant *et al.*, 1989) studies was effective in Sweden. However, in Paper IV a pheromone was identified, synthesized and their optimal doses (and ratios) for trap catches were determined. The *D. abietella* pheromone has also been identified (Löfstedt *et al.*, 2011), hence pheromones are now available for two of the three most serious *P. abies* cone and seed-infesting moth species. These pheromones might be useful for use in trap catching to decide if and when pest management measures should be applied, and thus minimize the use of insecticide in Integrated Pest Management.

In North American seed orchards IPM is used to decide when to spray insecticides, and this has reduced the use of insecticides substantially (DeBarr, 1993). Monitoring of *D. abietella* has been carried out in Sweden (Rosenberg *et al.*, 2010), but so far not in connection with insecticide treatment.

Results of trials in Canada (Trudel *et al.*, 2006) indicate that disrupting the mating of *C. strobilella* using pheromones could also be a good method for reducing damage in Swedish seed orchards. This is partly because the females emit extremely low amounts of pheromone and the male antennae are correspondingly sensitive, in both Canadian populations (Grant *et al.*, 1989) and Swedish populations (**Paper IV**). The fact that the adults also have short life-spans, of about three to four days (Bakke, 1963), probably also reduces the possibility of disoriented males finding "real" females.

5 Pest management strategies

In this section various pest management strategies, focusing on strategies of particular relevance to the context of Studies I-IV, are discussed.

5.1 Conventionally applied insecticides

Turex 50 WP (Btk) is the only insecticide permitted for use in Swedish seed orchards today. It can be effective against *D. abietella* and *E. abietaria* larvae (provided they eat it), and the optimum time for spraying is during late flowering, before the insects fly. Thus, pheromone traps cannot be used to determine if spraying is required in advance. Since at least *D. abietella* is present in most years and Btk has relatively weak effects against non-target species, it may be beneficial to spray Btk whenever there are sufficient flowers to give a harvestable cone crop. However, the effect of Btk on *D. abietella* may also be weak in some years, possibly because the insect's flight period is prolonged, or the weather during or after spraying may reduce its efficacy. Spraying should be avoided in sunlight (since ultra-violet light degrades Btk), at temperatures below about 13°C (which reduce larval activity) and if rain (which may wash the insecticide off the flowers) is likely to occur shortly after spraying. After the cone scales have closed, Btk may be protected from further degradation and from being washed off.

A commonly applied strategy in various agricultural and horticultural contexts is Integrated Pest Management (IPM), in which pest insects are monitored (e.g. using pheromone traps) and insecticides used if the catches suggest they are necessary. Use of insecticides that are effective at contact (none of which are registered for use in seed orchards today) in combination with pheromone-baited traps may be an option in such cases. Monitoring reveals if any pest insects are present in the locality, and when they start to fly. If sprayed during flowering (same as Btk) contact insecticide can reduce damage by larvae feeding on the scales and, if the timing is correct they may decrease the number of ovipositing females and possibly also the numbers of larvae eating cones and seeds.

5.2 Injectable insecticides

At present no injectable insecticide is permitted for use in Swedish seed orchards, but since injections have substantial advantages (minimizing possible effects on the surrounding environment), and their potential utility was assessed in Study III, strategies for their use are briefly outlined here. Such insecticides can be injected either in the same year as flowering or the year prior to expected flowering. Injections (by current techniques at least) are time-consuming and insecticides used today are quite expensive, therefore treatment of complete orchards is unlikely to be viable. However, injections could feasibly be applied to certain especially valuable trees in flowering years. In addition, when used in years prior to flowering, insecticide injections could be combined (at least in time) with injections of the flower-stimulating hormone gibberellin. Furthermore, both insecticide and gibberellin could be injected with the same equipment without pre-drilling holes, thus potentially increasing flowering, reducing damage to the cones and increasing the cost efficiency of the treatments.

Knowledge regarding the presence and abundance of pest species obtained using pheromone traps could not be exploited using any of these methods, since they are applied before flight begins.

5.3 Pheromones

Pheromones have only been identified for two of the major *P. abies* cone and seed pests as yet: *D. abietella* and *C. strobilella*. However, for these species (and others, if and when suitable substances are identified) pheromones could be used to determine if, when and for how long they fly. Pheromones can also be potentially used to disrupt the insects' mating, by releasing large amounts to disorientate males, thereby preventing them from finding conspecific females. As discussed above, results presented in Paper IV show that female *C. strobilella* moths release very small quantities of pheromone, and the males have extremely sensitive antennae, indicating that there are good prospects for using the identified pheromones for disrupting the species' mating. Like insecticides, any pheromones to be used for control purposes must be registered, but as yet no pheromone has been registered in Sweden for controlling cone- and seed-infesting insects.

5.4 A possible decision scheme

5.4.1 The year prior to flowering

With knowledge of the weather and previous flowering patterns in a specific seed orchard (which indicate the likely abundance of flowers in the coming year), the manager can decide if the flower-stimulating hormone gibberellin should be injected, and if any insecticide (if permitted) should be injected to reduce damage to the expected cones.

5.4.2 The year of flowering

Forecasts by Skogforsk, available at *http://www.skogforsk.se/sv/Verktyg/Froservice/Kott--och-froprognos/* will have provided the seed orchard manager with information about expected cone and seed yields, in time for planning appropriate actions to reduce damage.

Any spraying of Btk against *D. abietella* and *E. abietaria* should be done during late flowering, before any of these species start flying.

Injections can also be applied in flowering years, targeting specific, valuable trees rather than whole orchards (which is probably not economically viable), during early flowering before any flight is observed.

Fastac, and or (probably) some other pyrethroids, can be applied during late flowering to reduce damage (at least) by *D.abietella* and *S. anthracina*.

Pheromones can be used for trapping and monitoring the two species *C. strobilella* and *D. abietella*, and hence acquiring information about the abundance of males of the species in the orchard and their flight periods. Information acquired in this manner could also assist decisions regarding if and when some contact insecticide, e.g. Fastac, should be applied (although this strategy has not yet been tested). Improvements in the timing of Fastac applications may also reduce damage by *C. strobilella*.

Pheromones might also be useful for disrupting the mating of both *C. strobilella* and *D. abietella*. Further, combining mating disruption of *C. strobilella* and Btk treatment could potentially reduce damage caused by three of the four severe pest species substantially.

6 References

Almqvist, C., Simonsen, R. & Rosenberg, O. 2008. Norway spruce seed orchards- forestry´s golden nugget. Skogforsk, Resultat Nr. 4. pp. 4. (In Swedish with English summary).

Almqvist, C., Rosvall, O. & Wennström, U. 2007. Fröplantager- anläggning och skötsel [Seed orchards- establishment and management]. Skogforsk Handledning pp. 97

Almqvist, C., Wennström, U. & Karlsson, B. 2010. Improved forest regeneration material 2010-2050- Supply and needs, and measures to minimize shortage and maximize genetic gain. Skogforsk, Redogörelse Nr. 3. pp. 59. (In Swedish with English summary).

Annila, E. 1966. On the occurrence of *Plemeliella abietina* Seitn. (Dipt., Cecidomyidae) and *Megastigmus strobilobius* Ratz. (Hym., Chalciidae) in Finland. *Annales Entomologici Fennici* 32: 3-11.

Annila, E. (1973) Chemical control of spruce cone insects in seed orchards. *Communicationes Instituti Forest Fenniae*, 78, 1-25.

Annila, E. 1979. The life cycles of the cone-infesting *Dioryctria* species (Lepidoptera, Pyralidae) in Finland. *Notulae Entomologicae* 59:69-74.

Annila, E. 1981. Fluctuations in cone and seed insect populations in Norway spruce. *Communicationes Instituti Forest Fenniae* 101: 1-32. (In Finnish with English summary).

Annila, E. & Heliövaara, K. 1991. Chemical control of cone pests in a Norway spruce seed orchard. *Silva Fennica* 25: 59-67.

Bakke, A. 1955. Insects reared from spruce cones in northern Norway 1951. *Norsk Entomologisk Tidskrift* 9: 152-212.

Bakke, A. 1963. Studies on the spruce cone insects *Laspeyresia strobilella* (L.) (Lepidoptera: Tortricidae), *Kaltenbachiola strobi* (Winn.) (Diptera: Itonidae) and their parasites (Hymenoptera) in Norway- Biology, Distribution and Diapause. Reprint from *Reports of The Norwegian Forest Research Institute* No 67, pp. 151.

Brockerhoff, E.G. & Kenis, M. 1996. Parasitoids associated with *Cydia strobilella* (L.) (Lepidoptera: Tortricidae) in Europe, and considerations for their use for biological control in North America. *Biological Control* 6: 202-214.

Brockerhoff, E.G. & Kenis, M. 1997. Oviposition, life cycle, and parasitoids of the spruce cone maggot, *Strobilomyia anthracina* (Diptera: Anthomyiidae), in the Alps. *Bulletin of Entomological Research* 87: 555-562.

Brockerhoff, E.G. & Kenis, M. 1997b. Strategies for the biological control of insects infesting coniferous seed cones. In: Battisti, A. & Turgeon, J.J. *Proceedings of the 5th cone and seed insects working party conference*, September 1996, Monte Bondone, Italy. Padova: Institute of Agricultural Entomology, University of PAdova. pp. 49-56.

Cameron, R.S., DeBarr, G.L., Goodbee, J.F. & Taylor, J.W. 1987. Potential alternative insecticides for insect control in southern pine seed orchards. In: *Proceedings 19th Southern Forest Tree Improvement Conference* pp. 182-189.

DeBarr, G.L. 1993. Challenges for insect pest management in forest tree seed orchards. In: *Proceedings 22nd Southern Forest Tree Improvement Conference* pp. 464-473.

DeBarr GL, Hanula JL, Niwa CG, Nord JC, 2000. Synthetic pheromones disrupt male *Dioryctria* spp. moths in a loblolly pine seed orchard. *Canadian Entomologist* 132: 345–351.

Denno, R.F., McClure, M.S. & Ott, J.R. 1995. Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annual Review of Entomology* 40: 297-331.

Ebel, B.H. 1963. Insects affecting seed production of slash and longleaf pines- Their identification and biological annotation. U.S. Department of Agriculture *Forest Service Research Paper* SE – 6. pp. 24

Eidmann, H.H. & Klingström, A. 1990. *Skadegörare i skogen*. Andra upplagan. LTs förlag, Stockholm, Sweden.

Eriksson, U., Jansson, G. & Almqvist, C. (1998) Seed and pollen production after stem injection of gibberellin A4/7 in field-grown seed orchards of *Pinus sylvestris*. *Canadian Journal of Forestry Research*, **28**, 340-346.

Evenden, M.L. & McLaughlin, J.R. 2004. Factors influencing the effectiveness of an attracicide formulation against the oriental fruit moth, *Grapholita molesta*. Entomologia Experimentalis et Applicata 112: 89-97.

Gill, S., Jefferson, D.K., Reeser, R.M. & Raupp, M.J. (1999) Use of soil and trunk injection of systemic insecticides to control lace bug on hawthorn. *Journal of Arboriculture*, 25, 38-42.

Glynn, C. & Weslien, J. 2004. *Bacillus thuringiensis* var. *kurstaki* x *aizawai* applied to spruce flowers reduced *Dioryctria abietella* (Lepidoptera: Pyralidae) infestation without affecting seed quality. *Journal of Economic Entomology* 97: 1836-1841.

Grant, G.G., Fogal, W.H., West, R.J., Slessor, K.N. and Miller, G.E. 1989. A sex attractant for the spruce seed moth, *Cydia strobilella* (L.), and the effect of lure dosage and trap height on capture of male moths. *Canadian Entomologist* 121: 691-697.

Grosman, D.M., Upton, W.W. & McCook, F.A. 2002. Systemic insecticide injections for control of cone and seed insects in Loblolly pine seed orchards-2 year results. *Southern Journal of Applied Forestry* 26, 146-152.

Hannerz, M., Eriksson, U., Wennström, U. & Wilhelmsson, L. 2000. Scots pine and Norway spruce seed orchards in Sweden –a description with an analysis of future seed supply. *Skogforsk, Redogörelse* nr 1, 2000, 40 pp. (in Swedish with English summary)

Helson, B.V., Lyons, B.D., Wanner, K.W. & Scarr, T.A. 2001. Control of conifer defoliators with neem-based systemic bioinsecticides using a novel injection device. *Canadian Entomologist* 133: 729-744.

Holste, G. 1922. Fichtenzapfen- und fichtensamenbewohner Oberbayerns. *Zeitschrift für Angewandte Entomologie* 8: 125-160.

Johnson, N.E. & Rediske, J.H. 1965. A test of systemic insecticides to control Douglas-fir cone and seed insects. *Journal of Economic Entomology* 58: 1020-1021.

Karlsson, B. & Rosvall, O. 1995. Breeding programmes in Sweden. Skogforsk, Arbetsrapport Nr. 302.

Kobayashi, K. 1981. Cone and seed insects of Japanese conifers. In *Proceedings of 17th IUFRO World Congress*, Kyoto, 1980. pp. 451-461.

Li, B., McKeand, S. & Weir, R. 1999. Tree improvement and sustainable forestry – Impact of two cycles of Loblolly pine breeding in the U.S.A. *Forest Genetics* 6: 229-234.

Löfstedt, C., Svensson, G.P., Jirle, E.V., Rosenberg, O., Roques, A. & Millar, J.G. 2011. (3Z, 6Z, 9Z, 12Z, 15Z)-pentacosapentaene and (9Z, 11E)-tetradecadienyl acetate: sex pheromone of the spruce coneworm *Dioryctria abietella* (Lepidoptera: Pyralidae). *Journal of Applied Entomology* Online: doi: 10.1111/j.1439-0418.2011.01619.x

Mangini, A.C., Duerr, A. & Taylor, J.W. 2003. Seed and cone insect pest management: Challenges and solutions. *Proceedings of the Society of American Foresters National Convention* 2002. pp. 170-175.

Mansour, M. 2010. Attract and kill for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) control in Syria. *Journal of Applied Entomology* 134: 234-242.

Mattson, W.J. 1986. Competition for food between two principal cone insects of Red pine, *Pinus resinosa*. *Environmental Entomology* 15: 88-92.

McLeod, P.J. & Yearian, W.C. 1981. Insects associated with pine seed production in Arkansas. *Arkansas Experiment Station, Bulletin* 850. pp. 39.

McLeod, P.J., Yearian, W.C. & Young, S.Y. 1983. Persistence of *Bacillus thuringiensis* on second-year loblolly pine cones. *Environmental Entomology* 12: 1190-1192.

Merkel, E.P. 1964. Hydraulic spray applications of insecticides for the control of Slash pine cone and seed insects. U.S. Department of Agriculture, *U.S. Forest Service Paper* SE – 9. pp. 7.

Merkel, E.P. 1969. Control of insects in Slash pine cones with trunk implants if Bidrin® systemic insecticide-First-year results. U.S. Department of Agriculture *Forest Service Research Note* SE-109 pp. 4.

Nansen, C. & Phillips, T.W. 2004. Attractancy and toxicity of an attracticide for Indianmeal moth, Plodia interpunctella (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 97: 703-710.

Niwa, C.G. & Overhulser, D.L. 1992. Oviposition and development of *Megastigmus spermotrophus* (Hymenoptera: Torymidae) in unfertilized Douglas-fir seed. *Journal of Economic Entomology* 85: 2323-2328.

Nord, J.C., DeBarr, G.L., Barber, L.R., Weatherby, J.C. & Overgaard, N.A. 1985. Low-volume applications of azinphosmethyl, fenvalerate, and permethrin for control of coneworms (Lepidoptera: Pyralidae) and seed bugs (Hemiptera: Coreidae and Pentatomidae) in Southern pine seed orchards. *Journal of Economic Entomology* 78: 445-450.

Robertson, S.P., Hull, L.A. & Calvin, D.D. 2005. Tufted apple bud moth (Lepidoptera: Tortricidae) management model for processing apples based on early season pheromone trap capture. *Journal of Economic Entomology* 98: 1229-1235.

Rosenberg, O., Ylioja, T., Krokene, P., Ravn, H.P., Voolma, K., Harala, E., Savonen, E-.M. & Löfstedt, C. 2010. Monitoring of *Dioryctria abietella* Den. et. Schiff. (Lepidoptera:

Pyralidae) in northern Europe using pheromone traps. Abstract In: Parrotta, J.A. & Carr, M.A. *The International Forestry Review* 12: 391.

Rosvall, O., Jansson, G., Andersson, B., Ericsson, T., Karlsson, B., Sonesson, J. & Stener, L. –G. 2001. Genetic gain from present and future seed orchards and clone mixes. *Skogforsk, Redogörelse* nr 1, 2001. 41 pp. (In Swedish with English summary).

Roques, A.1983. *Les insectes ravageurs des cônes et grains de conifères en France*. INRA, Paris, France.135 pp.

Roques, A., Sun, J.-H., Zhang, X.-D., Philippe, G. & Raimbault, J.P. 1996. Effectiveness of trunk-implanted acephate for the protection of cones and seeds from insect damage in France and China. *Canadian Entomologist* 128: 391-406.

Rouault, G., Turgeon, J., Candau, J.-N., Roques, A. & von Aderkas, P. 2004. Oviposition strategies of conifer seed chalcids in relation to host phenology. *Naturwissenschaften* 91: 472-480.

Seifert, M., Wermelinger, B. & Schneider, D. 2000. The effect of spruce cone insects on seed production in Switzerland. *Journal of Applied Entomology* 124: 269-278.

Sclar, D.C. & Cranshaw, W.S. (1996) Evaluation of new systemic insecticides for elm insect pest control. *Journal of Environmental Horticulture*, 14, 22-26.

Skrzypczynska, M. & Roques, A. 1987. *Megastigmus strobilobius* Ratzeburg, 1848 (Hym., Torymidae): its morphology, life history and economic significance. *Journal of Applied Entomology* 104: 484-495.

Skrzypczynska, M., Koziol, M. & Kosibowicz, M. 1998. Pheromone trapping of the spruce seed moth Cydia strobilella L. (Lep., Tortricidae) in southern Poland in 1996-1997. *Anzaiger für Schädlingskunde, Pflanzenschutz, Umweltschutz* 71: 96-99.

Spessivtseff, P. (1924) Grankottmätarna (*Eupithecia abietaria* och *strobilata*) och deras skadegörelse [The spruce cone loopers (*Eupithecia abietaria* and *E. strobilata*) and their damage]. *Meddelanden från Statens Skogsförsöksanstalt*, 21, 295-310. (In Swedish with German summary).

Stadnitskiy, G.V. 1971. Characteristics of cones of Norway spruce (Picea abies (L.) Karst.) as habitat and the complex of insects infesting them. Entomological Review 50: 50-55.

Stein, J.D., Sandquist, R.E., Koerber, T.W. & Frank, C.L. (1993) Response of Douglas-fir cone and seed insects to implants of systemic insecticides in a Northern California forest and a Southern Oregon seed orchard. *Journal of Economic Entomology*, 86, 465-469.

Suckling, D.M., Gibb, A.R., Dentener, P.R., Seldon, D.S., Clare, G.K., Jamieson, L., Baird, D., Kriticos, D.J. & El-Sayed, A.M. 2005. Uraba lugens (Lepidoptera: Nolidae) in New Zealand: Pheromone trapping for delimitation and phenology. *Journal of Economic Entomology* 98: 1187-1192.

Timonin, M.I., Fogal, W.H. & Lopushanski, S.M. 1980. Possibility of using white and green muscardine fungi for control of cone and seed insect pests. *Canadian Entomologist* 112: 849-854.

Tripp, H.A. 1954. Description and habits of the spruce seedworm, (Laspeyresia youngana (Kft.) (Lepidoptera: Olethreutidae). *Canadian Entomologist* 9: 385-402.

Trudel, R., Guertin, C. and Grant, G.G. 2006. Potential for mating discruption to reduce cone damage by the spruce seed moth, *Cydia strobilella*, in spruce seed orchards. *Journal of Applied Entomology* 130: 245-250.

Trägårdh, I. 1917. Investigations into the insects injurious to the spruce and pine cones. *Meddelanden från Statens Skogsförsöksanstalt*, 13-14, 1141-1214 (In Swedish with English summary).

Turgeon, J.J., Roques, A. & de Groot, P. (1994) Insect fauna of coniferous seed cones: diversity, host plant interactions, and management. *Annual Review of Entomology* 39, 179-212.

Werner, M. 2010. Föreningen Skogsträdsförädling- från ursprunget 1936 och de första 70 åren. Föreningen Skogsträdsförädling, Uppsala.

Weslien, J. 1999. Biological control of the spruce coneworm *Dioryctria abietella*: Spraying with *Bacillus thuringiensis* reduced damage in spruce seed orchard. *Scandinavian Journal of Forest Research* 14:127-130.

Wiersma, N. 1978. Cone and seed insects in Europe. In: *Proceedings Flowering and seed development in trees: a symposium,* Mississippi State University, May 15-18, 1978 pp. 291-304.

Witzgall, P., Chambon, J.-P., Bengtsson, M., Unelius, C.R., Appelgren, M., Makranczy, G., Muraleedharan, N., Reed, D.W., Hellrigl, K., Buser, H.-R., Hallberg, E., Bergström, G., Tóth, M., Löfstedt, C. & Löfqvist, J. 1996. Sex pheromone and attractants in the Eucosmini and Grapholitini (Lepidoptera, Tortricidae). *Chemoecology* 7:13-23.

Witzgall, P., Kirsch, P. & Cork, A. 2010a. Sex pheromones and their impact on pest management. *Journal of Chemical Ecology* 36: 80-100.

Witzgall, P., Trematerra, P., Liblikas, I., Bengtsson, M. and Unelius, C.R. 2010b. Pheromone communication channels in tortricid moths: lower specificity of alcohol vs. acetate geometric isomer blends. *Bulletin of Entomological Research* 100: 225-230.

Acknowledgements

Tack till:
Jan Weslien, som varit min närmaste handledare under den här tiden och är den som jag har terroriserat mest med förslag och frågeställningar. Jan, du är duktig på att ge snabba och kloka återkopplingar på manus och idéer (även om kommentarerna emellanåt framkallat både transpiration och gråa hår).
-Göran Nordlander, även han handledare, för att ha tagit hand om alla praktikaliteter runt doktorerandet, samt för små men kärnfulla synpunkter på manus.
-Curt Almqvist, för att du alltid har tagit dig tid att svara på mina förädlingsinriktade frågor, och för att du läst och kommenterat delar av denna sammanläggning.
-Carolyn Glynn som tillsammans med Jan Weslien gjorde det möjligt för mig att börja studera kott- och fröätande insekter.
-Christer Löfstedt och hans forskargrupp för mycket bra samarbete vad gäller feromoner.
-mina arbetskamrater på Skogforsk, ingen nämnd och ingen glömd, som gör det väldigt trevligt att gå till arbetet, även en bister måndag morgon.

The work this thesis is based upon was mainly funded by The Swedish Forest Tree Breeding Association.

Linguistic revision of this thesis and papers I-III was conducted by Sees-editing, U.K.

För övrigt anser jag (helt utan att vara opartisk) att Hälsingland är ett av Sveriges absolut vackraste landskap.