

Oilseed Rape Transformed with a Pea Lectin Gene

**Target and Non-target Insects, Plant Competition, and
Farmer Attitudes**

ANNA LEHRMAN

*Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
Uppsala*

**Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2007**

Acta Universitatis Agriculturae Sueciae

2007: 95

ISSN 1652-6880

ISBN 978-91-576-7394-7

© 2007 Anna Lehrman, Uppsala

Tryck: SLU Service/Repro, Uppsala 2007

Abstract

Lehrman, A. 2007. Oilseed rape transformed with a pea lectin gene. Target and non-target insects, plant competition, and farmer attitudes. Doctor's dissertation.
ISSN 1652-6880, ISBN 978-91-576-7394-7

Plants genetically modified (GM) for insect resistance (IR) have to be tested and compared to their non-GM counterparts with respect to several aspects. This thesis summarizes the effects of three transgenic lines of oilseed rape (*Brassica napus*) expressing pea (*Pisum sativum*) seed lectin (PSL) in the pollen on a target pest: the pollen beetle (*Meligethes aeneus*), and a non-target insect: the honey bee (*Apis mellifera*). The competitive ability of the transgenic plants was tested to evaluate potential invasive characters. Finally, attitudes towards GM crops among Swedish farmers were surveyed.

Pollen beetle adults and larvae were exposed to three PSL expressing plant lines and two control lines without any PSL. Fourteen life history parameters were studied and significant differences between transgenes and controls were found for egg size and larval mortality. This means that the modification does not prevent direct damage to the attacked crop but the effects could, together with the action of natural enemies, lead to a reduced pollen beetle population.

The sensitivity of honey bee larvae to PSL containing pollen was tested by feeding them diets with high levels of transgenic or control pollen. The addition of pollen had a positive effect on developmental time and larval weight but no differences were detected between transgenic and non-transgenic pollen.

Competitive ability was tested by growing transgenic plants either in monoculture or mixed with control plants, with or without pollen beetles, and with or without pollinators (bumblebees). Plant characters related to plant fitness were measured but transgenic plants did not benefit from the transformation regarding pest damage. However, yield was higher on transgenic plants when grown mixed with control plants than when grown in monoculture, and the opposite was true for control plants.

A majority of the surveyed farmers were negative to GM crops and considered consumers' unwillingness to buy GM products as the largest drawback, while higher yield was considered the largest potential benefit from growing such crops.

Keywords: *Brassica napus*, *Meligethes aeneus*, pea lectin, *Pisum sativum*, *Apis mellifera*, transgene, performance, attitude, GMO.

Author's address: Anna Lehrman, Department of Ecology, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden
E-mail: Anna.Lehrman@ekol.slu.se

Raps modifierad med ärtlektin

Skadegörare, nyttodjur, växtkonkurrens och attityder bland lantbrukare

Växter genmodifierade (GM) för insektsresistens (IR) behöver testas och jämföras med motsvarande icke-GM gröda för flera egenskaper. Denna avhandling summerar effekten av tre GM-linjer av raps (*Brassica napus*), som uttrycker ärtlektin (*Pisum sativum*) (PSL) i pollenet, på dess skadegörare, rapsbagge (*Meligethes aeneus*), och en nyttoinsekt, honungsbi (*Apis mellifera*). De transgena plantorna testades även för att utreda om modifieringen inneburit ökad konkurrenskraft. Slutligen undersöktes attityderna till GM-grödor bland Sveriges lantbrukare.

Både larver och vuxna rapsbaggar fick äta på någon av de tre transgena linjerna eller på någon av två kontrollinjer som inte innehöll något ärtlektin. Fjorton olika parametrar testades men det var bara äggstorlek och larvdödlighet som skiljde sig signifikant mellan transgena och icke transgena plantor. GM-plantorna skulle därmed inte vara direkt skyddade mot skadegöraren, men om denna effekt skulle bestå under naturliga förhållanden skulle den transgena rapsen, tillsammans med rapsbaggens naturliga fiender, kunna begränsa en av de svåraste skadegörare på raps i norra Europa.

Möjliga negativa effekter av PSL-pollenet på bilarver testades genom att mata larverna med höga nivåer av antingen pollen från den transgena rapsen, eller pollen från kontrollplantor. Pollentillskottet i dieten hade positiv effekt på utvecklingstid och vikt men inga skillnader kunde påvisas mellan transgent och icke-transgent pollen.

Plantornas konkurrenskraft undersöktes genom att odla transgena plantor för sig eller i blandning med icke-transgena, med eller utan rapsbaggar och pollinatörer (humlor). Flertalet växtkaraktärer relaterade till fitness mättes men den transgena rapsen blev inte mindre skadad jämfört med kontrollplantorna. Däremot gav PSL-rapsen lägre avkastning när den odlades separat jämfört med den blandade odlingen medan det motsatta gällde för kontrollrapsen.

Majoriteten av lantbrukarna var negativa till GM-grödor. Konsumenternas negativa attityd sågs som den största nackdelen och en högre avkastning som den största fördelen om de skulle odlas en gröda modifierad för insektsresistens.

Content

Introduction, 7

Breeding for insect resistance, 8

Domestication of plants, 8

Plant defence, 9

Improving insect resistance in plants using biotechnology, 9

Global status of transgenic crops, 10

Risk/ benefit assessments, 10

Toxicity to non-target organisms, 11

Gene introgression, 11

Resistance development, 12

Additional effects of gene transfer, 12

Environmental benefits, 12

The plant and its pests, 13

Brassica history, 13

Rapeseed breeding and cultivation, 13

The major pest; the pollen beetle, 14

The making of a transgene, 16

Finding the gene, 16

Introduction of the gene and plant line production, 16

Transgene assays, 17

Lectin quantification, 17

Pollen beetle assays, 17

Honey bee assay, 20

Plant invasiveness, 22

The grower, 22

Economic benefits, 23

Farmer survey, 23

Conclusions, 24

References, 25

Acknowledgement – Tack, 30

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Lehrman, A., Åhman I & Ekbom, B. 2007. Influence of pea lectin expressed transgenically in oilseed rape (*Brassica napus*) on adult pollen beetle (*Meligethes aeneus*). *Journal of Applied Entomology* 131, 319-325.
- II. Lehrman, A., Åhman I & Ekbom, B. Effect of pea lectin expressed transgenically in oilseed rape on pollen beetle life history parameters. (Submitted)
- III. Lehrman, A. 2007. Does pea lectin expressed transgenically in oilseed rape (*Brassica napus*) influence honey bee (*Apis mellifera*) larvae? *Environmental Biosafety Research* 6 (in press).
- IV. Åhman, I., Lehrman, A. & Ekbom, B. Competitive ability of oilseed rape transformed for pollen beetle resistance. (Manuscript)
- V. Lehrman, A. & Johnson, K. Swedish farmers attitudes, expectations and fears in relation to growing genetically modified crops. (Manuscript)

Paper I is reproduced by kind permission of Blackwell Publishing. Paper III is reproduced by kind permission of EDP Sciences.

Introduction

The ability to introduce novel traits into plants by gene transformation has provided plant breeders with new opportunities to breed for higher yields, improved insect and disease resistance, tolerance to abiotic stress and herbicides, new qualities of plant products etc. The potential environmental impacts connected to the technology are in many ways similar to that of conventional crops, although the novelty of some transgenic crops may involve new challenges (Dale, Clarke & Fontes, 2002). More than 10 years have passed since the first commercial transgenic crop was introduced in agriculture (James, 2006) but this plant breeding technology is still not universally accepted. The potential risks of negative environmental effects have resulted in extensive pre-release tests of transgenic varieties where every new trait and crop combination and new environment into which the transgenic crop is introduced have to be assessed (Nap et al., 2003). However, benefits need to be acknowledged and weighed against the risks, and in the case of transgenic insect resistance the result can be beneficial both for the environment and the growers. Pest control today often relies on pesticide application, an alternative that often is harmful to organisms besides the targeted insect. At farm level the pesticides also expose the farmer to toxic chemicals and require economic input in purchase, equipment, and labour.

In this thesis I have evaluated some of the anticipated benefits and risks with a transgenic oilseed rape that express a protein from pea (*Pisum sativum*), pea seed lectin (PSL). This plant material was the first product of an effort to develop transgenic oilseed rape cultivars with resistance to an important insect pest in northern Europe, the pollen beetle (*Meligethes aeneus* Fab.). However, the commercial aspect was abandoned and the plant material is here used as model for testing issues related to a transgenic insect resistant crop.

I will begin by describing the domestication and breeding of plants that resulted in the crops we have today, continuing with the benefit and risk approach to transgenic plants. I also present the history of the *Brassica* crop, pollen beetle ecology, crop damage, and current control of the insect pest. The introduction ends with a description of how the transgenic plants were developed and the initial studies of their resistance to pollen beetles (Melander et al., 2003). The benefits from the introduced trait with respect to negative effects on the pollen beetle had previously only been studied to a limited extent. The damage from the pollen beetle is mainly attributed to the hibernated adults and therefore it was necessary to determine the effect of the transgenes on this life stage (paper I), but to be able to draw any conclusions about the effects on the population level the entire life cycle had to be considered (paper I and II) and in order to evaluate the potential effects at population level several life history parameters had to be studied. In this thesis I summarize results from pollen beetle assays with PSL oilseed rape (paper I and II).

In the following section I give a short introduction to one of the most important beneficial insects, the honey bee (*Apis mellifera* (L.)). Because the foreign protein is only expressed in the pollen of the plant, the number of species that would be

exposed to the transgenic protein is rather limited. However, honey bees also feed on pollen and any negative impact on this pollinator would be devastating. Here I present the effects found on bee larvae feeding on pollen from the transgenic oilseed rape (paper III).

The next section deals with the plant material and its potential increase in competitive ability from the traits introduced by transformation. Insect resistance reducing the damage to the resistant plant could give it a competitive benefit in contrast to plants not carrying the resistance trait, which in turn could result in increased invasiveness. Several wild relatives to oilseed rape can be found in the vicinity of the crop fields in Europe, and some of these can most likely hybridise with the transgenic crop. Feral populations of plants originating from *Brassica* oilseed cultivation can also be found in and outside the fields. Therefore it was important to test the competitive ability of the transgenic plants in comparison with their conventional counterpart (paper IV).

Finally, application of plant biotechnology at farm level will depend on attitudes among the producers. Surveys on attitudes often ask what position a person takes, but often not why. Therefore I performed a survey among Swedish farmers on their perception of potential benefits and drawbacks connected to insect resistant transgenic crops (paper V).

Breeding for insect resistance

Domestication of plants

Evolution among plants, as all organisms, is driven by the selection for individuals with the highest fitness; the ability to produce as many fertile offspring as possible in that specific environment. Plants are forced to cope with UV-radiation, draught, flooding, herbivores, and diseases in competition with other plant species as well as their relatives. Evolution would not be possible without reoccurring genomic changes; altered gene sequences, expressions, functions, or chromosome numbers, creating new traits that give the plant an advantage over its competitors. Depending on if the mode of reproduction is crossbreeding, self-fertilization or vegetative, the trait may or may not be transferred to other plants and combined with the genes of the receiving plants.

The domestication of plants started long before Darwin and Mendel enlightened us about evolution and inheritance. As soon as humans started collecting seeds to be cultivated more than 10 000 years ago (Balter, 2007), the man-made evolution of crops began. Preparation of the soil, removal of weeds, draining, irrigation etc., have changed the conditions for natural selection and today, many domesticated are plants more or less dependent on humans for their survival (Hyams, 1971). Uniform ripening and shatter resistance are traits that facilitate harvest. In addition, reduced toxicity for consumption and resistance to pests and pathogens are preferable in crops and have been more or less consciously selected for during the history of plant breeding. Breeding against toxicity to humans and livestock

and at the same time for resistance to pests can cause conflicts because many of the substances toxic or repellent to pests, such as alkaloids, have the same effect on humans. Selection against such traits often results in a crop more sensitive to pests and an increased need for pesticide application.

Plant defence

Resistance to insects can be defined as inherited plant characteristics that reduce the effect of insect attack compared to susceptible plants that are more severely damaged (Smith, 1989). The less damaged plant might be avoided by the insect (antixenosis) or be less suitable as a host (antibiosis). A third way to withstand pest attacks is tolerance. An ideal resistance is a combination of all three mechanisms where tolerance is most desirable since no pressure is put on the insect to adapt (Smith, 1989). For crops in modern agriculture, antixenosis may be insufficient since this may depend on what other choices the insects have, and several hectares of monoculture may force the insect to choose the less preferred host for feeding or oviposition. Large areas of susceptible host crops also increase the population growth of the pest if it is not hampered by natural enemies or other pest control methods.

Plants are protected against insect pests by both physiological and chemical traits. Thick tissues and epidermis, spines, thorns, and hairs, in some cases with glands, can prevent herbivory by insects and secondary metabolites, allelochemicals, can be toxic, deterrent or repellent to the herbivore (Dethier, 1970) and/or attract natural enemies to the pest (Dicke, van Poecke & de Boer, 2003). Both chemical and physical defence can be constitutively expressed or induced by herbivory, in the whole plant or restricted to specific plant parts.

Improving insect resistance in plants using biotechnology

It is often difficult to find resistance to insects in cultivars. Often one has to use wild relatives, and if the resistance is a quantitative trait (affected by many genes) crossings and back crossings to transfer the desired genes is very resource demanding. Less desirable genes may also be linked with the gene of interest and therefore difficult to get rid of by crossings and selections. Development in plant biotechnology has enabled not only selection of single genes but also the possibility to transfer genes from unrelated plants, other organisms, or even synthetic genes, to the genome of a crop plant. For a gene to be translated, a promoter is needed that is recognised by the transformed crop plant's RNA polymerases. The selection of promoter enables gene expression to be restricted to specific tissues of the plant. The gene construct also needs to contain a selectable marker that makes it possible to select successfully transformed plants. There are two major techniques to make the transfer, one biological using the bacterium *Agrobacterium tumefaciens* as a vector (Gheysen et al., 1985). Another, physical, is to use a "genegun" where micro particles coated with the gene construct are shot into living cells (Klein et al., 1987).

The first mission in the transformation procedure is to find a potential resistance factor and to isolate the gene(s) coding for it. Several plant proteins have been

tested as potential resistance factors to be used against insect pests; for example proteinase inhibitors (PIs) and lectins (Carlini & Grossi-de-Sá, 2002). But the only insect resistant (IR) transgene crops grown commercially today are plant varieties of maize and cotton resistant to insect pests by the transformation of genes coding for δ -endotoxin from the bacterium *Bacillus thuringiensis*. There are several hundreds of strains of *B. thuringiensis* coding for different δ -endotoxins, which are highly specific toxins to certain insect groups but harmless to mammals (Betz, Hammond & Fuchs, 2000).

Global status of transgenic crops

From the first commercialization in 1996 the global area of biotech/genetically modified (GM) crops has increased to 102 million hectares in 2006 of which 32% were IR GM crops (James, 2006). 19% of the IR GM crops contained “stacked” IR and herbicide tolerance genes, making the plant resistant both to insect pests and an herbicide. USA accounts for more than half of the acreage of transgenic crops (54 million ha), followed by Argentina (18 million ha), Brazil (11.5 million ha), Canada (6.1 million ha), India (3.8 million ha), and China (3.5 million ha) (James, 2006). The first commercial planting of GM crops in Europe took place in 1998 in Spain, which still is the major grower in the EU. However, the 65 000 ha planted with GM crops in Europe in 2006 (Brookes, 2007) make up less than 0.1% of the global area planted with transgenes.

Risk/ benefit assessments

A risk can be defined as the likelihood of an event multiplied by (negative) impact of that event (Conner, Glare & Nap, 2003). When addressing the introduction of a transgenic crop, risks and benefits have to include both human health and environmental effects. The countries that grow GM crops have different frameworks regulating the release, growing and monitoring of transgenic crops emphasising potential risks for humans and environment. However, the philosophy behind the regulation can differ; New Zealand and EU demand extensive information, focusing on the plant development process. This is in contrast to, for example, USA and Canada that focus more on the product (Conner, Glare & Nap, 2003).

The transgenic plant needs to be characterized, and the features by which the transgene differs from its corresponding conventional variety determined. A distinction has to be made between traits that occur in native plants and novel ones, or traits from exotic plants or other organisms. Although a trait is found in a native plant, expressing it in another plant species might expose new organisms to the novel trait. Also, the level of the potential toxin might be higher in the transgenic plant and expressed in other tissue than in the original plant species. Different transformation events might lead to different levels of gene expression or affect the expression of other genes. Therefore all transgenic crops have to be approved on a case by case basis (EU, 2001). Environmental as well as

agricultural benefits have to also be addressed, the latter will be dealt with in the section concerning the grower.

Toxicity to non-target organisms

Transgenes that are to be used for human and livestock food obviously have to be evaluated for potential toxicity to the same, but also the toxicity to other organisms that might come in contact with the plant, directly or indirectly, has to be evaluated. Risk of direct effects on non-target organisms depends on the level of exposure to the novel gene product i.e. if the organism feeds on the plant tissue where the transgene is expressed, and the potential hazard or toxicity of the same. The level of gene expression is also important for potential toxicity. The toxin can also be passed on in the food web to predators of the plant herbivores (Birch et al., 1999) and parasitoids (Romeis, Babendreier & Wäckers, 2003). The toxin might also end up in the environment if it persists in the soil after plant material or insects that ingested the toxin decay (Groot & Dicke, 2002). Plant proteins might also end up in the soil through root exudates which can affect soil bacteria, protozoa, nematodes, or earthworms (Groot & Dicke, 2002).

In the transgenic oilseed rape tested in my study, the expression of the pea lectin was restricted to the anthers and pollen and thereby only organisms feeding on those plant parts would be directly exposed to the novel protein. Oil from the seeds is the only part of the plant that is used for human consumption today and does not contain much protein, so even if the lectin were to be expressed in the whole plant the level of lectin in the oil would be very low. The by-product from oil extraction is, however, fed to livestock, which then would be exposed to the transgenic protein. However, the protein comes from a plant used as both human and animal feed, the garden pea, where it occurs at a concentration of about 2% of seed protein (Edwards et al., 1991) which is more than twice the levels found in the pollen of the studied oilseed rape (paper I). The only way that the pea lectin will end up in the environment is through the rapeseed pollen, but a much more important contribution to the environment should be from seeds of the pea plant, the origin of the transferred lectin.

Gene introgression

The advantage that a transgenic plant would have over other plants by the new added trait has also been widely debated. Concern has been expressed for the creation of new weedy plants, the risk of the transformed plants crossing with wild relatives, and that plants with the transformed trait could out-compete native populations (Ellstrand, Prentice & Hancock, 1999). Crop plants in general are rather poor at competing outside the fields; actually they are not strong competitors in the field either, which is the reason for the use of herbicides and tillage. But weedy plants that are the product of natural hybridization between a crop and their wild relatives do exist (Ellstrand, Prentice & Hancock, 1999) and this problem is not exclusive for transgenic plants.

For outcrossing to occur, the plants must be at least partially cross pollinated, flower at the same time, and be related in such way that they produce fertile

offspring (Conner, Glare & Nap, 2003). For a wild plant to gain increased fitness from crossing with the crop plant, traits that will improve the offspring's total fitness outside the field are required, or the gene has to be closely situated to other beneficial genes in the genome (Chapman & Burke, 2006). However, most of the crops traits will lower the fitness of the first hybrid generation. If conditions are good though, the offspring might survive to reproduce, this time back crossing with the wild plants and thereby regaining some of the traits needed to succeed in the wild. Selection pressure will then favour individuals that have the most favourable traits, of which one might be the transformed one. If this is at all probable, one has to address what the consequences would be. Plants with the gene would only benefit if the targeted herbivores are restricting the plant population, or if other organisms on that plant are affected by the trait.

Concerns have been raised regarding the risk of horizontal gene transfer (i.e. outside the reproductive process, across species barriers) of transgenic DNA or marker genes from the vector (Kleter, Peijnenburg & Aarts, 2005; Weaver & Morris, 2005). Whether or not transgenic DNA is more (or less) likely to be horizontally transferred than non-transgenic DNA has not been determined.

Resistance development

The agricultural success of *Bt* is unquestionable but irrespective of GM or conventionally bred resistance, the reliance on just one resistance factor makes it likely that resistance will be of short duration. Eventually, somewhere, there will be individuals that overcome the resistance. Precautions are taken to prevent pests overcoming plant resistance, for example by gene stacking, susceptible crop refuges and rotations of crops and insecticides.

Additional effects of gene transfer

The transformation process, tissue culturing, and gene inserts can have effects on other genes in the plant genome (Latham, Wilson & Steinbrecher, 2006). Changes in plant phenotype due to the transformation might affect the attractiveness and nutritional value of the plant which is why the transgene is screened for nutritional and known secondary compounds for comparison with the non-transgenic counterpart (EU, 2003). The transformed gene might also behave differently in its new environment (Prescott et al., 2005), such alteration could, however, also occur by natural mutations. When a gene is expressed in the new genome it may affect the expression of other genes, interact with those genes or function differently due to post-translational processes compared to when expressed in the organism of origin (Prescott et al., 2005).

Environmental benefits

Potential benefits must be identified in order to weigh them against the risks posed. The major environmental benefit from insect resistant crops is the reduction in insecticide application. Many insecticides persist in the environment affecting many other organisms and sometimes also end up in our food and water.

Reduction in insect diversity and natural enemies of the insect pest makes pest control even more dependent on insecticides. A positive side effect of reduced insecticide application is reduction in CO₂ emission from less machinery use (Brookes & Barfoot, 2006). In some areas the growers experienced less fungal disease in the *Bt* crops which may also lead to fewer fungicide applications (Brookes, 2007).

The plant and its pests

Brassica history

The oldest and most widespread Brassica crop is turnip rape (*Brassica rapa* syn. *B. campestris*), which grows both wild and cultivated in Europe and Asia. Both south-east Asia and south-west Europe have been suggested as sites of origin for the genus (Baranyk & Fábry, 1999). Seeds dating back to the Bronze Age have been found (Baranyk & Fábry, 1999), and rapeseed (*B. rapa* and *B. napus*) was cultivated in ancient Rome, mainly as green fodder (Fussel, 1955). During the 19th century rapeseed production increased in Sweden and peaked in 1866 when 3000 tons were produced (Andersson & Granhall, 1954). Cultivation then declined, probably due to access to imported mineral oil that could be used for lamps (Baranyk & Fábry, 1999) and other vegetable oils for margarine and soap production (Andersson & Granhall, 1954). The shortage of edible oils in Europe after World War II caused the production of oilseed crops to increase again (Appelqvist & Ohlson, 1972). Rapeseed cultivation was also adopted in Canada and later on in USA and Australia (Baranyk & Fábry, 1999).

Rapeseed breeding and cultivation

Breeding of oilseed crops has, apart from an increased yield, focused on glucosinolate and erucic acid content, resistance against pathogens and pests, frost resistance (Baranyk & Fábry, 1999; Meyer, 1997), and lately also on transgenic herbicide tolerance (Mazur & Falco, 1989). Today oilseed rape is grown for the oil which is used in food production, and for technical applications such as fuel and lubricants. A by-product is a protein rich meal which is used in fodder. Cultivation of rapeseed reached over 25 million ha globally in 2006 with India as the largest grower (7.3 million ha), followed by China (6.7 million ha), Canada (5.3 million ha) and Europe (4.7 million ha) (FAOSTAT, 2007). In 2006 transgenic rapeseed was grown on 4.8 million ha, but the only commercially available varieties are transformed for herbicide tolerance and are restricted to Canada and USA (GMO Compass, 2007). In Sweden rapeseed cultivation has shown an increase during recent years (SJV, 2006) and was, in 2006, grown on 90 760 ha (FAOSTAT, 2007), which accounts for about 3 % of the arable land (not including farms with less than 2 hectares). In contrast to the general decline in herbicide and fungicide use in Sweden, insecticide use increased by over 20% (active substance) from 1998 to 2006. The increased insecticide use was mostly due to increased use in peas and oilseed rape (SCB, 2007).

Brassica pests

A large number of insect pests cause damage to oilseed rape. For example: the seed weevil *Ceutorhynchus assimilis* (Payk.) (Free & Williams, 1978; Gould, 1975), the pod-infesting gall midge *Dasineura brassicae* Winn. (Gould, 1975), the aphid *Brevicoryne brassicae* L. and flea beetles of the genera *Phyllotreta* and *Psylliodes*. But the most severe pest in oilseed rape in northern Europe is the pollen beetle (*Meligethes aeneus* F.) (Alford, Nilsson & Ulber, 2003) which can cause up to 70% yield loss (Nilsson, 1987) and accounts for the main insecticide use in rapeseed in Sweden.

No insect resistant oilseed rape variety has been marketed although efforts have been made to breed for resistance against the aphid *Lipaphis erysimi* Kalt. (Sekhon & Åhman, 1992) and the flea beetle *Phyllotreta* spp. (Lamb 1989). In a search for resistance sources, more than 90 populations of spring oilseed rape were screened for insect resistance in south Sweden. Even though a couple of the most promising populations gave higher yields than standard varieties when no insecticide was applied, the highest yielding population only gave 36% of the yield compared to when insecticides were applied (Åhman, 1993).

The major pest; the pollen beetle

The dominate pollen beetle species in oilseed rape in Sweden is *M. aeneus* followed by *M. viridescens* (Karltopf & Nilsson, 1981). Among the 489 beetles that were examined in my study, not a single *M. viridescens* was found. Neither in the studies by Nilsson, was there any species other than *M. aeneus* detected in the same geographic area (Nilsson, 1994). *Meligethes aeneus* will hereafter be referred to as the pollen beetle.

Life cycle

The pollen beetle is univoltine (one generation per year) and hibernates as an adult (Fig. 1). As the name implies, the pollen beetles mainly feed on pollen, in the fall and early spring in a variety of plants. Later in spring they move to Brassica plants to feed and reproduce, and the eggs are laid in small clusters preferably in 2-3 mm large buds through beetle-made holes (Ekbom & Borg, 1996). Egg production can last up to 2 months (Fritzsche, 1957), or as long as plants suitable for oviposition are present (Hopkins & Ekbom, 1996), which can result in a production of several hundreds of eggs per female (Fritzsche, 1957).

Plant damage

The most severe damage to the crop occurs before the flowers are open when the beetles feed on the small buds, and if many eggs are laid in the same bud the larvae might also damage the ovaries of the flower. The plant can compensate for bud damage (Gould, 1975), or even overcompensate, by the production of side branches (Tatchell, 1983). But this makes harvest difficult because seeds on side branches ripen later than those on the main shoot. Pollen beetles do not normally

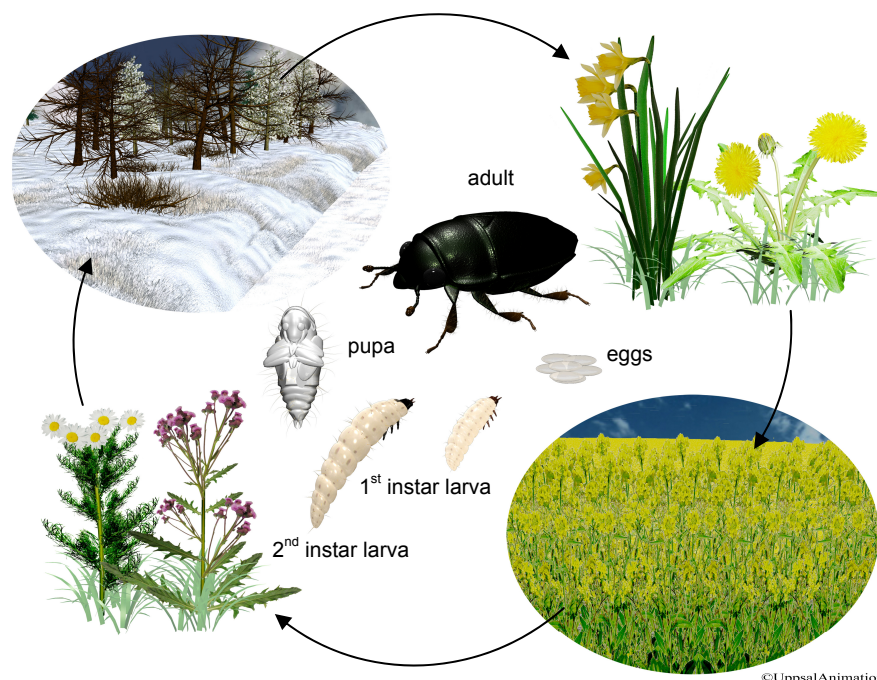


Fig. 1. The pollen beetle life cycle: The adults emerge in spring and feed on spring flowers before emigrating to Brassica plants to feed, mate and oviposit. Eggs are laid in the buds and the 1st instar larvae feed in the developing flower. The 2nd instar larvae continue to feed in the open flowers before dropping to the ground for pupation in the soil. The eclosing adults feed on a variety of flowers before overwintering in the top soil or leaf litter, usually in a forest nearby. (Graphics by UppsalAnimation©)

fly when the temperature is below 14°C (Fritzsche, 1957) which usually means that winter rape already has begun to flower before the beetles move to the crop, but spring rape flowers later and can suffer severely when the beetles move to those fields.

Control history

In the absence of rapeseed crops, the pollen beetle is restricted to its natural host plants; cruciferous weeds; and reproductive success is dramatically increased as soon as cultivation of Brassica is intensified (Hokkanen, 2000). During the 19th century damage was occasionally so severe in Sweden that the crop could only be used as green fodder or had to be ploughed under (Lampa, 1893 in Nilsson, 1994). The first efficient pest control became available after the Second World War in the form of DDT, which was replaced by organic phosphates (OP) in the 1960s, and at the same time control thresholds were adopted to minimize insecticide use (Nilsson, 1994). In the early 1980s the less noxious pyrethroids replaced the OP's. However, the extensive use of pyrethroids has resulted in development of resistance in pollen beetles in several countries (Derron et al., 2004; Ekbom & Kuusk, 2001; Hansen, 2003). In Sweden, farmers in such areas are granted permission to use OP's. From 2007 Swedish farmers are also allowed to spray with neonicotinides, which are harmless for mammals but the recommendation is

to alternate between these substances to prevent or delay further development of resistance. The economic threshold for spraying is close to the most favourable density for the beetles to maximize their offspring, and thereby an unwanted side effect of chemical protection is a high pest population in the following year (Hokkanen, 2000; Veromann et al., 2007).

The making of a transgene

Finding the gene

In order to find a resistance factor that could be transformed into oilseed rape several lectins, proteinase inhibitors (PIs) and patatin were tested in a feeding assay using pollen beetle larvae and two lectins also on adults (Åhman & Melander, 2003). The Concanavalin A (Con A) lectin from jackbean (*Canavalia ensiformis* L.) caused the highest larval mortality of the tested plant proteins. The problem with Con A is that it can be toxic to mammals, which makes the protein unsuitable for transformation of crops used for food and fodder. Lectins are a wide and heterogenic group of proteins with the common characteristic that they bind reversibly to carbohydrates (Van Damme et al., 1998). In plants the lectins are suggested to function as storage proteins, defence against herbivory and/or nodulation of root hairs (Kijne, 1996). Function and toxicity of lectins towards insects is highly variable and seems to be rather specific depending on the lectin and insect species combination (Murdock & Shade, 2002), even within the same insect order (Sétamou et al., 2002). Con A binds to mannose and glucose a feature shared with the pea (*Pisum sativum*) lectin (PSL) (Strosberg et al., 1986), however PSL is harmless to mammals (Grant, 1989). The posttranslational process of Con A is also more complex than that of the PSL, which might lead to changed protein structure when expressed in a different organism, and therefore the PSL was chosen for further testing (Melander et al., 2003). When pollen beetle larvae were fed oilseed rape anthers soaked in 1% solution of PSL, survival was reduced by 84% and the body mass by 79%. These results encouraged development of transgenic oilseed rape expressing the PSL (Melander et al., 2003).

Introduction of the gene and plant line production

The pollen specific promoter of the Sta44-4 gene from *B. napus* was combined with the PSL gene isolated from pea. This promoter was chosen in order to restrict the expression of the lectin gene to the developing pollen in anthers, which should minimize the impact of the transgene on both the plant and non-target organisms. The Sta44-4-pea lectin gene complex was then ligated with the NPTII marker gene (kanamycin resistance) to enable selection of transformed plants. The final construct was transferred to the spring rape cultivar Westar using *A. tumefaciens* as a carrier (Melander et al., 2003).

Self pollination of transformed plants (T_0) and their offspring (T_1) gave a second generation of inbred plants (T_2) and their anthers were analyzed for PSL content.

Twenty of the primary transformants were tested in a feeding assay on pollen beetle larvae; a slight reduction in survival and up to 46% weight reduction was recorded (Melander et al., 2003). A negative correlation between lectin concentration in anthers and larval weight was detected. In order to reduce the risk of segregation for transgeny in following generations, doubled haploid (DH) plants were generated from microspores (from T₃ plants) (Åhman et al., 2006). Plants from five transformation events were selected as microspore donors, of which two did not contain any PSL. Those zero plants were expected to be non-transgenic, since their ancestors were negative for the NPTII gene construct when tested in generations T₁ and T₂. The T₂ generation of the transgenic plant lines had been tested in a feeding assay with pollen beetle larvae that resulted in a reduction in larval growth by 13-24%. (Melander et al., 2003). Among the 171 DH lines produced, five plant lines (one from each transformation event) were selected for further testing (Åhman et al., 2006).

Transgene assays

Lectin quantification

Anthers from each of the five DH lines, designated T-17, T-83, T-132 (transgenes) and C-101 and C-112 (controls), used in my studies were sampled in 2004 for PSL quantification. The pollen specific promoter used in the construct results in gene expression late in the pollen development (Hong et al., 1997). Therefore both buds and recently opened flowers were selected to determine if there was any difference in PSL expression in those two stages of developing anthers, but no such differences were found (paper I). The PSL concentration in the anthers was 0.2, 0.6 and 0.7% (of total soluble protein) in T-83, T-132 and T-17 respectively. No lectin could be detected in the anthers of the control lines (paper I).

Pollen beetle assays

The initial test of PSL-expressing oilseed rape on pollen beetle larvae showed a negative effect; a reduction in body mass (Melander et al., 2003). A low adult weight, which could be the result of low larval weight, has been shown to reduce the beetle's winter survival rate (Hokkanen, 1993). The adult stage is also the most damaging to the rapeseed crop and therefore the effect of PSL on adults was of particular interest. Additionally, even though a substance is not acutely toxic, small effects on various life history parameters can lead to a decline of the population (Larsson, Ekbohm & Björkman, 2000; Sétamou et al., 2003). To be able to calculate the potential effects of the transgene on pollen beetles at the population level, effects of the five selected plant lines, were tested on all life stages of the pollen beetle.

Adults

Beetles were collected as soon as they emerged after hibernation and stored cold until used in the different tests. The effect on adult beetles when fed only anthers (where the PSL was expressed), was tested by controlling the weight change and feeding for individual beetles every third day over a three week period (paper I). No significant difference in feeding, weight or survival could be detected between the plant lines.

Oviposition was tested by first isolating groups of beetles in cages with one of the five plant lines to allow them to feed and mate (paper I). The beetles were then weighed and individually isolated on racemes, from the same plant as the pre-treatment, for 10 days. Every second day the beetles were moved to a new raceme and the number and length of the eggs laid in the buds recorded. No difference in insect mortality between the plant lines was found, neither could any difference in number of eggs laid be detected. However, the egg sizes tended to increase for beetles feeding on the control lines but were unchanged or decreasing for the transgenic lines. Egg size is known to be reduced on suboptimal hosts (Ekbohm & Popov, 2004) and it would have been interesting to continue to follow the females until they ceased to oviposit to see if egg size continued to diverge. From a resistance point of view egg size was of less interest since no correlation between egg size and survival, weight or development time was found in the following study on larval development (paper II). Females lost more weight than males during the raceme test. This is probably due to oviposition reducing the weight, which was supported by the fact that no difference in weight change could be detected in the anther study where the females had no opportunity to lay eggs. However, there was no correlation between weight loss and number of eggs laid.

Larvae

To obtain eggs for tests of larval development and survival (paper II) adults were isolated on the different plant lines in cages in order for them to feed and mate and then isolated smaller groups on individual plants for 24 h. Eggs were then dissected from the buds and the developing larvae were continuously fed anthers from the test plants. Hatching, development time until adult, larval weight and survival were recorded. The only difference in larval weight was found between C-112 and T-132; larvae feeding on C-112 weighed significantly more. The main effect was found on survival until pupation where larvae on all the transgenic lines had significantly higher mortality compared to the control lines (paper II).

Adult offspring

Groups of beetles from the previously described pre-treatment cages were isolated on several plants for 24 h. The emerging larvae fed on the same plant before pupating in the pot soil, and eclosing beetles were collected and weighed. This test was performed both in 2004 and 2005 and gave somewhat contradictory results; beetles developed on T-132 had the lowest mean weight in 2004 but the highest in 2005 (paper II). There was no consistent difference in weight between beetles from the group of transgenic and the group of control lines, instead a significant

difference was found between the two control lines in 2005. The result implies that there might be some other plant quality affecting offspring weights, which will be discussed below.

Overwintering

I also wished to follow beetles developed on the transgenes throughout the following winter and during the winters 2003-2006 I tested winter survival. Initially (2003-2004) I tested survival in groups divided by weight among beetles collected in the autumn. The proportion of survivors was higher in the groups with heavier beetles and the average weight among the hibernated beetles only increased among the small beetles. These results are in accordance with the results from the hibernation study by Hokkanen (1993) where the mean average weight among the pollen beetles increased from autumn to spring.

The following two years beetles produced in the test for adult weights were overwintered, but it was only in the second year any beetles survived to the following spring (paper II). The less successful hibernations during those last two years might be due to colder winters, but the 2.4% survivors collected in the spring in 2006 is within the range of the 2-15% survival recorded by Hokkanen (1993). The fact that no surviving beetles were found the previous spring might be due to the lower number of beetles tested that year (paper II). One might argue that the low number of surviving test beetles depends on the more or less artificial rearing, compared to beetles collected in the field, but in both years survival of the reared beetles was compared with survival of beetles collected outdoors a couple of weeks before the pots were placed in the ground, and mortality was equally high.

Population effects

Melander et al. (2003) suggested that there is a dose response to PSL in pollen beetle larvae, something I did not find clear evidence for in my tests. The lectin content in T-83 was an intermediate between control plants and the high PSL lines but not the results, something that would be expected if the effect was dependent on the level of PSL. Even though open pollinated rapeseed varieties are produced to be as uniform as possible to start with, they are not clones and also, during subsequent propagation steps individual plants may become more and more different from one another via crossings. Unfortunately, I had no control over the genetic uniformity of the seed lot of Westar used for the transformations. Furthermore, the transformation and tissue culturing might also have altered or disturbed the expression of other genes in the plants (Latham, Wilson & Steinbrecher, 2006), affecting the pollen beetle in unpredictable ways.

All parameters measured are summarized in table 1, and the results from the five plant lines are ranked according to beetle performance. When summarizing the effects shown in the table, certain patterns for transgenic and non-transgenic lines emerge, but far from all measured parameters differ significantly and therefore the ranking should be interpreted with caution. However, even if only considering the parameters in which the plant lines differed significantly, the effects of the PSL

expressing oilseed rape would reduce the pollen beetle population by half compared to the control plants (paper II).

Table 1. Summary of parameters measured on the pollen beetle when fed the five plant lines (transgenes T-17, T-83, T-132, and control lines C-101 and C-112). The ranking of plant lines corresponds to the beetle performance; highest feeding, weight gain, and survival, highest number of eggs laid, largest eggs, and least weight loss, highest larval weight, shortest development time, and highest percentage of beetles surviving the winter are given rank one (lowest rank = five). Different letters indicate significant difference between plant lines ($P < 0.05$ for specific tests *see* paper I and II). Mean ranking of plant lines when considering all parameters measured

Adults - Anther assay			Adults - Raceme assay				
feeding	weight change ¹	survival	ovi-position	egg size	weight change ^f	weight change ^m	survival
T-83 ^a	C-101	C-101*	T-132	C-101 ^a	T-83	C-101	C-112
C-112 ^{ab}	C-112	C-112*	C-101	C-112 ^{ab}	C-112	T-132	T-83
T-17 ^{ab}	T-132	T-17*	C-112	T-17 ^{ab}	C-101	T-17	T-17
C-101 ^{ab}	T-83	T-83*	T-17	T-83 ^{ab}	T-17	T-83	C-101
T-132 ^b	T-17	T-132	T-83	T-132 ^b	T-132	C-112	T-132

Larvae			Adult weight		Over-wintering	Plant line	Mean ranking
weight	dev. time	survival to adult	2004	2005	2005-2006		
C-112 ^a	T-83 ^a	C-112 ^a	C-112 ^a	T-132 ^a	C-101	C-112	2.3
T-17 ^{ab}	C-101 ^{ab}	C-101 ^a	T-17 ^{ab}	C-112 ^a	T-83	C-101	2.5
C-101 ^{ab}	C-112 ^b	T-132 ^{ab}	T-83 ^{ab}	T-17 ^{ab}	T-132	T-83	3.0
T-83 ^{ab}	T-17 ^b	T-83 ^{ab}	C-101 ^{ab}	T-83 ^b	T-17	T-17	3.3
T-132 ^b	T-132 ^b	T-17 ^b	T-132 ^b	C-101 ^b	C-112	T-132	3.9

¹ both sexes, ^f females, ^m males, * same value

Honey bee assay

Which insects besides the targeted pollen beetle might be affected by the transformed plants? Because the expression of the PSL gene is restricted to the anthers and pollen in the plant, other pollen feeders would be the major organisms directly exposed to the protein by feeding. The honey bee is one of the most important beneficial insects in agriculture and many crop plants over the world are more or less dependent on bees for reproduction (Klein et al., 2007). Moreover, oilseed rape yield and oil content in seed have shown to be higher when the apiary is close to the field (Fries & Stark, 1983). With wild bee species declining insect pollinated plants are even more dependent on the honey bee (Kearns, Inouye & Waser, 1998; Westerkamp & Gottsberger, 2000) and in the pre-release tests of transgenic crops, the honey bee is one of the first insects to be tested.

Exposure

Bees are social insects and the worker honey bees collect pollen and nectar which is brought to the hive. If the novel gene is expressed in the whole plant the bees could be exposed to the transformed protein through the nectar, but the amino acid

(protein) levels in nectar are very low and it is through the pollen the bees would come into contact with the transgenic protein. The nursing bees in the hive feed on pollen, not only to provide themselves with protein but also to produce royal jelly, which is fed to the larvae (Crailsheim et al., 1992; Haydak, 1963) during the first 3-4 days in larval development (Crailsheim, 1990). Later on the brood food contains less protein and more sugar from nectar and also some pollen (Babendreier et al., 2004; Crailsheim, 1990). The exact composition of the food depends on whether the larvae are raised to become a drone, worker or a queen. It has been suggested, but so far not proven, that proteins could be transferred to the larvae through the royal jelly produced in the hypopharyngeal glands. Many lectins are stable proteins, insensitive to insect proteases and variations in pH (Peumans & Van Damme, 1995), and could hypothetically pass through bee digestion without degradation. In the young bee larvae the peritrophic membrane that lines the midgut epithelium is not fully developed (Davidson, 1970), which probably makes them more sensitive to harmful or toxic substances in the food.

Selection of test plants and protein quantification

Three plant lines from the initial tests on pollen beetles were selected using results from the pollen beetle assays; plant line T-132 had the most negative effect on the beetles while the control C-112 had the most positive effect. Additionally line T-17 was also tested because this plant line has a similar level of PSL as T-132 (paper I). The PSL quantification in the dried pollen showed higher PSL content compared to the freshly frozen anthers, which was probably due to lower total protein content because of protein degradation (paper III). No lectin could be detected in the control lines.

Feeding assay

I wanted to create a worst case scenario where the larvae would receive as much pollen as possible during the early stages of their development (paper III). To test a potential toxic protein such as PSL on bee larvae under natural and, at the same time, controlled conditions is difficult because sick and dead larvae would be removed by nurse bees several days before being detected by the human eye (Brødsgaard, Ritter & Hansen, 1998). Methods have therefore been developed to culture bee larvae in the lab. In my study newly hatched larvae were collected from a comb and gently placed on the surface of the food, which was a mix of sugar solution, royal jelly and pollen. The amount of pollen fed to the larvae in the hive changes during development and the exact amount eaten by the larvae may vary with the protein content of the pollen. Before testing the transgenic pollen I therefore examined how much oilseed rape pollen could be fed to the larvae without increasing their mortality (paper III). The optimal test would have included a gradual increase in pollen as larvae developed, since the second instar might cope with higher pollen levels. However, the levels at which the larval mortality increased was approximately the same when tested from day 4 and onward (Brødsgaard pers. comm.).

Diet without pollen was used as an extra control for possible negative effect of pollen in general. Significant differences were found between treatments.

However those differences were not between control and transgenic plants but between diet with and without pollen. Larvae fed diets containing pollen developed faster and weighed more than larvae fed the control diet, although the control diet is supposed to contain the nutrition needed for the larvae. The mortality was rather high in all diets compared to other studies on bee larvae (Aupinel et al., 2005; Genersch, Ashiralieva & Fries, 2005), which was probably due to handling of the larvae, but there was no difference in mortality between diets until pupation. Although only tested on larvae and with dried pollen, my results indicate that PSL at 1.2% of total soluble protein poses no hazard to the honey bee. Adult bees, especially the nursing bees, would eat more pollen compared to the larvae who receive the pollen in the brood food mix. Thus if a PSL containing crop variety were to be considered for commercial cultivation adult bees would also have to be tested.

Plant invasiveness

The invasiveness of a plant, its ability to spread to, establish and expand at new locations, depends on many factors such as mode of pollination, seed dispersal, competitive ability, and environment (Chapman & Burke, 2006; Conner, Glare & Nap, 2003). If wild relatives to the plant are growing in the range of pollen dispersal hybrids might form that could out-compete the native population.

In order to test if the oilseed rape had gained a higher competitive ability from the transgenic character in our case, a greenhouse study was performed where the transgenic plant lines were grown alone and in mixtures with the control lines, with and without bumblebees (*Bombus terrestris* (L.)), and with and without pollen beetles (paper IV). When the transgenic plants were grown in mixture with control plants, in the experiment with pollinators but without pollen beetles, they produced more viable seeds than when grown alone. There was an opposite trend for the control plants, which implies that there might be lower viability in the pollen from the transgenic plants. This might be due to the expression of the foreign protein affecting the pollen or that the transformation caused rearrangements in the genome (Forsbach et al., 2003). The PSL did not seem to give the plants any competitive benefit with respect to reduced pollen beetle damage. In fact, in the absence of bumblebees the pollen beetle had a positive effect on the plant yield, presumably due to increased self-pollination of the plant by the beetle.

The grower

Apart from the potential changes in plant ecology of the transgene there might also be changes in farming practices that have to be included in a risk/benefit assessment. There have been several benefits at farm level from growing IR GM crops. Reports from the first 10 years of commercial use of IR GM crops show economic benefits from decreased insecticide and machinery use, as well as

reduced labour costs including scouting. Reduced pesticide use means less damage to other organisms in the field and reduced exposure to the farmer who handles it.

At farm level the risk of GM crops crossing with non-GM crops can be important for both the GM farmer and the non-GM farmer. In the EU, products that contain more than 0.9% GM (unintentional mix) have to be labelled (EU, 2003). A non-GM farmer may receive a lower price if transgenes are mixed in his harvest at higher levels than 0.9% and thereby suffer economic loss.

Economic benefits

Of the 10.3 million farmers growing GM crops in 2006, 9.3 million were small, resource poor farmers in developing countries (James, 2006). Non governmental organisations (NGOs) often state that the only beneficiaries from GM-crops are the large seed companies. However, calculations show that, besides the seed companies, both consumers and growers have benefited economically from the application of the technology (Fernandez-Cornejo & Caswell, 2006). Among the growers in developing countries not only economic benefits are important. Due to poor application techniques and equipment they are often highly exposed to pesticides and thereby have much to gain in improved health from growing IR crops.

The expenses for GM seeds and technological fees need to be weighed up by increased yield or higher quality products and/or reduced production costs in order to be beneficial for the grower. Additionally, there must be retailers willing to buy the product. Other benefits such as less exposure to insecticides and reduced impact on beneficial insects and environment may also motivate a grower to select a GM crop.

No GM crops are grown for commercial use in Sweden so far, and the IR GM crop approved in the EU; *Bt* maize resistant to the European corn borer (*Ostrinia nubilalis*) and the Mediterranean stemborer (*Sesamia nonagroides*), is currently of no interest to Swedish farmers. Maize is, however, increasing as a crop in the south of Sweden and with a warmer climate one can expect an increase of pests on maize. With GM products beginning to enter the Swedish market and with the approval of GM soy-meal as feed in Swedish meat production, farmers already have to make choices about GM technology, despite the fact that they do not yet grow them in their fields.

Farmer survey

What do the Swedish farmers believe are the potential benefits and drawbacks if they were to grow IR GM crops? In a survey in 2005, Swedish farmers were asked first about their attitude towards GM crops in general, and then asked two questions where they selected statements about potential benefits and drawbacks of an IR GM crop (paper V). This meant that we could connect their general attitude to their perception of benefits and risks, and also get an idea about how selections of specific benefits were connected to perceived risks. A majority, 57%, was negative to GM crops but as many as 30% were neither positive nor negative.

A higher number of growers with large farms were surveyed than what is representative for Swedish farmers, and the response frequency was also higher among owners of large farms. Owners of large farms were more positive towards GM crops in general, which means that the actual percentage of farmers in Sweden that are negative to GM crops probably is higher than that in the survey (paper V). The difference in attitude between owners of small and large farms might be due to the fact that larger farms generally use more insecticides and therefore have more to gain from growing an IR GM crop. Furthermore, owners of large farms more often had higher education in agriculture which was correlated to a more positive attitude towards GM crops.

The major concern among all farmers in the survey was scepticism among consumers, which may result in an unwillingness to buy the product. Only 20% of Swedish consumers are willing to buy GM foods. However, more than twice as many would buy such food if the crop was grown in a more environmentally friendly way compared to conventional crops (Fjæstad, Olofsson & Öhman, 2003), something that the farmers might not be aware of.

The relation between attitude and selected statements reveal four groups of farmers with different concerns and expectations (paper V). A general positive attitude to GM crops was connected to potential for reduced damage to other organisms, reduced insecticide costs, and reduced health risk for the grower as well as concerns about more expensive seeds. The contrasting group saw no benefits from growing IR GM crops and believed that the crop could be dangerous for both humans and other organisms. A third group saw higher yields as the most probable benefit but were, at the same time, concerned about consumer attitudes to GM products. The fourth group (contrasting the third) was concerned about genes spreading from the GM crop to both wild relatives to the plant and to conventional crops. The farmers could also point out additional potential benefits and drawbacks that they found important in connection with the questions. The most frequently mentioned concern was the lack of knowledge about the technology. This might reflect why as many as 30% claimed to be neither positive nor negative to GM crops. The selection of statements by “neutral” farmers often coincided with the selection by positive farmers, which may be interpreted as many farmers acknowledging the benefits but at the same time experiencing high uncertainty about the potential risks.

Conclusions

I have developed a system for testing the effects of *Brassica* host characters on performance of pollen beetles and found that PSL oilseed rape can reduce the rate of population increase. The transgenic plants did not gain any competitive benefit by their negative effect on the pollen beetle; neither did the feeding assay with the honey bee larvae indicate any negative effects. All my assays were conducted under greenhouse and/or lab conditions. Although such studies provide essential

knowledge, it is not until field trials are performed that the full impact can be determined.

Furthermore, my studies show the importance of testing more than one transgenic and one control line to be able to draw conclusions about the effects of the transgene. It may be important to screen for alterations in the genome introduced by the transformation, doubled haploid production, or tissue culturing, and to control for possible genetic heterogeneity in the starting material for transformations. Also negative effects of the transgene on the plant, such as the reduced pollen quality need more attention.

The question is if we can find another toxin, as effective and precise as *Bt*, for the control of pests. We may have to accept some infestations in the field to make crop production as environmentally friendly as possible. Although pollen beetle larvae showed an increased mortality, the PSL oilseed rape by itself is probably not the single solution to the pollen beetle problem. If PSL oilseed rape were to be grown at larger scales it might, together with natural enemies, which under current pest control are hampered by insecticides (Veromann, Luik & Kevvää, 2006; Veromann et al., 2006), prevent rapid population growth of pollen beetles. This is, of course, contingent on there being no negative effects of the GM crop on the natural enemies. It should also be kept in mind that regardless of method used to control the pest, if the pest population is reduced, it will affect the biological community (Shelton, Zhao & Roush, 2002).

The goal for future agriculture should be to use farm practices that have as little environmental impact as possible for the attained yield. A sustainable agricultural practice should ideally implement all the available knowledge, and this might include the usage of transgenic crops. When decisions are to be made regarding new crop varieties, improved by gene transformation or through other plant breeding techniques, both risks and benefits need to be addressed, and be based on scientific knowledge.

References

- Alford, D.A., Nilsson, C. & Ulber, B. 2003. Insect pests of oilseed rape crops. In *Biocontrol of oilseed rape pests*. Edited by D.A. Alford. Blackwell Science. Oxford. 9-42. pp.
- Andersson, G. & Granhall, I. 1954. *Odling av olje- och spånadsväxter*. LT. Stockholm. 191 pp.
- Appelqvist, L.-Å. & Ohlson, R. 1972. *Rapeseed - cultivation, composition, processing and utilization*. Elsevier Publishing Co. New York. 391 pp.
- Aupinel, P., Fortini, D., Dufour, H., Tasei, J.-N., Michaud, B., Odoux, J.-F. & Pham-Delègue, M.-H. 2005. Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. *Bulletin of Insect Ecology* 58, 107-111.
- Babendreier, D., Kalberer, N., Romeis, J., Fluri, P. & Bigler, F. 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie* 35, 293-300.
- Balter, M. 2007. Seeking agriculture's ancient roots. *Science* 316, 1830-1835.

- Baranyk, P. & Fábry, A. 1999. History of the rapeseed (*Brassica napus* L.) growing and breeding from middle age Europe to Canberra. In *International Rapeseed Congress*, Edited by N. Wratten & P. Salisbury. Canberra: The Regional Institute Ltd.
- Betz, F.S., Hammond, B.G. & Fuchs, R.L. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regulatory Toxicology and Pharmacology* 32, 156-173.
- Birch, A.N.E., Geoghegan, I.E., Majerus, M.E.N., McNicol, J.W., Hackett, C.A., Gatehouse, A., M. R. & Gatehouse, J.A. 1999. Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. *Molecular Breeding* 5, 75-83.
- Brookes, G. (2007). The benefits of adopting genetically modified, insect resistant (Bt) maize in the European Union (EU): First results from 1998-2006 plantings. PG Economics Ltd.
- Brookes, G. & Barfoot, P. 2006. GM Crops: The first ten years - Global socio-economic and environmental impacts. *ISAAA Brief No. 36*
- Brødsgaard, C.J., Ritter, W. & Hansen, H. 1998. Response of in vitro reared honey bee larvae to various doses of *Paenibacillus larvae* spores. *Apidologie* 29, 569-578.
- Carlini, C.R. & Grossi-de-Sá, M.F. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon* 40, 1515-1539.
- Chapman, M.A. & Burke, J.M. 2006. Letting the gene out of the bottle: the population genetics of genetically modified crops. *New Phytologist* 170, 429-443.
- Conner, A.J., Glare, T.R. & Nap, J.-P. 2003. The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment. *The Plant Journal* 33, 19-46.
- Crailsheim, K. 1990. The protein balance of the honey bee worker. *Apidologie* 21, 417-429.
- Crailsheim, K., Schneider, L.H.W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R. & Schöffmann, B. 1992. Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function. *Journal of Insect Physiology* 38, 409-419.
- Dale, P.J., Clarke, B. & Fontes, E.M.G. 2002. Potential for the environmental impact of transgenic crops. *Nat Biotech* 20, 567-574.
- Davidson, E.W. 1970. Ultrastructure of peritrophic membrane development in larvae of the worker honey bee (*Apis mellifera*). *Journal of Invertebrate Pathology* 15, 451-454.
- Derron, J.O., LeClech, E., Bezençon, N. & Goy, G. 2004. Résistance des méligèthes du colza aux pyrèthrinoides dans le bassin lémanique. *Revue Suisse d'Agric* 36, 237-242.
- Dethier, V.G. 1970. Chemical interactions between plants and insects. In *Chemical Ecology*. Edited by E. Sondheimer & J.B. Simeone. Academic Press. New York. 83-102. pp.
- Dicke, M., van Poecke, R.M.P. & de Boer, J.G. 2003. Inducible indirect defence of plants: from mechanisms to ecological functions. *Basic and Applied Ecology* 4, 27-42.
- Edwards, G.A., Hopher, A., Clerk, S. & Boulter, D. 1991. Pea lectin is correctly processed, stable and active in leaves of transgenic potato plants. *Plant Molecular Biology* 17, 89-100.
- Ekbom, B. & Borg, A. 1996. Pollen beetle (*Meligethes aeneus*) oviposition and feeding preference on different host plant species. *Entomologia Experimentalis et Applicata* 78, 291-299.
- Ekbom, B. & Kuusk, A.-K. 2001. Rapsbaggar och resistens mot pyretrorider. *Växtskyddsnotiser* 65, 39-42.
- Ekbom, B. & Popov, S.Y.A. 2004. Host plant affects pollen beetle (*Meligethes aeneus*) egg size. *Physiological Entomology* 29, 118-122.
- Ellstrand, N.C., Prentice, H.C. & Hancock, J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30, 539-563.
- EU (2001). Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms and repealing Council directive 90/220/EEC. The European parliament and the council of the European Union

- EU (2003). Regulation (EC) No 1829/2003 of the European parliament and of the council of 22 September 2003 on genetically modified food and feed. The European parliament and the council of the European Union.
- FAOSTAT 2007. Food and agriculture organization of the United Nations. <http://faostat.fao.org/site/408/DesktopDefault.aspx?PageID=408>. Accessed 2007 August 27.
- Fernandez-Cornejo, J. & Caswell, M. (2006). The first decade of genetically engineered crops in the United States, *Economic information bulletin Number 11*. United States Department of Agriculture.
- Fjæstad, B., Olofsson, A. & Öhman, S. (2003). Svenskarna och gentekniken - Rapport från 2002 års Europabarometer om bioteknik. Mitthögskolan.
- Forsbach, A., Schubert, D., Lechtenberg, B., Gils, M. & Schmidt, R. 2003. A comprehensive characterization of single-copy T-DNA insertions in the *Arabidopsis thaliana* genome. *Plant Molecular Biology* 52, 161-176.
- Free, J.B. & Williams, I.H. 1978. A survey of the damage caused to crops of oil-seed rape (*Brassica napus* L.) by insect pests in south-central England and their effect on seed yield. *Journal of Agricultural Science* 90, 417-424.
- Fries, I. & Stark, J. 1983. Measuring the importance of honeybees in rape seed production. *Journal of Apicultural Research* 22, 272-276.
- Fritzsche, R. 1957. Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Zeitschrift für angewandte Entomologie* 40, 222-280.
- Fussel, G.E. 1955. History of cole (*Brassica* sp.). *Nature* 176, 48-51.
- Genersch, E., Ashiralieva, A. & Fries, I. 2005. Strain- and genotype-specific differences in virulence of *Paenibacillus larvae* subsp *larvae*, a bacterial pathogen causing American foulbrood disease in honeybees. *Applied and Environmental Microbiology* 71, 7551-7555.
- GMO Compass 2007. GM Crop Production: Global Cultivations Exceed 100 Million Hectares in 2006. http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/257.global_gm_planting_2006.html. Accessed 2007 August 27.
- Gould, H.J. 1975. Surveys of pest incidence on oil-seed rape in south central England. *Annals of Applied Biology* 79
- Grant, G. 1989. Anti-nutritional effects of dietary lectins. *Aspects of applied biology* 19, 51-74.
- Groot, A.T. & Dicke, M. 2002. Insect-resistant transgenic plants in a multi-trophic context. *Plant Journal* 31, 387-406.
- Hansen, L.M. 2003. Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. *Pest Management Science* 59, 1057-1059.
- Haydak, M.H. 1963. Influence of storage on the nutritive value of pollen for brood rearing by honeybees. *Journal of Apicultural Research* 2, 105-107.
- Hokkanen, H.M.T. 1993. Overwintering survival and spring emergence in *Meligethes aeneus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. *Entomologia Experimentalis et Applicata* 67, 241-246.
- Hokkanen, H.M.T. 2000. The making of a pest: recruitment of *Meligethes aeneus* onto oilseed Brassicas. *Entomologia Experimentalis et Applicata* 95, 141.
- Hong, H.P., Gerster, J.L., Datla, R.S.S., Albani, D., Scoles, G., Keller, W. & Robert, L.S. 1997. The promoter of a *Brassica napus* polygalacturonase gene directs pollen expression of β -glucuronidase in transgenic Brassica plants. *Plant Cell Reports* 16, 373-378.
- Hopkins, R.J. & Ekbohm, B. 1996. Low oviposition stimuli reduce egg production in the pollen beetle, *Meligethes aeneus*. *Physiological Entomology* 21, 118-122.
- Hyams, E. 1971. *Plant in the service of man: 10,000 years of domestication*. J.M. Dent & Sons Ltd. London. 222 pp.
- James, C. (2006). Global status of commercialized biotech/GM crops: 2006. *ISAAA Brief* No. 35. ISAAA: Ithaca, NY. ISAAA.
- Karltorp, M. & Nilsson, C. 1981. Rapsbaggar i mellansvenska våroljevåxtodlingar. *Växtskyddsnotiser* 45, 146-154.

- Kearns, C.A., Inouye, D.W. & Waser, N.M. 1998. Endangered mutualism: The Conservation of plant-pollinator Interactions. *Annual Review of Ecology and Systematics* 29, 83-112.
- Kijne, J.W. 1996. Function of plant lectins. *Chemtracts - Biochemistry and molecular biology* 6, 180-187.
- Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274, 303-313.
- Klein, T.M., Wolf, E.D., Wu, R. & Sanford, J.C. 1987. High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature* 327, 70-73.
- Kleter, G.A., Peijnenburg, A.A.C.M. & Aarts, H.J.M. 2005. Health considerations regarding horizontal transfer of microbial transgenes present in genetically modified crops. *Journal of Biomedicine and Biotechnology* 2005, 326-352.
- Larsson, S., Ekbom, B. & Björkman, C. 2000. Influence of plant quality on pine sawfly population dynamics. *Oikos* 89, 440-450.
- Latham, J., Wilson, A.K. & Steinbrecher, R.A. 2006. The Mutational Consequences of Plant Transformation. *Journal of Biomedicine and Biotechnology* 2006, Article ID 25376, 25377 pages.
- Mazur, B.J. & Falco, S.C. 1989. The Development of Herbicide Resistant Crops. *Annual Review of Plant Physiology and Plant Molecular Biology* 40, 441-470.
- Melander, M., Åhman, I., Kamnert, I. & Strömdahl, A.-C. 2003. Pea Lectin Expressed Transgenically in Oilseed Rape Reduces Growth Rate of Pollen Beetle Larvae. *Transgenic Research* 12, 555-567.
- Meyer, J. 1997. Olje- och spånadsväxter. In *Den svenska växtförädlingens historia: jordbruksväxternas utveckling sedan 1880-talet*. Edited by G. Olsson. Kungliga Skogs- och lantbruksakademien. Stockholm. 241-252. pp.
- Murdock, L.L. & Shade, R.E. 2002. Lectins and Protease Inhibitors as Plant Defenses against Insects. *Journal of Agricultural and Food Chemistry* 50, 6605 -6611.
- Nap, J.-P., Metz, P.L.J., Escaler, M. & Conner, A.J. 2003. The release of genetically modified crops into the environment. Part I. Overview of current status and regulations. *The Plant Journal* 33, 1-18.
- Nilsson, C. 1987. Yield losses in summer rape caused by pollen beetles (*Meligethes spp.*). *Swedish Journal of Agricultural Research* 17, 105-111.
- Nilsson, C. (1994). *Pollen beetles (Meligethes spp) in oilseed rape crops (Brassica napus L.): Biological interactions and crop losses*, Swedish University of Agricultural Sciences.
- Peumans, W.J. & Van Damme, E.J.M. 1995. Lectins as Plant Defense Proteins. *Plant Physiology* 109, 347-352.
- Prescott, V.E., Campbell, P.M., Moore, A., Mattes, J., Rothenberg, M.E., Foster, P.S., Higgins, T.J.V. & Hogan, S.P. 2005. Transgenic Expression of Bean α -Amylase Inhibitor in Peas Results in Altered Structure and Immunogenicity. *Journal of Agricultural and Food Chemistry* 53, 9023-9030.
- Romeis, J., Babendreier, D. & Wäckers, F., L. 2003. Consumption of snowdrop lectin (*Galanthus nivalis* agglutinin) causes direct effects on adult parasitic wasps. *Oecologia* 134, 528.
- SCB (2007). Plant protection products in agriculture and horticulture. Use in crops. Statistics Sweden.
- Sekhon, B.S. & Åhman, I. 1992. Insect resistance with a special reference to mustard aphid. In *Breeding Oilseed Brassicas*. Edited by K.S. Labana, S.S. Banga & S.K. Banga. Narosa publishing house. New Delhi. 206-221. pp.
- Sétamou, M., Bernal, J.S., Legaspi, J.C., Mirkov, T.E. & Legaspi Jr, B.C. 2002. Evaluation of lectin-expressing transgenic sugarcane against stalkborers (Lepidoptera: Pyralidae): Effects on life history parameters. *Journal of Economic Entomology* 95, 469-477.
- Sétamou, M., Bernal, J.S., Mirkov, T.E. & Legaspi, J.C. 2003. Effects of snowdrop lectin on mexican rice borer (Lepidoptera: Pyralidae) life history parameters. *Journal of Economic Entomology* 96, 950-956.

- Shelton, A.M., Zhao, J.Z. & Roush, R.T. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annual Review of Entomology* 47, 845-881.
- SJV (2006). Yearbook of Agricultural Statistics. Swedish Board of Agriculture.
- Smith, C.M. 1989. *Plant resistance to insects: a fundamental approach*. Wiley. New York. 286 pp.
- Strosberg, A.D., Buffard, D., Lauwereys, M. & Fories, A. 1986. Legume Lectins: a large family of homologous proteins. In *The lectins: properties, functions, and applications in biology and medicine*. Edited by I.E. Liener, N. Sharon & I.J. Goldstein. Academic press Orlando. 600. pp.
- Tatchell, G.M. 1983. Compensation in spring sown oilseed rape (*Brassica napus* L.) plants in response to injury to their flower buds and pods. *Journal of Agricultural Science* 101, 565-573.
- Van Damme, E.J.M., Peumans, W.J., Barre, A. & Rougé, P. 1998. Plant Lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Critical Reviews in Plant Sciences* 17, 575-692.
- Weaver, S.A. & Morris, M.C. 2005. Risks associated with genetic modification: an annotated bibliography of peer reviewed natural science publications. *Journal of Agricultural and Environmental Ethics* 18, 157-189.
- Veromann, E., Kevvää, R., Luik, A. & Williams, I.H. 2007. Do cropping system and insecticide use in spring oilseed rape affect the abundance of pollen beetle (*Meligethes aeneus* Fab.) on the crop? *International Journal of Pest Management (In press)*
- Veromann, E., Luik, A. & Kevvää, R. 2006. Oilseed rape pests and their parasitoids in Estonia. *IOBC/wprs Bullentin* 29, 165-172.
- Veromann, E., Tarang, T., Kevvää, R. & Luik, A. 2006. Insect pests and their natural enemies on spring oilseed rape in Estonia: impact of cropping systems. *Agricultural and Food Science* 15, 61-72.
- Westerkamp, C. & Gottsberger, G. 2000. Diversity pays in crop pollination. *Crop Science* 40, 1209-1222.
- Åhman, I. 1993. a search for resistance to insects in spring oilseed rape. *IOBC wprs bullentin* 16, 36-46.
- Åhman, I. & Melander, M. 2003. Potato proteins, and other plant proteins, as potential transgenic resistance factors to pollen beetles in oilseed rape. *Annals of Applied Biology* 143, 253-260.
- Åhman, I.M., Kazachkova, N.I., Kamnert, I.M., Hagberg, P.A., Dayteg, C.I., Eklund, G.M., Meijer, L.J. & Ekbom, B. 2006. Characterisation of transgenic oilseed rape expressing pea lectin in anthers for improved resistance to pollen beetle. *Euphytica* 151, 321-330.

Acknowledgements – Tack!

För en som är så självcentrerad som jag, så passar det väl bra att börja acknowledgements med att prata om mig själv. Jag är kanske inte lat, men väldigt bekväm. Så Barbara och Inger, jag vet inte hur ni burit er åt men nu är jag alltså i mål. Barbara, du har all min respekt och det är väl lika bra att jag erkänner att jag var lite rädd för dig i början. Du har en fantastisk förmåga att känna när jag behövt uppmuntran, eller när jag behöver en knuff, eller ja en spark framåt. Trots många andra uppdrag har du alltid tid för dina studenter. Inger! Om det inte varit för dig så hade jag kanske inte doktorerat överhuvudtaget. Och att kalla dig biträdande handledare känns inte helt rätt då du varit delaktig i mer eller mindre allt jag företagit mig under åren. Jag hoppas att jag smittats av din noggrannhet, även om jag inte alltid verkat helt nöjd när mina manus kommit tillbaka med mer röd än svart text... Alla goda ting lär ju vara tre och så även i detta fall. Johan, mitt molekylärbiologiska skyddsnät! Även om vi inte jobbat ihop någon längre tid har det varit väldigt skönt att du funnits till hands och jag uppskattar att du tagit dig tid när frågorna hopat sig.

Det finns fler som bidragit till att det finns några artiklar och manus i avhandlingen. Plötsligt på västkanten kom en låda från Danmark och jag kastades in i en värld av hypop..hypofaryngeala körtlar, grafting tools och drottninggelé, som förresten INTE är gott (borde vetat bättre än att fråga en rättsentomolog?). Som tur var fick jag guidning av ett gäng med koll på bin. Ingemar, till slut blev det rätt i alla fall! Tusen tack för att du tog dig tid. Eva, utan dig hade isoleringen i källaren i 30 graders värme nästan känts tråkig. Tack för all hjälp & lycka till med avhandlingen! Och Anders, hur många strängar har du egentligen på din lyra (har han någon lyra överhuvudtaget? Kanske en tung bas?)? Jag saknar B-korridoren med Anders trummande i skrivbordet & Ingemars svärande över dåliga manus (men det här med Mac...).

One of the papers would definitely be of questionable quality without the efforts of England's most lovely girl Katy, clever and annoyingly young! Those 11 days in the Boldrewood bunker was without doubt efficient, but never underestimate the scientific value of shopping! Just so you know, I see it as my mission to hook you up with a certain football player..

Per ”formell” Hydbring! Ibland har man tur. Om jag inte varit sådär trött på doktorandrådsrådet hade jag antagligen inte fått tillfälle att på kort tid och med bästa tänkbara hjälp, göra det molekylära jobb som krävdes för att mina artiklar skulle bli publicerbara. Tur också att jag hann utnyttja LG's grupp innan ni försvann till KI! Se nu till att lösa cancertgåtan så att jag kan skryta med att jag jobbat med dig!

Under de första tre åren spenderade jag somrarna i Svalöv och där blev ni, Helena, Yvonne och Lillemor min andra familj. Stort tack till övriga på oljevästavdelningen på SW som gjorde att jag alltid kände mig välkommen.

I am very grateful for being part of the department of Entomology, to me you guys was everything a new PhD student could ask for. A big thank you to ALL the

people in the house, who contribute to the positive atmosphere. Ingen nämnd, ingen glömd brukar det ju heta men det finns speciell person på institutionen som var väldigt viktig för mig som ny och förvirrad student, Hjördis! Nu har du ju fått sällskap av ett par som inte heller är så tokiga men din hjälp och omtänksamhet har betytt otroligt mycket! Tack till alla sköna doktorander på institutionen! Sandra, du var den första jag träffade på entomologen och du har liksom Kakan blivit en av mina bästa vänner i huset. Du är den mest omtänksamma och osjälviska person jag träffat, men lite mer ego har du kanske ändå blivit i mitt sällskap? Kakan, min roommate. Det har hänt en hel del under de här åren eller hur? Nu har ju jag varken gift mig eller blivit gravid men ändå. Något som inte förändrats är prasslet av kak- & godispapper framåt eftermiddagen och att jag fortfarande tror på allt du säger! Nu får du ju dras med mig ett tag till men passar på att tacka för sällskapet och att du hållit kvar mig på marken när jag varit på väg upp i det blå. Ska nog ta och köpa den där Kalle Jularbo-skivan ändå.

Thank's to the chemical ecology group in Alnarp, especially Bill and Marcus. You were the ones who introduced me to research and made me believe that this might be something for me!

Jag har även haft ett liv utanför arbetstid (!) och där finns det en så lång rad av människor så det nästan skulle krävas en separat avhandling för att tacka alla! Mina vänner sedan barndomen, Anna & Helena, och Linda som, trots att vi bor en bit ifrån varandra alltid finns där. Det fantastiska Lundagänget (utan inbördes ordning!); Sussi, Matilda, Astrid, Manne, Micke, Siv, Johan, Åsa, Nina m fl. Tack Micke för att du vågade sammanföra dina två mest envisa & bestämda vänner! Det har lett till både det ena & andra. Nyår i Hassela x 5, charter i Halmstad med mina partners in crime Tina (det är inte aktuellt) & Nina (jag sitter på bussen). På köpet fick jag också lära känna Norrlands Nations antagligen galnaste gäng, bl. a. Marita (var är min väska?), jag hoppas att du flyttar (eller jag kanske?) lite närmare för nu ses vi på tok för sällan.

Tack till Tannér-Persson's, min extra familj i Skåne, som jag alltid lyckades hälsa på när det vankades mat! Men är det inte lite skumt att jag fått två bilar lagade otaliga gånger men aldrig sett mekanikern? Misstänker att du Jan är som Stålmannen, fast istället lagar du bilar i hemlighet..

Lugnet infinner sig alltid när jag får chans att besöka mitt barndomshem Bäckäng. Karin, Christian & Fredrik, tack för att ni tar så bra hand om vårt gamla hus! Ser fram emot att fira Lille-Jul med er i minst 18 år till!

Tack till min underbara familj. Mamma, den bästa som finns. Vad jag än tar mig för så är du alltid på min sida, även om det inte varit lätt att hänga med i svängarna (läs humör). Pappa! Det här trodde du väl inte (och inte jag heller) när jag hoppade av gymnasiet efter 2 månader? Vi är ju lika du & jag, vilket tog tid att erkänna, och du förstår mig så väl. Marianne, tack för att du från dag ett fått mig att känna mig välkommen! På köpet fick jag Lundahlbröder x 3! Tack också till min bror Mats med familj som jag är otroligt stolt över men träffar allt för sällan.

Sist, killen som bidragit till att göra det sista året till en roligare resa och som envisats med att jag kunde klara av det här. Tack Daniel, jag tror på dig också.