LAST FIVE DEFENCES

PILLE SOOVÄLI
INTEGRATED PLANT DISEASE MANAGEMENT IN SPRING BARLEY AND OAT PRODUCTION
INTEGREERITUD TAIMEKAITSE KASUTAMINE ODRAL JA KAERAL
Professor Marika Mänd
Associate Professor *emer* Ülle Lauk
Mati Koppel (PhD)
December 21, 2011

PRIIT PÄKK
ALTERATIONS OF EPIDERMAL CELLS` FUNCTIONAL ACTIVITY IN FISH DUE TO INFECTION
NAKKUSTE POOLT PÕHJUSTATUD EPIDERMISE RAKKUDE TALITUSLIKU AKTIIVSUSE MUUTUSED KALADEL
Professor Tiit Paaver
Associate Professor Piret Hussar
February 14, 2012

SIRJE VÄRV
MARKER-BASED GENETIC CHARACTERIZATION OF THE ESTONIAN DAIRY BREEDS
EESTI PIIMAVEISETÕUGUDE ISELOOMUSTAMINE GENEETILISTE MARKERITE ALUSEL
Professor Haldja Viinalass
Professor Juha Kantanen
March 20, 2012

KÄTLIN BLANK
DYNAMICS AND INTERACTIONS OF PHYTO- AND ZOOPLANKTON AS INDICATORS OF THE STATUS OF LAKE PEIPSI
FÜTO- JA ZOOPLANKTONI DÜNAAMIKA JA NENDE OMAVAHELISED SUHTED KUI PEIPSI JÄRVE SEISUNDI INDIKAATORID
Senior Researcher Ellen-Juta Haberman
Senior Researcher Reet Laugaste
Senior Researcher Külli Kangur
April 23, 2012

ANTS VAIN
CORRECTING AND CALIBRATING AIRBORNE LASER SCANNING INTENSITY DATA USING NATURALLY AVAILABLE TARGETS
AEROLASERSKANEERIMISE INTENSIIVSUSE PARANDAMINE JA KALIBREERIMINE LOODUSLIKKE PINDASID KASUTADES
Associate Professor Natalja Liba
Professor Kalev Sepp
June 15, 2012

ISBN 978-9949-484-37-9

roheline trükis
Trükitud taastoodetud paberile looduslike trükivärvidega. ©Ecoprint

ENELI VIIK THE IMPACT OF SPRING OILSEED RAPE FERTILIZATION AND PESTICIDE APPLICATION ON BEES (APOIDEA)

Eesti Maaülikool
Estonian University of Life Sciences

THE IMPACT OF SPRING OILSEED RAPE FERTILIZATION AND PESTICIDE APPLICATION ON BEES (APOIDEA)

VÄETAMISE JA PESTITSIIDIDE KASUTAMISE MÕJU MESILASELAADSETELE (APOIDEA) SUVIRAPSIL

ENELI VIIK

A Thesis
for applying for the degree of Doctor of Philosophy
in Agricultural Sciences in Entomology

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse õppekava entomoloogia erialal

Tartu 2012

EESTI MAAÜLIKOOL
ESTONIAN UNIVERSITY OF LIFE SCIENCES

Eesti Maaülikool
Estonian University of Life Sciences

THE IMPACT OF SPRING OILSEED RAPE FERTILIZATION AND PESTICIDE APPLICATION ON BEES (APOIDEA)

VÄETAMISE JA PESTITSIIDIDE KASUTAMISE MÕJU MESILASELAADSETELE (APOIDEA) SUVIRAPSIL

ENELI VIIK

A Thesis
for applying for the degree of Doctor of Philosophy
in Agricultural Sciences in Entomology

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse õppekava entomoloogia erialal

Tartu 2012

Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

According to the verdict No 108 of May 14, 2012, the Doctoral Committee of the Agricultural and Natural Sciences of the Estonian University of Life Sciences has accepted the thesis for the defence of the degree of Doctor of Philosophy in Agricultural Sciences in Entomology.

Opponent: **Prof. Juha Helenius**
Department of Agricultural Sciences
University of Helsinki
Helsinki, Finland

Supervisors: **Prof. Marika Mänd**
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

Prof. Anne Luik
Vice-rector of research
Estonian University of Life Sciences

Defence of the thesis:
Estonian University of Life Sciences, Karl Ernst von Baer House, Veski 4, Tartu, on June 19, 2012, at 10.00.

The English language was edited by Ingrid H. Williams and the Estonian by Luule Metspalu.

Publication of this thesis is supported by the Estonian University of Life Sciences and by the Doctoral School of Earth Sciences and Ecology created under the auspices of European Social Fund.

ISBN 978-9949-484-37-9

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is a summary of the following papers, which are referred to by Roman numbers in the text. The papers are reproduced by kind permission of the following journals or publishers: Springer (I), Žemdirbystė=Agriculture (II) and Pest Management Science (III).

I Mänd, M., Williams, I. H., **Viik, E.**, Karise, R. 2010. Oilseed rape, bees and integrated pest management. In: (Ed. Williams, I. H.) Biocontrol-based integrated management of oilseed rape pests. Springer Dordrecht Heidelberg, London, New York, p. 357-379.

II **Viik, E.,** Mänd, M., Karise, R., Lääniste, P., Williams, I. H., Luik, A. 2012. The impact of foliar fertilization on the number of bees (Apoidea) on spring oilseed rape. Žemdirbystė=Agriculture, 99 (1), 41-46.

III Karise, R., **Viik, E.**, Mänd, M. 2007. Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape *Brassica napus* flowers in field conditions. Pest Management Science, 63, 1085-1089.

IV Muljar, R., Karise, R., **Viik, E.**, Kuusik A., Mänd, M., Williams, I. H., Metspalu, L., Hiiesaar K., Luik, A., Must, A. Effects of Fastac 50 EC on bumble bee *Bombus terrestris* L. respiration: DGE disappearance does not lead to increasing water loss. Journal of Insect Physiology. (submitted)

Authors' contribution to the papers:

Paper	Idea and study design	Data collection	Analysis of data	Manuscript preparation
I	MM, IHW, **EV**, RK	**EV**, IHW, MM, RK		MM, IHW, **EV**, RK
II	**EV**, MM, RK	**EV**	**EV**	**EV**, MM, IHW, AL
III	**EV**, RK, MM	**EV**	**EV**, RK	RK, **EV**, MM
IV	RM, **EV**, MM, RK	RM, RK, AK, **EV**	RM, **EV**, RK, AM	RM, RK, **EV**, MM, LM, KH, AK, AL, IHW

AK – Aare Kuusik; AL – Anne Luik; AM – Anne Must; EV – Eneli Viik; IHW – Ingrid H. Williams; KH – Külli Hiiesaar; LM – Luule Metspalu; MM – Marika Mänd; RK – Reet Karise; RM – Riin Muljar

ABBREVIATIONS

a.i.	active ingredient
C	closed
CFO	closed-flutter-open
CGE	cyclic gas exchange
CFV	closed-flutter-ventilation
DGE	discontinuous gas exchange
F	flutter
FV	flutter-ventilation
GS	growth stage of (oilseed rape) plants
IR	infrared
IRGA	infrared gas analyser
O	open
ppm	parts per million
VCO_2	rate of CO_2 emission
WLR	water loss rate

1. INTRODUCTION

Spring oilseed rape (*Brassica napus* L. var. *oleifera* subvar. *annua*) is an important oilseed crop, the area of which has increased significantly in northern countries of Europe (Treu, Emberlin, 2000), including Estonia (Veromann *et al.*, 2006a). This has resulted in an increased number of pests as monocultures offer unlimited food resources and reproduction opportunities (Hokkanen, 2000; Cook, Denholm, 2008). Due to the increased occurrence of pests in oilseed rape, the use of pesticides has become an almost inevitable part of cultivating these crops (Alford *et al.*, 2003). In addition, oilseed rape, as a fast growing crop, needs a high amount of nutrients and thus needs more fertilizers than graminaceous crops (Holmes, 1980; Barraclough, 1989; Grant, Bailey, 1993; Orlovius, 2003).

Spring oilseed rape is predominantly autogamous and visits of insect pollinators are not essential for the final seed yield (Williams *et al.*, 1987). Despite this, adequate pollination of spring oilseed rape can have positive effects such as reduction of the flowering period and raceme production, acceleration of ripening, increases in seed weight (Williams *et al.*, 1987; Rosa *et al.*, 2011; Bommarco *et al.*, 2012), seed oil content (Free, 1993; Bommarco *et al.*, 2012) and seed yield (Steffan-Dewenter, 2003; Morandin, Winston, 2005; Sabbahi *et al.*, 2005; Chifflet *et al.*, 2011). Hence, it is profitable to encourage a high number of pollinators in oilseed rape fields.

The economically most important and abundant pollinators of spring oilseed rape are bees (Klein *et al.*, 2007; Ali *et al.*, 2011a). However, a general and widespread shortage of bee-pollinators is predicted in agricultural areas of America (Kremen *et al.*, 2004; Currie *et al.*, 2010), Asia (Klein *et al.*, 2003) and Europe (Williams *et al.*, 1991; Williams, 1996; Giray *et al.*, 2010). The main reason for this is probably the loss and degradation of habitats and food resources due to changes in land-use and agricultural practice (Williams *et al.*, 1993; Mänd *et al.*, 2002; Sepp *et al.*, 2004; Goulson *et al.*, 2005; Öckinger, Smith, 2007; Potts *et al.*, 2010), including the intensive use of pesticides (Osborne *et al.*, 1999; Miranda *et al.*, 2003; Maini *et al.*, 2010), as well as changes in climate and the effects of predators and parasites (Williams, 1986).

Oilseed rape, as a mass flowering crop, provides highly rewarding resources of both nectar and pollen for bees and therefore promotes colony growth

and bee abundance (Westphal *et al.*, 2003, 2009). Thus, it is vital that husbandry of the oilseed rape crop helps to sustain and not to diminish bee populations. Unfortunately, the application of pesticides to the crop may contribute to the decline of wild bees and honey bees (Miranda *et al.*, 2003; Laurino *et al.*, 2011; Krupke *et al.*, 2012) as they may come into contact with poisoning compounds (Gels *et al.*, 2002; Schneider *et al.*, 2012). This may happen especially when pesticides are applied during flowering when bees are foraging on the crop (Thompson, Maus, 2007). Pesticide effects on bees may be lethal or sub-lethal: in certain circumstances, the sub-lethal effects may cause more harm than lethal doses since they affect the survival of the brood and colony (Thompson, 2001).

To avoid the toxic effects of pesticides on bees the application of insecticides is often not permitted during the flowering period of a given crop, but, despite this, the residues of the compounds still contaminate nectar and pollen in sub-lethal doses via both active and passive transport (Thompson, 2001). In addition, pyrethroids, most often sprayed on flowering oilseed crops, have been reported to be repellent to honey bees (Rieth, Levin, 1988; Thompson, 2001) but there is evidence that, in some instances, the attractiveness of a food resource may override the repellent effect (Thompson, 2003).

The results of honey bee studies are often extrapolated to native pollinators, including bumble bees, although their foraging behaviour is different and they are more vulnerable: colonies are smaller, they do not have the trophallaxis which could diminish the amount of pesticide residues possibly reaching the larval food and, for a long period in late spring, only the queens are collecting food, and thus are exposed to pesticides (Alford, 1975). Hence, special studies are needed to explain the impact of sub-lethal effects of pesticides on bumble bees. Further, the sub-lethal effects of pesticides on adult foragers may not be observable without special methods used in experiments of insect physiology. For example, the patterns of discontinuous gas exchange have been used for characterizing the physiological state of an insect, while several stress factors, including chemical ones, can affect it (Kestler, 1991).

So, taking into account several benefits of oilseed rape cross-pollination (Rosa *et al.*, 2011; Chifflet *et al.*, 2011; Bommarco *et al.*, 2012) it is profitable to encourage high number of bees in oilseed rape fields. On

the other hand, the bees visiting oilseed rape flowers need to be protected against the negative effects of pesticides. In order to favour and protect the pollinators enough knowledge is needed – unfortunately there are still many unanswered questions. To fill the gap in our knowledge the current thesis examines the impact of oilseed rape (as a crop with a high nutrient demand) foliar fertilization with different microfertilizers on the number of flower visiting bees (honey bees, bumble bees and solitary bees) through the number of flowers and food resources (nectar and pollen production). In addition, we examined the repellent effect of the non-systemic insecticide Fastac 50 EC (a.i. alpha-cypermethrin) on the number of foraging honey bees on spring oilseed rape fields and the effect of low concentrations of Fastac 50 EC on the metabolic rate, respiratory pattern and total water loss rate of bumble bee *Bombus terrestris* foragers.

2. REVIEW OF THE LITERATURE

2.1. Oilseed rape

Spring oilseed rape (*Brassica napus* L. var. *oleifera* subvar. *annua*) is an important oilseed crop; its growing area has increased significantly in northern countries of Europe, including Estonia (Veromann *et al.*, 2006a), in recent decades (Treu, Emberlin, 2000). Oilseed rape has spring and winter forms but wintering conditions for the latter are usually not favourable in the Estonian climate. Today oilseed rape is cultivated and processed for many different purposes: oil for human nutrition, a renewable raw material for the chemical industry, a source of regenerative energy, a source of high energy and protein content for animal nutrition in the form of rape cake and meal, a catch crop for green manuring and as a forage crop (Orlovius, 2003).

Oilseed rape is a typical cruciferous plant with yellow (or in some cultivars, white) flowers arranged in elongated terminal racemes. Each flower has four sepals, four petals and, usually, six stamens, four of which are longer and two shorter than the style. The flower bears four partly-hidden nectar glands (nectaries) at the base of the six stamens, two at the inner bases of the short stamens and two outside the ring of stamens (Eisikowitch, 1981; Rosa *et al.*, 2010). Flowering extends from 22 to 45 days (Radchenko, 1964; Free, 1993; Delaplane, Mayer, 2000) depending on weather conditions.

Oilseed rape seeds are small and the colour varies from black to dark brown. Several studies have shown that the average yield of oilseed rape is affected by fertilization (Haneklaus *et al.*, 1999; Sidlauskas, Bernotas, 2003; Szulc *et al.*, 2003; Rathke *et al.*, 2006). Oilseed rape as a fast growing crop needs a high amount of nutrients – more than graminaceous crops (Holmes, 1980; Barraclough, 1989; Grant, Bailey, 1993; Orlovius, 2003). Crop production handbooks in Estonia (Kärblane, 1996; Kaarli, 2004) recommend complex fertilizers together with microelements for oilseed rape fertilization.

The expansion of the oilseed rape growing area in Europe has created good conditions for pests, as monocultures offer unlimited food resources and reproduction opportunities – thus, the number of pests has been increasing (Hokkanen, 2000; Cook, Denholm, 2008). There are

several pests of oilseed rape in Europe, but the pollen beetle, *Meligethes aeneus* (Fab.) (Coleoptera: Nitidulidae) and the cabbage seed weevil, *Ceutorhynchus obstrictus* (Marsh.) (Coleoptera: Curculionidae) are the two most important pests of the flowering phase (Alford *et al.*, 2003), also in Estonia (Veromann *et al.*, 2006a), causing yield loss through damage to flower buds and pods, respectively. Due to the increased occurrence of pests in oilseed rape, the use of pesticides has become an almost inevitable part of cultivating these crops (Alford *et al.*, 2003). However, it has been found that intensive growing technology of spring oilseed rape, based on pesticide application, enhances the new generation of pollen beetles (Veromann *et al.*, 2008). In addition, the frequent use of pyrethroids has resulted in resistance of pollen beetle to this pesticide in many European countries (Richardson, 2008; Zimmer, Nauen, 2011).

2.1.1. Pollination requirements of oilseed rape (I)

Pollination is a mutually beneficial relationship which in most cases takes place between a plant and insect: the pollen from the anthers will be transported to the stigmas of the same or different flowers and as a reward the insect gets food – mostly nectar and pollen. Oilseed rape is commonly considered to be a self-pollinating species but actually has entomophilous flowers capable of both self- and cross-pollination – nevertheless, insect pollinators are not essential for the final seed yield (Williams *et al.*, 1987). However, flower morphology and behaviour of the oilseed rape flower encourage cross-pollination at first, but self-pollination later (Delaplane, Mayer, 2000; Rosa *et al.*, 2010). Before the corolla fully expands, the four long stamens dehisce and release pollen outwards. Anthers on the two short stamens release pollen below the stigma which lengthens during flowering to reach the height of the anthers of the long stamens. When the flower is old, the long stamens bend towards the flower centre so that they become directed towards the stigma, and self-pollination can occur (Eisikowitch, 1981; Williams, 1985; Free, 1993; Bell, Cresswell, 1998; Delaplane, Mayer, 2000; Rosa *et al.*, 2010). In the case of cross-pollination, more pollen can reach the stigmas, particularly pollen from the short stamens (Free, 1993).

Bees are the economically most important and abundant pollinators of spring oilseed rape (Klein *et al.*, 2007; Ali *et al.*, 2011a) – all bee species successfully transfer rape pollen from anthers to stigmas. There are several

advantages of adequate pollination of spring oilseed rape: reduction of the flowering period and of raceme production, acceleration of ripening and increases of seed weight (Williams *et al.*, 1987; Rosa *et al.*, 2011; Bommarco *et al.*, 2012) and seed oil content (Free, 1993; Bommarco *et al.*, 2012). Cross-pollination with pollen from short stamens is significantly superior to that from long stamens, and gives a 14% greater weight of seed per pod (Free, 1993; Steffan-Dewenter, 2003). Cross-pollination also raises seed yield (Steffan-Dewenter, 2003; Morandin, Winston, 2005; Sabbahi *et al.*, 2005; Chifflet *et al.*, 2011). Altogether, the seed yield of oilseed rape could be increased by up to 25 – 46 % (Delaplane, Mayer 2000; Sabbahi *et al.*, 2005). Thus, a high number of pollinators in oilseed rape fields should be favoured.

2.1.2. The composition of pollinators on oilseed rape

The composition of flower visitors to oilseed rape varies according to different authors. Langridge and Goodman (1982) found that, of the total insects counted on oilseed rape flowers, 71.4% were honey bees, 14.3% hoverflies, 12.1% small Diptera and the remaining 2.2% were made up of blowflies, native bees, Lepidoptera, one Hemiptera and one Coleoptera. Rosa *et al.* (2011) found that Hymenoptera representatives were the most prevalent (92.3%, among which 99.8% were honey bees) but some Diptera, Lepidoptera and Coleoptera also visited oilseed rape flowers. According to Delaplane and Mayer (2000), 64% of bee visitors on oilseed crop were honey bees and 36% different wild bees. Karise *et al.* (2004) found that 54% of oilseed rape flower visitors were bees (Apoidea), 45% dipterans (Diptera) and 1% butterflies (Lepidoptera) and bugs (Hemiptera).

On the basis of different studies it can be said that honey bees are the main pollinators of oilseed rape and can account for up to 95% of all insect pollinators of this crop (Mesquida *et al.*, 1988; Adegas, Couto, 1992; Blight *et al.*, 1997; Koltowski, 2001; Rosa *et al.*, 2011). According to Koltowski (2001), solitary bees can account for about 4%, or sometimes 9%, of all insect pollinators on oilseed rape flowers. Bumble bees being important pollinators of many agricultural crops, however, make up only 2% of all insect pollinators in rape crops (Cresswell, 1999; Koltowski, 2001). So, although many species of bumble bee and solitary bee may visit a crop, their proportion is often quite low (Free, 1993; Varis, 1995; Karise *et al.*, 2004).

2.2. Bees (Apoidea)

2.2.1. Foraging behaviour of bees

Bees are the most important pollinators as they need to feed not only themselves but also their colony; they have to gather a large amount of food fast – that means they need to visit a high number of flowers (Corbet *et al.*, 1991). Most bees collect only two food items from flowers: nectar, which provides bees with energy, and pollen, which provides them with protein necessary for the growth of larvae (Rasheed, Harder, 1997). Bumble bees consume pollen throughout their entire development whereas honey bee larvae are fed, during their early development, by glandular secretions of adult workers, which eat pollen both to feed the larvae (Dobson, Peng, 1997; Hrassnigg, Crailsheim, 1998; Babendreier *et al.*, 2004) and to satisfy their own protein needs (Smeets, Duchateau, 2003). Nectar as a liquid substance serves as an attraction for bees and reward for their pollination service (Baker, Baker, 1973) and is a proper medium for supporting the digestion of pollen grains (Roulston, Cane, 2000). In addition, nectar is important as an easily assimilable energy source (Faegri, van der Pijl, 1979).

According to the optimal foraging theory, bees try to maximize the benefit and minimize the costs (Pettersson, Sjödin, 2000). Hence, the food collected from the flower – the reward – has to exceed the energy spent on receiving it. Bees have some specialities which help to maximize the benefit – flower constancy, a well developed memory and good learning ability. Both, memory and the learning capacity of insects are usually under-estimated. Laboratory studies as well as those in nature have demonstrated that honey bees learning is fast and comprises various levels of cognitive processing, such as generalization, context-dependency, concept formation, configuration and categorization (Menzel, Giurfa, 2001; Gegear, Thomson, 2004; Gross *et al.*, 2009; Sandoz, 2011). Well developed learning and memory helps the honey bee forager to find her way back to the hive (Dyer *et al.*, 2008; Cruse, Wehner, 2011; Pahl *et al.*, 2011). Raine and Chittka (2008) demonstrated that the learning speed of bumble bees is correlated with foraging success: colonies that learn faster achieve greater fitness.

Foraging bees often show a kind of flower constancy favouring some and bypassing others that might offer a reward (Free, 1970; Gegear,

Thomson, 2004) although the level of flower constancy may vary even among species of the same genera (Free, 1970). Bees test various flower types until they find one that offers a reward (Pohtio, Teräs, 1995) developing in that way a flower colour preference which is claimed not to be an innate characteristic (Waser, Price, 1983; Gumbert, Kunze, 2001). Flower constancy, which probably can occur due to their well developed memory and learning, make bees the best pollinators from the plant's point of view as it ensures that less pollen is wasted.

The most important signals for the bees' recognition of a food source while foraging are thought to be olfactory signals (Menzel *et al.*, 1993; Leonard *et al.*, 2011). The chemical signals may function as indirect cues: young bees remember the smell of the food they ate inside the hive and search for it during their first foraging trip; or directly as long or short distance attractants. Bees are able to differentiate a large number of olfactory signals and learn to predict which flowers offer rewards and which do not (Laska *et al.*, 1999; Gumbert, Kunze, 2001) but they still restrict the number of scent components they use in their searching (Pham-Delègue *et al.*, 1997; Laloi *et al.*, 2000). It has been found that olfactory learning performance depends on the circadian rhythmicity being the highest in the morning (Lehmann *et al.*, 2011). During a honey bee's first foraging trip in the morning, it has to learn the most profitable food source for that day which it can then later harvest. Odours are also used by homing foragers to advertise profitable food sources (Farina *et al.*, 2007). It is considered possible that bees may avoid plants treated with pesticides due to the repellent odours of the compounds present (Shires *et al.*, 1984).

2.2.2. Oilseed rape as food resource for bees (I)

Large fields of oilseed rape in flower are important food resources for bees enhancing both nectar and pollen resources abundantly (Westphal *et al.*, 2009). Flowering at a time when there are few other cultivated food plants available for honey bees, a rape crop may be visited from a distance of 3.5–4 km from bee hives and fruit trees may be neglected in favour of rape (Free, 1993). Furthermore, many beekeepers move their colonies onto or near to oilseed rape crops to benefit from the nectar and pollen it produces (Williams, 1980; Williams, Cook, 1982; Williams *et al.*, 1993; Carreck *et al.*, 1997). There has even been a concern that oilseed rape crops lead to reduced wild plant pollination (Diekötter *et al.*, 2010; Holzschuh *et al.*, 2011).

Oilseed rape is an especially attractive food plant for bees because of the high nectar production of its flowers and its high sugar content. The nectar of oilseed rape flowers contains carbohydrates, such as sucrose, glucose, fructose and ribose (Pierre *et al.*, 1999). Nectar volume can vary greatly from 0.2 µl per flower up to 6 µl per flower (Free, 1993; Davis *et al.*, 1994; Pierre *et al.*, 1999) and may be affected by genotype (Pierre *et al.*, 1999), cultivar (Davis *et al.*, 1994), flower age (Williams, 1980) and local environmental conditions (Williams, 1985; Rathcke, 1992). Nectar production has been reported to be greater in the morning and early afternoon than midday (Williams, 1985) and to decrease towards the end of the day, while the sugar concentration increases toward the end of the day (Meyerhoff, 1958; Radchenko, 1964). The flowers are able to replenish the level of nectar completely within 30 min of being emptied which makes them very attractive to bees. Nectar production even increases if bee density is high, and flowers are visited more than three times per day (Williams, 1985).

Oilseed rape flowers produce a lot of pollen which contains proteins, lipids, carbohydrates, starch, sterols, vitamins and minerals (Day *et al.*, 1990; Herbert, 1992). All are important nutrients for brood rearing and development of young worker bees, particularly the protein content (Winston, 1987; Hrassnigg, Crailsheim, 1998). The pollen of oilseed rape contains more of the three most important amino acids (leucine, valine and isoleucine) for bee survival and development than other field crops flowering at the same time (Cook *et al.*, 2003).

The growing of mass-flowering oilseed rape crops greatly enhances nectar and pollen resource availability in agricultural areas and, when appropriately managed, has potential to promote the abundance, as well as the fitness, of bee populations (Westphal *et al.*, 2003, 2009).

2.3. Pollinator decline in agricultural landscapes (I)

Bees are the most important pollinators of almost all terrestrial ecosystems because they provide a vitally important ecosystem service as pollinators for a wide range of agricultural, horticultural and wild plants (Corbet *et al.*, 1991; Williams 1994, 1996; Klein *et al.*, 2007; Kasina *et al.*, 2009; Pauw, Hawkins, 2011). At the same time, there is clear evidence of recent declines in both wild and domesticated bee-pollinators (Potts *et al.*, 2010) which has been observed in different regions of the world – America

(Kremen *et al.*, 2004; Currie *et al.*, 2010), Asia (Klein *et al.*, 2003) and Europe (Williams *et al.*, 1991; Williams, 1996; Giray *et al.*, 2010). The decrease in the number of bees is an alarming tendency (Thompson, 2001; Biesmeijer *et al.*, 2006; Gabriel, Tscharntke, 2007). In 2005, the economic value of insect pollination in Europe was estimated at 22 billion and in the world at 153 billion € per annum (Gallai *et al.*, 2009).

Several factors have been suggested as possible contributors to this decline (Potts *et al.*, 2010), e.g. changes in climate and the effects of predators and parasites (Williams, 1986). However, the main reason is thought to be the intensification of agriculture, including changes in land-use causing decrease in food resources and habitats (Osborne *et al.*, 1991; Williams *et al.*, 1993; Mänd *et al.*, 2002; Sepp *et al.*, 2004; Goulson *et al.*, 2005; Öckinger, Smith, 2007; Potts *et al.*, 2010), and increasing application of pesticides (Osborne *et al.*, 1999; Miranda *et al.*, 2003; Maini *et al.*, 2010; Stokstad, 2012). The supply of nectar and pollen is now often insufficient in European agricultural landscapes to support healthy bee populations (Goulson *et al.*, 2005; Öckinger, Smith, 2007). Thus, necessary steps need to be taken to halt the loss of pollinators (Moritz *et al.*, 2010; Pettis, Delaplane, 2010; Winfree, 2010).

2.3.1. The sub-lethal effects of pesticides on bees (I)

Bees foraging on the crop are vulnerable to the toxic effects of pesticides applied to the crop and this may contribute to the decline of wild bees as well as honey bees (Corbet *et al.*, 1991; Miranda *et al.*, 2003; Laurino *et al.*, 2011; Krupke *et al.*, 2012).

Bees are especially vulnerable to the toxic effects of insecticides applied during flowering when they are foraging on the crop. They may come into contact with poisoning compounds through contaminated flower resources, direct contact with poison or exposure to residues (Gels *et al.*, 2002; Schneider *et al.*, 2012). Further, insecticides are often applied in tank-mixes with fungicides; this may change the effects of both products on non-target organisms; the toxicity of the insecticide may be greater when applied in a tank-mix (Thompson, Wilkins, 2003; Muranjan *et al.*, 2006). In addition, Free and Ferguson (1980) found that, even when 90% or more of the rape flowers had shed their petals, neither the weight or percentage of pollen collected, nor the honeybee population on the crop decreased greatly. So, it must never be assumed that, as the end

of flowering approaches, pesticides may be applied without danger to beneficial insects.

Pesticide effects on bees may be lethal or sub-lethal; most studies have assessed lethal effects, while only a few have addressed sub-lethal effects. Chemical companies are obliged to provide mortality data for their products for all larger organism groups but again less attention has been paid to the sub-lethal effects. In recent years, this has been an increasing area of study (Desneux *et al.*, 2007; Aliouane *et al.*, 2009; Cresswell, 2011; Schneider *et al.*, 2012) and a subject of discussion between scientists and regulatory authorities (Thompson, Maus, 2007). In addition, many insecticides have been described as safe to bees because they do not kill them, although sub-lethal doses may affect pollinators by decreasing their foraging and navigation abilities (Gels *et al.*, 2002).

Sub-lethal doses affect also the survival of the brood and colony and may thus, under certain circumstances, cause even more harm than lethal doses. Application of insecticides is often not permitted during the flowering period of a given crop. Even when insecticides are not sprayed on flowers but on flower buds, the residues of the compounds still contaminate nectar and pollen in sub-lethal doses via both active and passive transport (Thompson, 2001). Contamination may occur after application of the compounds to other parts of plants (Ferguson, 1987), to the soil (Jaycox, 1964; Krupke *et al.*, 2012) or on seeds (Sur, Stork, 2003). Contaminated nectar and pollen poses a potential danger not only to forager bees but also to bees in the hive and to brood. The toxicity of pesticides to brood has been investigated far less than their toxicity for adults (Alix, Vergnet, 2007).

Sub-lethal doses may affect bees' division of labour (Tasei, 2001; reviewed by Thompson, 2003), development and longevity (Wu *et al.*, 2011), foraging behaviour (Thompson, 2003; Koskor *et al.*, 2009; Decourtye *et al.*, 2011; Schneider *et al.*, 2012), discrimination of odours (Decourtye *et al.*, 2005; Aliouane *et al.*, 2009), communication and orientation abilities (Cox, Wilson, 1984; Vandame *et al.*, 1995; Bortolotti *et al.*, 2003; Yang *et al.*, 2008), learning capacity (Decourtye *et al.*, 1999, 2003; Guez et *al.*, 2001; Ramirez-Romero *et al.*, 2005; Aliouane *et al.*, 2009), reproduction (Stoner *et al.*, 1985; Johansen, Mayer, 1990; reviewed by Thompson, 2003), thermoregulation (Jagers op Akkerhuis *et al.*, 1999a; Belzunces *et al.*, 2001) and susceptibility to pathogens (Alaux *et al.*, 2010; Vidau

et al., 2011; Pettis *et al.*, 2012). Some pesticides do not affect adult bees, but affect brood so that young adults emerging from cocoons may have malformed wings or other deformations (Tasei, 2001).

Pyrethroids, which are the insecticides most often sprayed on flowering oilseed crops, contain a repellent substance which should keep honey bees away from treated fields for some time after application (Rieth, Levin, 1988; Thompson, 2001) but this is still in question in the field situation. In conventional farming, application of many insecticides (e.g., pyrethroids), considered to be safe for honey bees, is permitted to the oilseed rape crop whilst it is in flower. Despite this, 57 out of 117 honey bee poisoning incidents in the UK during 1994-2003 resulted from spray applications to flowering crops; 17 of these incidents were through approved use of the products (Barnett *et al.*, 2007). So, some insecticides may be regarded as safe because they repel bees, although in some instances, the attractiveness of a food resource may override the repellent effect (Thompson, 2003). The repellency of pyrethroids may also decrease when they are mixed with fungicides (Thompson, Wilkins, 2003).

It is also problematic to distinguish repellency from a sub-lethal effect. Bees feel a strong irritation when they come into contact with pyrethroids and, trying to get rid of it, comb the chemical on to their mouthparts and antennae. On receiving a small dose of poison they return to the nest to heal themselves, thereby avoiding contact with a lethal dose (Thompson, 2003). Thus, the repellent effect of pesticide to bees does not appear in repellency but in a small dose of sub-lethal disease-causing effect.

In addition, the results of studies carried out with honey bees are often extrapolated to native pollinators, including bumble bees. However, bumble bees are more vulnerable than honey bees as their colonies are smaller and they do not have the trophallaxis that could diminish the amount of pesticide residues possibly reaching larval food. In addition, bumble bee queens are exposed to pesticides for a long period in late spring while collecting food and establishing their nests. Bumble bees' foraging behaviour is also different from that of honey bees (Alford, 1975; Thompson, Hunt, 1999). There is a need to protect foraging bumble bees from direct overspray during the early morning and late evening when pesticides, which are repellent but highly toxic, are applied (i.e. pyrethroids) as the restrictions for application are often imposed on

the base of the foraging timetable of honey bees although it is different from that of bumble bees (Thompson, 2001).

Pesticide risk assessments for honey bees are based on hazard ratios which rely on application rates and toxicity data that are unlikely to be appropriate for bumble bees. Bumble bees are active at different times and on different crop species and are, therefore, likely to have different exposure profiles. Unlike honey bees, deaths of bumble bees due to pesticides are unlikely to be reported, since the bees are not kept domestically and will die in small numbers (Thompson, Hunt, 1999).

Sub-lethal doses of insecticides can be more harmful to bees than lethal doses as they appear to have no effect; in reality it has lead to a serious pollination crisis which is currently sharply raised in England and USA (Stokstad, 2006). To utilise fully the native pollinator service the use of pesticides should be corrected accordingly.

2.3.2. The sub-lethal effects of insecticides on respiration (I)

The effect of sub-lethal doses can sometimes be observed and proved only through physiological changes in insects. The physiological state of an insect is commonly estimated by its metabolic rate and respiratory patterns. In the case of bees, it is difficult to examine the effects of insecticides on respiration patterns because there is little data on their normal respiration patterns. However, this has been an area of increasing interest during the past decade.

Since water is a key element in every living organism, most insects have probably evolved mechanisms to prevent excessive water loss (Klowden, 2002). Resting insects often exhibit discontinuous gas exchange (DGE) cycles, a function of which may be the reduction of respiratory water loss (Levy, Schneiderman, 1966; Lighton, 1994; White *et al.*, 2007; Schimpf *et al.*, 2009; Williams *et al.*, 2010) through the large inner surface of the tracheal system.

According to Kestler (1971, 1985), in the state of DGE, the spiracles are closed most of the time. At low oxygen rates inside the trachea the spiracular valves flutter, allowing oxygen to enter the tracheal system. As larger amounts of carbon dioxide accumulate in the tracheae and haemolymph (Wobschall, Hetz, 2004), the spiracles open and allow the

gas to escape. So, DGE cycles consist of three phases: closed (C) phase during which spiracles are closed and there is no external gas exchange; flutter (F) phase where spiracles rapidly open and close, allowing bulk inflow of air, and open (O) phase where spiracles are open to allow unrestricted gas exchange (Chown *et al.*, 2006). As compared with continuous respiration, loss of carbon dioxide along with evaporated water occurs only discontinuously during the brief open phases of the spiracles. Cyclic gas exchange (CGE) (described by Lighton, 1996; Marais, Chown, 2003; Gibbs, Johnson, 2004; Marais *et al.*, 2005) has no closed phase, still, the opening of spiracles is alternated by a F period with a low level of CO_2 release. In this, the cycle length is shorter and CO_2 release rarely decreases to zero.

There are different views about the origin of DGE, as reviewed by Chown (2002) and Chown *et al.* (2006). In addition to the water retention, there are also hypotheses that DGE serves as an adaptation for coping with hypercapnia and/or hypoxia in soil-living insects (Lighton, 1998; Vogt, Appel, 2000; Lighton *et al.*, 2004) and protection against the oxidative damage during the periods with low metabolic cost (Hetz, Bradley, 2005; Terblanche *et al.*, 2008). Boardman *et al.* (2012) suggested a possible signalling role for reactive oxygen species rather than the previous idea of DGE protecting the organism against the oxidative damage. Probably the newest neural hypothesis claims that DGE results as a consequence of energy-saving once the brain relinquishes control of gas exchange to the segmental ganglia (Chown, 2011; Matthews, White, 2011). So, although the phenomenon of discontinuous gas exchange has been extensively studied in insects, its adaptive significance is a subject of considerable debate.

The existence and the precise pattern of DGE depends on the species (Lighton, 1994, 1996; Slama, 1999; Chown *et al.*, 2006; Chown, 2011), individual characteristics (Marais, Chown, 2003; Gibbs, Johnson, 2004; Karise *et al.*, 2010; Woods, 2011), life stage of the individuals (Beekman, van Stratum, 1999; Mänd *et al.*, 2005, 2006), metabolic rate (Moerbitz, Hertz, 2010) and environmental conditions like temperature (Lighton, Lovegrove, 1990; Lighton 1996; Vogt, Appel, 2000; Kovac *et al.*, 2007; Karise *et al.*, 2010), relative humidity (Duncan *et al.*, 2002; Lighton, 2007; Slama *et al.*, 2007; Schimpf *et* al., 2009) and the amount of oxygen or carbon dioxide in the air (Lighton, 1998; Vogt, Appel, 2000; Lighton *et al.*, 2004). It has been found that DGE cycles are longer in

species from xeric environments (White *et al.*, 2007), while cyclic and continuous patterns are more prevalent in mesic habitats (Marais *et al.*, 2005).

DGE patterns have been used to characterize the physiological state of an insect, as several stress factors, including chemical ones, can affect them (Kestler, 1991). Although knowledge of the sub-lethal effects of pesticides on insect physiology is poor, it is known that treatments of arthropods with pyrethroids cause neurotoxic effects in parts of the nervous system, including the central nervous system and sensory, motor or neurosecretory neurons (Corbett, 1974; Jagers op Akkerhuis *et al.*, 1995). Because the closing and opening of spiracular valves is controlled by the nervous system, the neurotoxic effects may also include interference by DGE cycles. In pupae of the cabbage butterfly *Pieris brassicae*, after treatment with original pyrethrum, the DGE cycles disappeared and metamorphosis was disrupted (Harak *et al.*, 1999; Jõgar *et al.*, 2008).

Pyrethroids, as well as many other insecticides, can induce increased water loss rate (WLR) in arthropods (Gerolt, 1976, 1983), due to production of diuretic hormones (Jagers op Akkerhuis *et al.*, 1999b). This process could be reversible if the insect could replenish its water reserves. Since the pyrethroids often affect motion as well, causing a knockdown effect, death may come through desiccation (Jagers op Akkerhuis *et al.*, 1995; Jagers op Akkerhuis *et al.*, 1999b; Thompson, 2003).

3. AIMS AND HYPOTHESES OF THE STUDY

1) Oilseed rape is a crop with high nutrient demand and thus needs fertilization (Holmes, 1980; Barraclough, 1989; Grant, Bailey, 1993; Orlovius, 2003). On the other hand, the crop provides highly rewarding food resources for bees (Westphal *et al.*, 2003, 2009) and, at the same time, benefits from cross-pollination, which improves both the quantity and quality of the seed produced (Free, 1993; Sabbahi *et al.*, 2005; Chifflet *et al.*, 2011; Bommarco *et al.*, 2012). Therefore, the foraging activities of bees have significant economic consequences for seed production, and, because of this, it is extremely important that factors that lower their pollinating activity are minimised (Thompson, Maus, 2007). Hence, it is profitable for growers to encourage a high number of pollinators in oilseed rape fields.

The aim: To explain the effect of foliar fertilization with different microfertilizers on flower density, nectar productivity and the number of pollen grains produced per flower of spring oilseed rape, and, through these factors, on the number of flower visiting bees (Apoidea). (**II**)

The hypothesis: we assume that additional foliar fertilization with microfertilizers increases bees' food resources (nectar and pollen production) and the number of flowers on spring oilseed rape and thus also the number of flower visiting bees (Apoidea).

2) Due to the increased occurrence of pests in oilseed rape, the use of pesticides has become an almost inevitable part of cultivating these crops (Alford *et al.*, 2003). Unfortunately, bees foraging on the crop are vulnerable to the toxic effects of insecticides, especially when applied during flowering when bees are foraging on the crop (Thompson, Maus, 2007). To avoid the toxic effects of pesticides on bees a repellent has been added to pesticides – e.g. pyrethroids, which are most often sprayed on flowering oilseed crops (Thompson, 2001). However, there is a need to evaluate pesticide effects on foraging behaviour of bees to guarantee crop pollination, because results obtained in the laboratory may not match with those obtained in the field (Thompson, Maus, 2007). Repellent effect of pyrethroids to bees is still in question in the field situation.

The aim: To examine if honey bees (*Apis mellifera* L.) avoid the repellent insecticide Fastac 50 EC (a.i. alpha-cypermethrin) in their food plant choice. (**III**)

The hypothesis: owing to the repellency of insecticide, the number of honey bees (*Apis mellifera* L.) is lower on insecticide-treated oilseed rape than on untreated oilseed rape.

3) Effects of insecticides on non-target organisms may be lethal or sub-lethal. At present, there is still little knowledge about the sub-lethal impacts of insecticides on the physiological state of bees. Therefore, special studies are needed to explain possible sub-lethal effects of insecticides on bees, since these effects may not be observable without special methods used in experiments of insect physiology. Although knowledge about the sub-lethal effects of insecticides on insect physiology is poor, it is known that treatment of arthropods with pyrethroids cause neurotoxic effects in parts of the nervous system, including the central nervous system and sensory, motor or neurosecretory neurons (Corbett, 1974; Jagers op Akkerhuis *et al.*, 1995). Because the closing and opening of spiracular valves is controlled by the nervous system, the neurotoxic effects may also include interference of gas exchange cycles.

The aim: To examine the effect of low concentrations of Fastac 50 EC (a.i. alpha-cypermethrin) on the metabolic rate, respiratory pattern and total water loss rate of bumble bee (*Bombus terrestris* L.) foragers. (**IV**)

The hypothesis: insecticides cause lethal water loss through respiratory failure in the bumble bee (*Bombus terrestris* L.) as a non-target organism.

4. MATERIAL AND METHODS

4.1. Field experiments

4.1.1. Study sites and subjects

Small-scale field experiments were conducted at the experimental station of the Estonian University of Life Sciences (58º21' latitude 26º 39' longitude) (**II, III**). The field experiments were conducted on the seed production fields of Pilsu farm (**III**) in Tartu County, Estonia (58º14' latitude 26º 16' longitude), in 2003-2005.

In 2004 and 2005, small-scale field experiments were conducted on the experimental station of spring oilseed rape *Brassica napus* L. var. *oleifera* subvar. *annua* to test the effect of microfertilizers on the number of flowers and food resources for bees: honey bees, bumble bees and solitary bees (Apoidea) (**II**).

To test the effect of Fastac 50 EC (a.i. alpha-cypermethrin 50 g l^{-1}) on honey bees (*Apis mellifera* L.) field experiment and small-scale field experiments were conducted on spring oilseed rape *B. napus* L. var. *oleifera* subvar. *annua* fields in 2003, 2004 and 2005 (**III**).

In all experiments, the spring oilseed rape cultivar ´Maskot`, bred and produced by the Swedish company Weibull, was used. Technical data of the variety is as follows: raw fat content 40–43%, 1000 seed weight 3.5–4.5 g, glucosinolates 20 μmol g^{-1}, lodging resistance 6–8 points, height of plant 98–108 cm, growth period 90–108 days (Velička, 2003).

4.1.2. Experimental design

4.1.2.1. Fertilization treatments

The impact of microfertilizers on the number of flower visiting bees through the number of flowers and bee food resources on spring oilseed rape was studied in small-scale field experiments in two years, 2004 and 2005 (**II**). The soil in the experimental field was slightly acidic (pH_{KCl} 6.2) Stagnic *luvisol* (FAO classification LV st, 2006) with a loamy texture: humus content 2.4%, P – 77.66 mg kg^{-1}, K – 169.8 mg kg^{-1}, Ca – 5648 mg kg^{-1}, S – 13.54 mg kg^{-1}. In 2004, spring oilseed rape seeds were sown

on 5 May, and, in 2005, on 9 May at 200 germinating seeds per m^2, a sowing depth of 2–3(4) cm, and after a pre-crop of potato.

In both years, the experiment consisted of 32 plots (10 m^2 each), eight treatments with four replicates of each. Control plots received no fertilizer, the other plots received a complex fertilizer alone (Amsterdam Fertilizers B.V., The Netherlands), or the complex fertilizer plus one of six different microfertilizers (Phosyn P.L.C., York, United Kingdom). The treatments were:

1. 0 (no mineral fertilizers);
2. OptiCrop (Opti) (only the mineral complex fertilizer OptiCrop NPK 21-08-12 + S + Mg + B + Ca, the amount of nitrogen applied 120 kg ha^{-1});
3. Opti + HydroPlus™ Boron (Opti + B) (consumption rate of B 2 l ha^{-1});
4. Opti + HydroPlus™ Micro Copper (Opti + Cu) (consumption rate of Cu 0.5 l ha^{-1}).
5. Opti + Hydromag 300 (Opti + Mg) (consumption rate of Mg 7 l ha^{-1});
6. Opti + HydroPlus™ Micro Manganese (Opti + Mn) (consumption rate of Mn 1 l ha^{-1});
7. Opti + HydroPlus™ Micro Molybdenum (Opti + Mo) (consumption rate of Mo 0.25 l ha^{-1});
8. Opti + Sulphur F3000 (Opti + S) (consumption rate of S 7 l ha^{-1}).

Prior to sowing, the whole field was sprayed with the soil-applied herbicide EK Trifluralin (0.15 l ha^{-1}). The mineral complex fertilizer OptiCrop was used (except for treatment 0). Liquid microfertilizers (spray volume 400 l ha^{-1}) were sprayed on to the oilseed rape leaves when the plants had reached the growth stage (GS) 27-31 according to the BBCH scale (Lancashire *et al.*, 1991). The flowering period of the crop lasted from 5 to 22 July in 2004, and from 28 June to 18 July in 2005.

4.1.2.2. Insecticide treatments

To study the repellent effect of the insecticide Fastac 50 EC on the density of the honey bee, two experiments were carried out on spring oilseed rape crops in 2003-2005 (**III**). A commercial formulation of alpha-cypermethrin (Fastac 50 EC, a.i. 50 g l^{-1}; BASF, Limburgerhof, Germany) was used at a rate of 0.15 l ha^{-1}.

Experiment 1: effect of Fastac 50 EC treatment intensity on the number of honey bees in small-scale field experiments

The first experiment with the insecticide was performed on small patches of spring oilseed rape treated once or twice with the insecticide to determine whether honey bees discriminate between differently treated plants. The design of the experiment was a randomized block with twelve 1 x 10 m^2 plots with a distance of 1 m between each. The observation area was surrounded with a 5 ha field of summer wheat. Three treatments were used: unsprayed, once sprayed and sprayed twice, each replicated 4 times. In the sprayed-once treatment, the insecticide was applied when rape plants were at the growth stage 2–4 true leaves (GS 10, according to Lancashire *et al.*, 1991). For the twice-sprayed treatment, the first spray was applied at the same time as the once-sprayed plots with an additional application at the stage of first flowers (GS 61–62). The lengths of flowering periods differed according to weather conditions and lasted from 2 weeks (2004) to 3.5 weeks (2005).

Experiment 2: honey bee abundance before and after Fastac 50 EC treatment in field experiment

The second experiment with the insecticide was carried out on a seed production field of spring oilseed rape to test the abundance of honey bees before and after insecticide application. The experiment was conducted in July 2003. A spring oilseed rape field (4 ha) was divided into two parts (approximately 2 ha): one part was treated with Fastac 50 EC at the mid-flowering stage (GS 65–66, according to Lancashire *et al.*, 1991) and the other was left untreated. Within both fields, seven 1 × 10 m^2 observation plots were marked 8 days before the treatment. Six honey bee colonies were brought close to the crops (200 m away) 2 days before flowering started (late bud stage, GS 60). To prevent direct poisoning of honey bees, the hives were closed before the insecticide application and kept closed for 24 h.

4.1.3. Counting of bees and flowers

In the small-scale field experiments, the flower visiting bees (honey bees **II, III**, bumble bees **II**, solitary bees **II**) and flowers (**II, III**) were counted twice a week during the flowering period of the crop, in case of the insecticide treatment study (**III**) starting at 24 h after the second

spray application. In the field experiment at Pilsu, flower and bee counts were made 8 days before and 1 day and 8 days after insecticide treatment (**III**).

During bee counts, the observer walked slowly along the plot and recorded all bees foraging on the oilseed rape on each 10 m^2 plot. Flowers were counted simultaneously with flower visiting bees on an area of 1 m^2 within each plot. The observations were made on sunny days when there was no rain or fog between 11:00 and 16:00 (around midday) when temperature was above 16°C and wind speed did not exceed 6 m s^{-1}. (**II, III**)

4.1.4. Measurement of nectar and pollen production

In order to evaluate the impact of microfertilizers on food resources of bees on spring oilseed rape, pollen and nectar production were quantified (**III**).

Nectar production was measured from five flowers in each plot three times during the flowering period of the crop in 2004. The measurement was carried out in late morning at full flowering of the plants. Each flower was previously covered with a voile bag for 24 h to exclude floral visitors and to prevent nectar consumption the day before nectar measurement. Nectar production was measured in the field by inserting a 1 µl capillary into the flower corolla tube. It should be noted that nectar productivity can only be measured when there is no precipitation during 24 h. As, in 2005, there was little rain on almost all days of the flowering period of spring oilseed rape, nectar production was analysed only for 2004.

In 2004 and 2005, after anthesis, pollen production was quantified for 5 flowers in each plot at the same time as flower visiting bees and flowers were counted. The flowers were collected randomly from the plant main raceme and stored separately. These racemes were previously isolated to avoid consumption of the pollen by pollen beetles (*Meligethes* sp.). The flowers with pollen were later acetolysed (Faegri, Iverson, 1989) to digest both the floral tissue and pollen content, leaving pollen exines intact. The separated pollen was dispersed in distilled water (1 ml). The pollen grains were counted with a light microscope using a Fuchs-Rosenthal chamber (3.2 mm^3). These data were used to calculate the number of pollen grains per flower.

4.2. Laboratory experiments

4.2.1. Subjects

Colonies (Natupol hives) of the bumble bee *Bombus terrestris* L. were purchased from Koppert Biological Systems B.V. (Berkel en Rodenrijs, The Netherlands) to study the effect of low concentrations of Fastac 50 EC (a.i. alpha-cypermethrin 50 g l^{-1}; BASF SE, D-67056 Ludwigshafen, Germany) on the metabolic rate, respiratory pattern and total water loss rate of bumble bee *B. terrestris* foragers (**IV**).

4.2.2. Laboratory equipment and measurements

Bumble bees
The hives were kept at room temperature and the bees fed with dried honey bee pollen and a sugar solution (30%). The bees used in the experiment were caught as they emerged naturally from the hive entrance tunnel; this ensured that all of them were foragers.

Respirometry
An infrared gas analyser (IRGA, Infralyt-4, VEB, Junkalor, Dessau), adapted for entomological research, was used in the first experiment, to record the CO_2 signals and metabolic rates (VCO_2 ml h^{-1}) at 8°C. The IRGA was calibrated at different flow rates using calibration gases (Trägergase, VEB, Junkalor, Dessau) with gas injection (Kuusik *et al.*, 2002; Martin *et al.*, 2004; Mänd *et al.*, 2005, 2006). The rate of carbon dioxide release was measured (VCO_2 ml h^{-1}) at an air flow rate of 120 ml min^{-1}, a pressure compensated URAS 26 (ABB Analytical, Frankfurt, Germany), covering a measuring range of 0 to 500 ppm. The data from the analyser were sampled at a rate of 10 Hz to PC via the analog output. The CO_2 and H_2O were eliminated from the flow-through system air by DRIERITE and a molecular sieve.

The LI-7000 differential CO_2/H_2O Analyser (LiCor, Lincoln, Nebraska, USA), designed for laboratory and field research applications, was used in the second experiment to record water loss (VH_2O µl h^{-1}) parallel to the bursts of CO_2 releases in bumble bee foragers at 18°C. Air flow in LI-7000 was regulated at 166 ml min^{-1} (10 l h^{-1}). The CO_2 and H_2O were eliminated from the air used in the flow-through system by NaOH and $Mg(ClO_{42})$. The IRGA was calibrated using NIST-traceable standard gases (for CO_2).

Infrared-actography

The LI-7000 was combined with an infrared (IR) actograph to record abdominal movements. The actograph has also been used as an insect IR cardiograph or optocardiograph (Hetz, 1994; Hetz *et al.*, 1999; Mänd *et al.*, 2006; Karise *et al.*, 2010). Two IR-emitting diodes (TSA6203) were placed on one side (ventral side of the insect abdomen) and two sensor diodes (BP104) were placed on the opposite side of the insect chamber. Abdominal movements caused changes in the light transmitters, which were converted into voltages and recorded as spikes.

Treatments

Fastac 50 EC was used to measure its effect on bumble bee respiratory patterns and water loss. We diluted the Fastac 50 EC to 0.04% (20 parts per million (ppm) of alpha-cypermethrin), which corresponds to the registered field rate in Estonia of 20 g a.i. ha^{-1}. For our experiments, the field dosage of Fastac 50 EC was diluted with distilled water to 0.004% (2 ppm of alpha-cypermethrin) and 0.002% (1 ppm of alpha-cypermethrin) which are accordingly 10 and 20 times lower concentrations than recommended for treating flowering rape fields against pests. The bumble bees were dipped into the Fastac 50 EC solution or distilled water as control for 10 seconds (Saba, 1971). Following dipping, each bee was air-dried on filter paper. This dipping method is widely used in various insect toxicology experiments with differing solvents or submergence times (5 sec to 1 min) by both insect larvae (Isayama *et al.*, 2005; Cetin *et al.*, 2006; Erler *et al.*, 2010) and adults (Sibul *et al.*, 2004; Azimi *et al.*, 2009). In the case of bumble bees, the dipping method has been used as an alternative method in contact tests (van der Steen, 2001).

The measurements

The measurements lasted for six hours per individual bumble bee. All individuals were measured in the flow-through respirometer for three hours after which the insect chamber was opened and the bumble bee taken out for treatment. The treatment, according to the prescribed scheme (different concentrations of Fastac 50 EC or distilled water), was carried out immediately and the bee then returned to the insect chamber for the next three hours.

In the first experiment, the metabolic rate and the frequency of bursts of CO_2 releases of *B. terrestris* foragers were measured at 8°C. Bumble bees are very active insects and tend to maintain high body temperature

by shivering and contracting their flight muscles. The temperature was chosen to prevent flight muscle activity in bees (Goller, Esch, 1990; Kuusik *et al.*, 2002) and eventually the regular DGE appeared in most of individuals.

In the second experiment, muscle activity, respiration rate and WLR were measured at 18°C. Bumble bees often experience this temperature when foraging. For bumble bees it is important to keep their thoracic temperature high for several reasons: to minimise pre-flight warm-up time when exploiting different inflorescences and to minimise escape time when avoiding predators (Nieh *et al.*, 2006). That is why many bumble bee individuals shorten the length of the DGE cycles or do not show DGE at all at 18°C. Therefore, we did not count the clear cycles of discontinuous gas exchange at this temperature; instead, we examined the change in the respiratory and abdominal activity patterns. The higher metabolic rate also increases the WLR of the insect; therefore the differences in WLR should be more easily detectable.

The dose of alpha-cypermethrin bumble bees received (measured from ground-up bumble bee bodies) was 0.995 ± 0.227 μg g^{-1} (0.004%) and 0.87 ± 0.18 μg g^{-1} (0.002%) (analysed by Agricultural Research Centre, Laboratory for Residues and Contaminants, Teaduse 4/6, Saku, 75501 Harjumaa, Estonia). The method used in the chemical analysis was EN 12393-1,2,3: 1998 GC-ECD/NPD, GC-MS, LC-MS/MS; Norwegian Crop Research Institute Pesticide Lab, M04.

The longevity of bumble bees

Bumble bees treated with Fastac 50 EC solutions of both concentrations or distilled water, as described above, were kept at room temperature in the dark. Each bee was placed in a separate chamber and provided with 30% sugar solution as food. The bumble bees were checked daily until death. They were considered dead when they did not move antennae or legs and did not respond to tactile stimulation. Then death was confirmed using LI-7000 (Jõgar *et al.*, 2008).

4.3. Data acquisition and statistics

Statistical analyses were performed using the software package STATISTICA (StatSoft, Inc., Tulsa, Oklahoma). To determine the correlations between two factors Pearson (**II**) and Spearman (**III**)

correlations were used. To compare the mean values between the groups t-test (**III**), paired t-test (**IV**), Kruskal-Wallis test (**IV**) and ANOVA (**II, III**) were used. Differences between means were inspected using Fisher's protected significant difference post hoc analysis (**II**). Data were normalised where necessary (**II**).

Computerised data acquisition and analysis were performed using the DAS 1401 A/D (analog-digital) hardware and the software TestPoint (Keitley, Metrabyte, USA) with a sampling rate of 10 Hz (**IV**). The LI-7000 analyser was connected to a computer to record CO_2 production in ppm using LiCor software. Mean metabolic rates were automatically calculated by a statistical program by averaging data over 3 h periods after excess CO_2 and H_2O, which had entered the system during handling, had left the system.

5. RESULTS

5.1. The impact of foliar fertilization on the number of flowers, food resources and on the number of bees (Apoidea) on spring oilseed rape (II)

5.1.1. Flower density

In both years (2004 and 2005), the abundance of flowers was significantly higher on fertilized than on unfertilized plots ($F_{7,184}$ = 2.83, p = 0.01 in 2004; $F_{7,216}$ = 2.85, p = 0.01 in 2005). No significant differences between differently fertilized plots, including plots fertilized only with the complex fertilizer OptiCrop, were found (**II** Figure 1).

5.1.2. Food resources for bees

Except for fertilization with manganese or only with the complex fertilizer OptiCrop, the production of nectar in 2004 was significantly higher on fertilized than on unfertilized plots ($F_{7,312}$ = 2.48, p = 0.02). Plots fertilized with OptiCrop plus manganese had significantly lower nectar production than plots fertilized with OptiCrop plus one of the other five microfertilizers (**II** Figure 2). Even plots fertilized with OptiCrop alone resulted in more flowers than plots fertilized with OptiCrop plus manganese.

The production of pollen was in both years, especially in 2005, higher on fertilized than on unfertilized plots (**II** Figure 3) but the differences were not statistically significant ($F_{7,248}$ = 1.15, p = 0.33 in 2004; $F_{7,344}$ = 2.02, p = 0.05 in 2005). However, when summarizing over these two years, the effect of treatment became significant (**II** Table 1). In addition, there was no statistically significant interaction between year and treatment on the number of pollen grains produced per flower which means that the impact of different treatments followed the same trend in both years, being higher on fertilized than on unfertilized plots.

5.1.3. The number of flower visiting bees

In both years, the number of flower visiting bees was higher on fertilized than on unfertilized plots (**II** Figure 4) but the difference was statistically significant only in 2004 ($F_{7,184}$ = 2.62, p = 0.01 in 2004; $F_{7,216}$ = 1.24,

p = 0.28 in 2005). When summarizing over two years, the effect of treatment was significant (**II** Table 2). Moreover, there was no statistically significant interaction between the year and treatment on the number of flower visiting bees which means that the impact of different treatments followed the same trend in both years being higher on fertilized than on unfertilized plots.

5.1.4. Correlations between flower visiting bees and the number of flowers and food resources of spring oilseed rape

A significant positive correlation between the number of flower visiting bees and the number of flowers was found in both years (r = 0.59, p < 0.01 in 2004; r = 0.69, p < 0.01 in 2005). The number of flower visiting bees correlated also moderately with nectar production (r = 0.41, p < 0.01) and, in 2005, weakly with pollen production (r = 0.21, p < 0.01).

5.2. The impact of Fastac 50 EC on bees

5.2.1. The impact of Fastac 50 EC treatment intensity on the number of honey bees in small-scale field experiments (III)

The total number of bees differed significantly between experimental years ($F_{2,177}$ = 3.7, p = 0.03). Nevertheless, there was no significant difference in the number of honey bees per 1000 flowers between treatments either during the whole observation period ($F_{2,57}$ = 0.3, p = 0.8 in 2003; $F_{2,33}$ =

Table 1. The Spearman Rank Order Correlations between the number of honey bees and the number of flowers on the experimental plots (10 m^2). In bold letters statistically significant correlations at $p \leq 0.05$ are indicated.

Year	Trial	N	Spearman R	p
2003	Untreated	20	0.44	0.06
	Once-treated	20	0.33	0.16
	Twice-treated	20	0.68	**<0.01**
2004	Untreated	12	0.26	0.39
	Once-treated	12	0.14	0.66
	Twice-treated	12	0.68	**0.02**
2005	Untreated	28	0.10	0.63
	Once-treated	28	0.11	0.59
	Twice-treated	28	0.74	**<0.01**

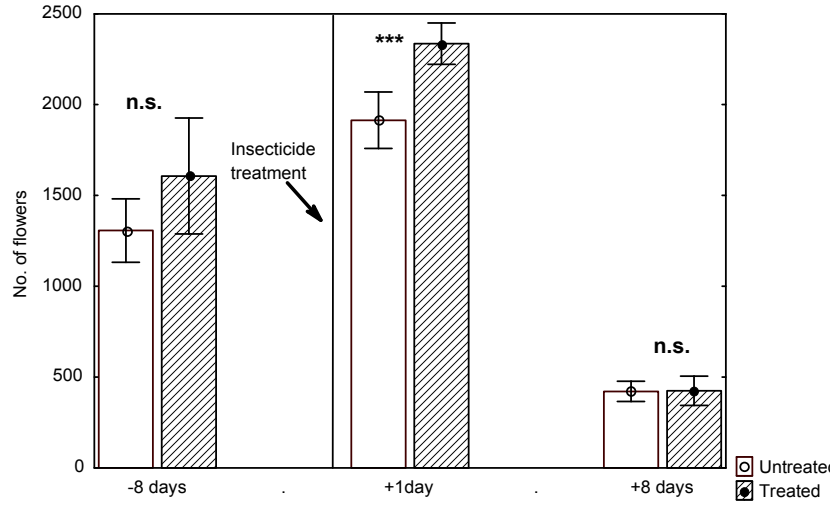


Figure 1. The number of flowers per 1 m^2 on three observation days on seed production crops adjacent to each other. The dots indicate the mean value and the whiskers indicate the standard error. *** – $p \leq 0.05$, n.s. – statistically not significant.

0.7, $p = 0.5$ in 2004; $F_{2,81} = 0.04$, $p = 0.9$ in 2005) (**III** Figure 1) or 24 h after spraying of flowers (GS 61-62) ($F_{2,9} = 0.5$, $p = 0.6$ in 2003; $F_{2,9} = 1.6$, $p = 0.3$ in 2004; $F_{2,9} = 0.2$, $p = 0.8$ in 2005) in any of the years. However, flower densities differed significantly between the treatments in all years ($F_{2,57} = 5.2$, $p < 0.01$ in 2003; $F_{2,33} = 8.4$, $p < 0.01$ in 2004; $F_{2,81} = 8.2$, $p < 0.01$ in 2005). There were positive correlations between flower densities and the abundance of honey bees on the flower-rich patches (Table 1, **III** Figure 2).

5.2.2. Honey bee abundance before and after Fastac 50 EC treatment in field conditions (III)

In field conditions, 24 h after spraying, the number of honey bees per 1000 flowers on the Fastac 50 EC treated crop was significantly higher than on the untreated crop ($t = 4.4$, df = 12, $p < 0.01$). However, 8 days before and 8 days after the insecticide application the number of honey bees per 1000 flowers between the Fastac 50 EC treated and untreated crops did not differ significantly (accordingly $t = 1.7$, df = 12, $p = 0.12$ and $t = 0.2$, df = 12, $p = 0.9$) (**III** Figure 3). The insecticide application took place during the peak flowering period, when the differences in flower numbers appear most clearly, the food resource was significantly higher on the treated field than on the untreated field (24 h after treatment: $t =$

2.2, df = 12, p = 0.048) (Figure 1). These differences did not appear at the beginning and at the end of flowering (accordingly t = 1.5, df = 12, p = 0.2 and t = 0.04, df = 12, p = 0.9).

5.2.3. The impact of Fastac 50 EC on the DGE of bumble bees (*Bombus terrestris*) (IV)

5.2.3.1. The experiment at 8°C

At low temperature the untreated resting bumble bee foragers exhibited rhythmic gas exchange patterns. 0.004% Fastac 50 EC solution changed the respiratory patterns of bumble bees. The numbers of bursts of CO_2 releases and the mean metabolic rates decreased significantly (**IV** Table 1). Treating the bees with 0.002% solution also caused a decrease in the numbers of bursts of CO_2 releases, although the difference was not statistically significant. The mean metabolic rate decreased significantly. Dipping the bumble bees into distilled water as a control affected neither the frequency of bursts of CO_2 releases nor the mean metabolic rate (**IV** Table 1).

5.2.3.2. The experiment at 18°C

The time for bumble bees to calm down and show CGE or DGE cycles were longer at 18°C – activity was higher than at low temperature. Depending on the activity type which the certain specimen belonged to (R. Karise, unpublished) the bumble bees showed different patterns of muscle activity (not locomotor activity) before the treatment. Some bumble bees showed the DGE pattern already 10-30 minutes after insertion into the insect chamber, whilst others needed more time to calm down before showing regular CGE or DGE. Longer or shorter periods of CGE or DGE usually interchanged the periods of active ventilation.

Treating the bees with 0.004 % Fastac 50 EC solution caused rapid disappearance of both rhythmic release of CO_2 and muscle activity (**IV** Figure 1A, B). In DGE, the bumble bee uses muscle work only during the short O period to aid gas exchange; after treatment, regular cycles disappeared and a long-lasting muscle tremor appeared. As a result of the treatment with the 0.004% solution the metabolic rate in one individual increased and in others decreased significantly. However, no significant effect on WLR was found (**IV** Table 2).

Treating the bees with 0.002% Fastac 50 EC solution did not disrupt either the regular bursts of CO_2 releases or muscle activity (**IV** Figure 2A, B) but the DGE was replaced by CGE (**IV** Figure 3A, B). In the case of CGE, the level of CO_2 release does not fall to near zero as happens during DGE. The treatment resulted in significantly lower metabolic rates but no significant effect on WLR was found (IV Table 2).

Treating the bees with distilled water did not disrupt either the DGE, if it had been present before the treatment, nor the muscle activity of the bumble bee foragers. The metabolic rate and WLR also did not change significantly (**IV** Table 2).

The simultaneous measurement ensured the exact coincidence of the bursts of CO_2 and H_2O release (**IV** Figure 4). However, during activity, the H_2O release was not recognisably higher compared to the WLR in the C-phase. Respiratory transpiration constituted only a small part, less than 10% of total transpiration in the bumble bee foragers.

5.2.3.3. The effect of Fastac 50 EC on bumble bee longevity

The mortality rate of bumble bees treated with different Fastac 50 EC solutions was affected by the solution concentration ($H (2, N = 30) = 11.73$, $p < 0.01$). Treatment with 0.002% solution did not shorten the life span of the bees significantly compared to those treated with distilled water ($p > 0.05$) (**IV** Figure 5). However, most individuals treated with the higher concentration solution (0.004%) died within 1-3 days, although individual variation was observed (one specimen lived for 8 days, another 16 days). The bee which lived for 16 days after the treatment was also repeatedly controlled in the respirometer, which showed that the normal DGE or CGE recurred 48 hours after treatment and this pattern persisted at least until day 4. Also muscle activity recurred on day 3. On day 6, there was neither DGE nor regular CGE.

6. DISCUSSION

6.1. The impact of spring oilseed rape foliar fertilization on the number of flowers and food resources provided for bees

Our results showed that oilseed rape fertilization increased the number of flowers as well as the production of nectar and pollen per flower. However, foliar fertilization with different microfertilizers in addition to the mineral complex fertilizer OptiCrop had no effect on the number of flowers and pollen compared to the plots fertilized only with OptiCrop, but affected the production of nectar.

The positive effect of fertilization on the number of flowers can be explained by the fact that oilseed rape, as a fast growing crop, needs a high amount of nutrients; otherwise its growth will slow down and, as a result, the number of flowers produced is also lower. In the case of resource deficiency, oilseed rape plants probably preserve the size of flowers rather than the number of flowers (Cresswell *et al.*, 2001). Although the application of microfertilizers had no additional effect on the number of flowers and pollen production, the relevance of micronutrients should not be underestimated – their importance for plant physiology lies in their influence on enzyme reactions and therefore deficiencies may severely limit crop yields (Orlovius, 2003). Balanced nutrition of oilseed rape is important to ensure optimum seed yield and quality as well as the most economic response to applied fertilizer (Grant, Bailey, 1993).

In both years, especially in 2005, the production of pollen was higher on fertilized than on unfertilized plots but the difference was not statistically significant probably because of high variability of pollen production. When summarizing over the years, the effect of treatment became significant being higher on fertilized plots than on unfertilized plots. Such an effect of fertilization can probably again be explained by the fact that oilseed rape, as a fast growing crop, needs a high amount of nutrients – otherwise its viability decreases. Recently, considerable attention has been paid to pollen dissemination by pollinators (Hayter, Cresswell, 2006; Chifflet *et al.*, 2011) and the influence of other factors on pollen transfer and gene flow (Beckie *et al.*, 2003; Devaux *et al.*, 2008; Sausse *et al.*, 2012) in connection with potential problems associated with the adoption of genetically modified oilseed rape. However, the effect of fertilization on pollen production has not received attention so

far, although this could affect the number of effective pollinators, bees, on oilseed rape.

Nectar production per flower in our study appeared to be inhibited by additional manganese. Manganese increases plant height, leaf area per plant and dry weight of the aerial parts (Ali *et al.*, 2011b), and apparently, plants contribute less to nectar production. Several authors have studied the dependence of nectar production of oilseed rape flowers on varietal (Mohr, Jay, 1990; Kotowski, 2001) and genetic differences (Pierre *et al.*, 1999) but not the effect of fertilization on nectar production. As several factors have been found to affect nectar production and nectar standing crop, e.g. evaporation and absorption (Corbet, 2003), final conclusions cannot be done on the basis of one study year, although a preliminary trend is evident. The topic of the effect of fertilization on nectar production needs further research.

6.2. The number of flower visiting bees on spring oilseed rape with different fertilization treatments

Our results showed that, in 2004, the number of flower visiting bees was significantly higher on fertilized than on unfertilized plots. In 2005, a similar trend appeared but the difference was not statistically significant. However, when summarizing over two years, the effect of treatment was significant. These results can be explained by the number of flowers and food resources offered on differently fertilized plots. The density of flower visiting bees – honey bees, bumble bees and solitary bees – on spring oilseed rape correlated strongly with flower density. Rosa *et al.* (2011) also found a significant positive correlation between the number of oilseed rape flowers and the number of honey bees. In addition, the number of flower visiting bees in our study correlated moderately with nectar production and weakly with pollen production (but only in 2005).

Most bees collect only two food items from flowers: nectar, which provides bees with energy, and pollen, which provides them with protein necessary for growth of larvae (Rasheed, Harder, 1997). According to the optimal foraging theory, bees try to maximize the benefit and minimize the costs (Pettersson, Sjödin, 2000). Hence, the food collected from the flower – the reward – has to exceed the energy spent on searching for food. The positive correlation between the number of flower visiting bees and the number of flowers found in this experiment concurs with this

theory. It is energetically more profitable to choose denser flower areas in order to expend less energy in flying between flowers (Cartar, Real, 1997). As the nectar of oilseed rape flowers can be replenished within half an hour of depletion (Pierre *et al.*, 1999), encountering empty flowers is unlikely.

6.3. Impact of Fastac 50 EC on the honey bee abundance

The number of foraging honey bees in small-scale field experiments did not differ between the patches treated with Fastac 50 EC once or twice and those not treated with the insecticide. No repellent effect of the insecticide on honey bees was found even 24 h after spraying although Fastac 50 EC has been reported to maintain repellency to bees for 48 h after treatment (Thompson, 2001). These results persisted through three observation years regardless of varying flower and honey bee densities. We also found that on flower-rich observation plots, the numbers of bees and flowers were positively correlated, whereas on sparse patches no such correlation was found. According to the theory of optimal foraging, animals distribute themselves among differently rewarding food resources so that the average amount of food per individual remains equal (Alonso *et al.*, 1995). Despite the theory, flower-rich patches of oilseed rape were even more attractive for the bees. Thus, the density of oilseed rape flowers most likely played a major role in choice of foraging area. The large scale field experiment confirmed this result. The field with the higher food resource attracted more bees regardless of the Fastac 50 EC treatment.

Fastac 50 EC (a.i. alpha-cypermethrin) did not show repellency to honey bees in small-scale field experiments or in the field experiment. Instead, flower density seemed to be the main signal for honey bees probably overriding the possible repellent effect. Fastac 50 EC has been reported to be repellent for honey bees at least for 48 h. Most studies on repellency have been performed in the laboratory or in semi-field conditions but these may not reflect the real situation in field conditions (Thompson, 2003). Mayer and Lunden (1999) found no repellent effect of alpha-cypermethrin on bees applied at the field rate to flowering oilseed rape. Evidence for repellency may also be questioned by the detection of cypermethrin residues in honey and wax (Pareja *et al.*, 2011).

Fastac 50 EC is commonly used to control pollen beetles in oilseed rape which contributes to higher flower densities as the damage caused by the

larvae to the flowering structures is prevented. So, treated crops may be even more attractive to bees than untreated crops as these may often have higher flower densities. Residues of alpha-cypermethrin on oilseed rape leaf surfaces have been shown to be toxic for more than 3 days following insecticide application and may kill up to 25 % of bees that come into contact with them (Cox, 1996). Choudary and Sharma (2008) proved the persistence of another pyrethroid, lambda-cyhalothrin, residues in the nectar and pollen of mustard at least 72 h after treatment. Thus, in field conditions, honey bees can become contaminated with the residues of insecticides even if the hives have been kept closed for some time after spraying as suggested by chemical companies.

6.4. Impact of Fastac 50 EC on the respiration of bumble bees

Our results show that Fastac 50 EC has a dose dependent after-effect on bumble bee respiratory rhythms, metabolic rate and muscle activity but has no effect on WLR. The regular periods of discontinuous or cyclic gas exchange disappeared during the first 30 minutes after treatment with 0.004% Fastac 50 EC solution. This treatment also shortened the lifespan of bumble bees. Contact with 0.002% Fastac 50 EC solution did not provoke that kind of drastic disappearance of rhythmic gas exchange and the longevity of bumble bees did not change compared to control bees treated with distilled water.

We found a decline in metabolic rates of bumble bees after contact with Fastac 50 EC, a pyrethroid insecticide. Some other researches also interpret the reduction in metabolic rate as a generalized response to stressors (e.g., toxins, insecticides, heat and cold) that could lead to a reduction in respiratory water loss (Hoffmann, Parsons, 1989; Chown, Gaston, 1999). By contrast, Kestler (1991) claims that negative stressors raise standard metabolic rate of resting insects. Jõgar *et al.* (2006) also described the rise in metabolic rates after treatment with Neem EC in Colorado potato beetles. Sibul *et al.* (2004), however, did not see any change in metabolic rates of pine weevils after contact with Neem EC. These results suggest that the effect of pesticides on metabolic rates of insects depends largely on both insect species and pesticide formulation.

Meanwhile, the existence and nature of carbon dioxide emission patterns also depends on many factors which include environmental conditions (Kestler, 1971; Dingha *et al.*, 2005; Terblanche *et al.*, 2008; Karise *et*

al., 2010), metabolic rate (Kestler, 1991; Sibul *et al.*, 2004; Jõgar *et al.*, 2006), the life stage of the insect (Beekman, Stratum, 1999; Mänd *et al.*, 2005, 2006) and several stress factors (Kestler, 1991; Lighton, Lovegrove, 1990; Kovac *et al.*, 2007). Normally bumble bees show DGE cycles as a sign of calming down or resting. The events of calming down are clearly observed on the respirograms of bumble bees (Karise *et al.*, 2010).

According to Kestler (1991), the pathological CO_2 release patterns can be divided into phases: latency phase with closed-flutter-ventilation (CFV), followed by continuous respiration with small irregular bursts of CO_2 releases. Kestler considers this to be a reversible excitation phase being a typical stress index for sub-lethal doses of neurotoxic pesticides. The reversible excitation phase devolves to an irreversible excitation phase with no bursts of cyclic CO_2 release. At that time, the spiracles stay open and are paralysed.

The respiratory rhythms of bumble bees altered clearly after treatment with alpha-cypermethrin, the neurotoxic active ingredient of Fastac 50 EC. Contact with the 0.004% solution caused rapid disappearance of the respiration cycles in most of the foragers. Contact with the 0.002% solution of Fastac 50 EC changed the classical CFO (closed-flutter-open) cycles to FV (flutter-ventilation) cycles within about the first 30 minutes; later the bouts of CO_2 releases disappeared. If the large bouts of CO_2 releases occurred after treatment, these were rather FV cycles instead of CFO cycles. Two specimens out of six showed large bursts of CO_2 releases after the treatment, others showed varying rates of released CO_2 of a relatively low but smooth level. We saw the shift from cyclic towards continuous respiratory behaviour along with decreasing metabolic rate due to non-ability of bumble bees to keep the spiracles closed. The diminishing muscle work after the treatment with the neurotoxic chemical (Zafeiridou, Theophilidis, 2006; Woodman *et al.*, 2008) is most likely the result of paralysis, not the result of calming down. In unstressed insects, the decreasing metabolic rate is a sign of calming down and therefore the shift towards classical DGE should appear (Bradley, 2007; Gray, Chown, 2008; Moerbitz, Hetz, 2010).

It seems reasonable to conclude that a dose of 0.004% Fastac 50 EC is not sub-lethal, but lethal. For most individuals, the symptoms of intoxication were irreversible. The fact that at least two individuals lived for longer (8 and 16 days), shows that this concentration must be near the lethal dose

for bumble bees but indicates also the heterogeneity of the *B. terrestris* population in the context of alpha-cypermethrin immunity. We interpret that, according to Kestler´s (1991) classification, the bumble bees must have been in reversible excitation phase only. The three hour period must have been too short to see total recovery from the intoxication. We saw the reappearance of the regular DGE in the bumble bees which survived the higher dose and lived for 8 or 16 days after treatment.

Total water loss did not differ significantly after dipping the bees into distilled water or into the Fastac 50 EC solution of either concentration although metabolic rate decreased significantly after the insecticide treatments. However, the WLR showed a tendency to increase after treatment of the bees with the 0.004% solution, while decreasing after treatment with 0.002% solution or distilled water. The decreasing WLR is normal when metabolic rate decreases. At lower metabolic rate the gas exchange including WLR is lower. The slightly higher WLR after the treatment with 0.004% Fastac 50 EC solution was not caused by muscular excitation, since this would have been seen on the actograph recordings. We suppose that, due to paralysis, the spiracles of the bumble bees may have been open (continuous CO_2 release) after treatment and along with the outflow of CO_2, the water vapour was also washed out from the tissues of moribund insects. Total water loss has been showed to be higher during continuous, compared to discontinuous, CO_2 release (Matthews, White, 2012).

Several studies reveal that respiratory water loss comprises mostly a small fraction of total water loss, even when the spiracles are open (Quinlan, Hadley, 1993; Quinlan, Lighton, 1999; Chown, 2002; Gibbs, Johnsson, 2004; Lighton *et al.*, 2004). We suppose that, for bumble bees, respiratory water loss probably does not play a very important role and the non-ability to DGE and desiccation thereafter was not the direct cause of death. The importance of respiratory water loss differs between insect species (Lamprecht *et al.*, 2009) depending more or less on water permeability of the cuticle. Bumble bees feed mostly on liquid food and therefore they need to discharge excess water, and the water permeability of their cuticle is high (Nicolson, 2009). A characteristic of bee water balance is the rapid mobilisation of ingested dietary water from the crop to the haemolymph, allowing rapid correction of haemolymph osmotic pressure (Willmer, 1986). Besides, in larger bees like *Xylocopa* and *Bombus sp*, the metabolic water may be in excess during flight and

occasionally these bees eliminate water by spitting or by defaecation (Bertsch, 1984; Willmer, Stone, 1997). Because of these characteristics of bumble bee physiology, which allow them to be less judicious about respiratory patterns, and based on our results, we do not believe that death resulted from desiccation, even if the pyrethroid had increased the diuretic event. Still, the DGE cycles may confer a fitness benefit to the bumble bee *B. terrestris*. We did not find proof for the theory of DGE cycles functioning as a water saving mechanism; rather our results support the oxidative damage hypothesis (Hetz, Bradley, 2005). Probably, the intoxicated bumble bees were paralysed and their spiracles were open: the freely entering oxygen could have been the key factor diminishing their fitness. This kind of research may benefit from precise observation under the microscope on the behaviour of the spiracles during intoxication.

6.5. Implications to promote and protect bees on spring oilseed rape

There have been several agricultural changes in Europe during recent decades, e.g. homogenization of farmland landscapes and increase in application of chemicals, one of the main reasons for that being policy changes (Stoate *et al.*, 2009). The intensification of agriculture has brought along several environmental problems, including loss of biodiversity (Benton *et al.*, 2003). One of the concerns of biodiversity is the pollination crisis in the world. There is clear evidence of recent declines in both wild and domesticated bee-pollinators (Potts *et al.*, 2010) which have been observed in different regions of the world – America (Kremen *et al.*, 2004; Currie *et al.*, 2010), Asia (Klein *et al.*, 2003) and Europe (Williams *et al.*, 1991; Williams, 1996; Giray *et al.*, 2010). The principal factor is likely to have been the loss and degradation of habitats and of food resources due to changes in land-use and agricultural practice (Osborne *et al.*, 1991; Williams *et al.*, 1993; Mänd *et al.*, 2002; Sepp *et al.*, 2004; Goulson *et al.*, 2005; Öckinger, Smith, 2007; Potts *et al.*, 2010), including the intensive use of pesticides (Osborne *et al.*, 1999; Miranda *et al.*, 2003; Maini *et al.*, 2010). At the same time, bees are the most important pollinators of almost all terrestrial ecosystems because they provide a vitally important ecosystem service as pollinators for a wide range of agricultural, horticultural and wild plants (Corbet *et al.*, 1991; Williams 1994, 1996; Klein *et al.*, 2007; Pauw, Hawkins, 2011).

To mitigate the negative effects of agriculture on pollinators, necessary steps need to be taken but this requires enough knowledge. Oilseed rape is a very attractive food plant for bees offering ample additional food resources (Westphal *et al.*, 2003, 2009) and, at the same time, benefits from cross-pollination, including improving both the quantity and quality of the seed produced (Free, 1993; Sabbahi *et al.*, 2005; Chifflet *et al.*, 2011; Bommarco *et al.*, 2012). Therefore, the foraging activities of bees have significant economic consequences for seed production, thus factors that lower their pollinating activity on oilseed rape should be minimised (Thompson, Maus, 2007). As spring oilseed rape is a crop with high nutrient demand and, on the other hand, often needs the application of pesticides, the effect of these factors on bees, as the most important pollinators of oilseed rape, needs to be explained.

The results of our study showed that, to secure a higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollinating, spring oilseed rape should receive proper complex fertilization. Applied microfertilizers turned out to be useless in terms of increasing the number of pollinators. In addition, our study tends to confirm that Fastac 50 EC does not show repellency for honey bees in field conditions although it has been reported to maintain a repellent effect to bees for 48 h after treatment. It seems that the attractiveness of high flower density overrides the repellent effect. Thus, oilseed rape fields treated with Fastac 50 EC against pollen beetles contribute to higher flower densities and are even more attractive to bees. Our results also showed that Fastac 50 EC has a dose dependent effect on bumble bee respiratory rhythms, metabolic rate and muscle activity but has no effect on water loss rate. Even solutions with lower concentrations of Fastac 50 EC (solution with 0.004% Fastac 50 EC, 2 ppm of alpha-cypermethrin) than the registered field rate in Estonia (20 ppm) affected significantly the physiology of bumble bees.

The sub-lethal doses of pesticides bees encounter do affect the physiological state of the pollinators, being thus one possible reason for global pollinator decline. Pollinators have evolved to recognize different signals and react respectively. As the application of pesticides is a quite new phenomenon from the evolutionary perspective, no co-evolving has occurred and the pollinators are not able to recognize the hazards. It is obligatory for chemical companies to provide mortality data for their products for all larger organism groups. Unfortunately, the results

of laboratory and semi-field studies do not reflect the situation in field conditions as additional factors may affect the choices of bees, e.g. high flower density which seemed to be the main signal for bees in our studies.

One of the possibilities to mitigate the negative effects of pesticides would be to close honey bee hives during pesticide treatment but it has been shown that the residues on leaf surfaces can be toxic for more than 3 days following insecticide application (Cox, 1996; Thompson, 2003). So, honey bees may still come into contact with sub-lethal doses of pesticides which have multiple negative effects (Yang *et al.*, 2008; Aliouane *et al.*, 2009; Decourtye *et al.*, 2011; Wu *et al.*, 2011; Pettis *et al.*, 2012; Schneider *et al.*, 2012). In addition, the nests of native pollinators cannot be closed.

The management of pests on oilseed rape throughout Europe relies heavily on chemical pesticides, most often applied routinely and prophylactically, often without regard to pest incidence (Williams, 2004). This leads to the over-use of pesticides, which reduces the economic competitiveness of the crop and threatens biological diversity. Thus, the need for pesticide application on oilseed rape should certainly be previously monitored. In addition, the pests of oilseed rape have several natural enemies which have the potential to contribute to biological control (Veromann *et al.*, 2006b; Veromann *et al.*, 2006c; Ekbom, 2010). The protection of pollinators against the negative effects of agriculture, including pesticides, should be supported by policy, e.g. through appropriate measures like agri-environment schemes of the European Union common agricultural policy (Köster *et al.*, 2009).

7. CONCLUSIONS

Spring oilseed rape (*Brassica napus* L. var. *oleifera* subvar. *annua*) is an important oilseed crop, the area of which has increased significantly in northern Europe, including Estonia. Spring oilseed rape is predominantly autogamous but cross-pollination can have several positive effects, including higher seed yield and better quality. Hence, it is profitable to encourage a high number of pollinators in oilseed rape fields. On the other hand, the bees visiting oilseed rape flowers need to be protected against negative effects of pesticides which have become an almost inevitable part of cultivating these crops. In order to favour and protect pollinators of oilseed rape appropriate knowledge is needed. Thus, as spring oilseed rape is a crop with high nutrient demand and, on the other hand, often needs the application of pesticides, the effect of these factors on bees, as the most important pollinators of oilseed rape, needs to be explained. The results of the current work shed light on some of these issues:

- The density of flower visiting bees – honey bees, bumble bees and solitary bees – on spring oilseed rape correlated strongly with flower density (**II**). In addition, the number of flower visiting bees correlated moderately with nectar production and weakly with pollen production (but only in 2005).

- Oilseed rape fertilization increased the number of flowers as well as the production of nectar and pollen per flower thus increasing food reserves of bees (**II**). However, foliar fertilization with different microfertilizers, in addition to the mineral complex fertilizer OptiCrop, had no effect on the number of flowers and pollen production compared to the plots fertilized only with OptiCrop, but affected the production of nectar (which appeared to be inhibited by additional manganese).

- To secure a higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollination spring oilseed rape should receive correct complex fertilization (**II**). Applied microfertilizers turned out to be useless in terms of increasing the number of pollinators.

- The number of foraging honey bees did not differ between the patches treated with the pyrethroid insecticide Fastac 50 EC (a.i. alpha-

cypermethrin) and those not treated with the insecticide. Thus, the results of our study tend to confirm that Fastac 50 EC does not show repellency for honey bees in field conditions (**III**). No repellent effect of the insecticide on honey bees was found even 24 h after spraying although Fastac 50 EC has been reported to maintain repellency to bees for 48 h after treatment.

- Our experiments (**III**) indicate that honey bees foraging on spring oilseed rape were most attracted by high flower density which probably overrided the repellent effect of the insecticide. Controlling pollen beetles in oilseed rape with the Fastac 50 EC may contribute to higher flower densities.

- Treating bumble bees at 8ºC with 10 times lower concentrations of insecticide (solution with 0.004% Fastac 50 EC, 2 ppm of alpha-cypermethrin) than the registred field rate in Estonia (20 ppm) changed the respiratory patterns of bumble bees: the number of bursts of CO_2 releases and the mean metabolic rates decreased significantly (**IV**). 20 times lower concentrations also decreased significantly the mean metabolic rates.

- Treating bumble bees at 18ºC with 10 times lower concentrations of the recommended field dosage the rythmic release of CO_2 and muscle activity disappeared (**IV**). Treatment with 20 times lower concentrations of the recommended field dosage did not disrupt either regular bursts of CO_2 releases or muscle activity but discontinuous gas exchange was replaced by cyclic gas exchange. Both concentrations, at 18ºC, changed the mean metabolic rates significantly.

- We found no significant effect of the solutions with 0.004% or 0.002% Fastac 50 EC on water loss rate in bumble bees although the treatments changed their respiratory patterns (**IV**). Thus, we did not find evidence for the theory of discontinuous gas exchange functioning as a water saving mechanism.

- An after-effect of 0.004% Fastac 50 EC solution was a significant decrease in the longevity of bumble bees (**IV**).

The results of the current research lead us to conclude that to favour bees as the main pollinators of spring oilseed rape the crop should receive

correct complex fertilization to assure sufficient food resources for bees. It is obligatory for chemical companies to provide mortality data for their products for all larger organism groups. Unfortunately, the results of laboratory and semi-field studies do not reflect the situation in field conditions as additional factors may affect the choices of bees, e.g. high flower density which seemed to be the main signal for bees in our studies. In addition, the sub-lethal dose of pesticides bees encounter do affect the physiological state of the pollinators, being thus one possible reason for global pollinator decline. Pollinators have evolved to recognize different signals and react respectively. As the application of pesticides is quite a new phenomenon from the evolutionary perspective, no co-evolving has occurred and the pollinators are not able to recognize the hazards.

Pesticides should not be applied routinely and prophylactically without regard to pest incidence but the need for pesticide application should be previously monitored. The protection of pollinators against negative effects of pesticides should be supported by policy, e.g. through appropriate measures like agri-environment schemes of the European Union common agricultural policy. In addition to decreasing the amount of pesticides used further research on the ecotoxicity, after-effects and sub-lethal effects of pesticides is needed and more environmentally friendly growing technologies should be developed (e.g. entomovector-technology and biopesticides).

REFERENCES

Adegas, J. E. B., Couto, R. H. N. 1992. Entomophilous pollination in rape (*Brassica napus* L var *oleifera*) in Brazil. Apidologie, 23 (3), 203-209.

Alaux, C., Brunet, J.-L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L. P., Le Conte, Y. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology, 12 (3) , 774–782.

Alford, D. V. 1975. Bumblebees. London, 352 p.

Alford, D. V., Nilsson, C., Ulber, B. 2003. Insect pests of oilseed rape crops. In: (Ed. Alford, D. V.) Biocontrol of Oilseed Rape Pests. Blackwell Science, Oxford, UK, p. 9-42.

Ali, M., Saeed, S., Sajjad, A., Whittington, A. 2011a. In search of the best pollinators for canola (*Brassica napus* L.) production in Pakistan. Applied Entomology and Zoology, 46 (3), 353-361.

Ali, S. O. M., El-Hiti, S. M. J., Rezazadeh, S., Yekta, M. 2011b. Effect of potassium with or without micronutrients on plant growth and alkaloids content in deadly nightshade (*Atropa bella-donna* L.). Part 1. Plant growth and biomass. Zeitschrift Fur Arznei- und Gewurzpflanzen, 16 (2), 70-74.

Aliouane, Y., el Hassani, A. K., Gary, V., Armengaud, C., Lambin, M., Gauthier, M. 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behaviour. Environmental Toxicology and Chemistry, 28 (1), 113-122.

Alix, A., Vergnet, C. 2007. Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds. Pest Management Science, 63 (11), 1069-1080.

Alonso, J. C., Alonso, J. A., Bautista, L. M., Muñoz-Pulido, R. 1995. Patch use in cranes: a field test of optimal foraging predictions. Animal Behaviour, 49 (5), 1367-1379.

Azimi, M., Pourmirza, A. A., Safaralizadeh, M. H., Mohitazar, G. 2009. Studies on the lethal effects of pinosad on adults of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) with two bioassay methods. Asian Journal of Biological Sciences, 2 (1), 1-6.

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 (3), 293-300.

Baker, H. G., Baker, I. 1973. Amino-acids in nectar and their evolutionary significance. Nature, 241, 543-545.

Barnett, E. A., Charlton, A. J., Fletcher, M. R. 2007. Incidents of bee poisoning with pesticides in the United Kingdom, 1994-2003. Pest Management Science, 63 (11), 1051-1057.

Barraclough, P. B. 1989. Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. Plant and Soil, 119 (1), 59-70.

Beckie, H. J., Warwick, S. I., Nair, H., Séguin-Swartz, G. 2003. Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecological Applications, 13 (5), 1276-1294.

Beekman, M., van Stratum, P. 1999. Respiration in bumblebee queens: effect of life phase on the discontinuous ventilation cycle. Entomologia Experimentalis et Applicata, 92 (3), 295-298.

Bell, S. A., Cresswell, J. E. 1998. The phenology of gender in homogamous flowers: temporal change in the residual sex function of flowers of oil-seed rape (*Brassica napus*). Functional Ecology, 12 (2), 298-306.

Belzunces, L. P., Vandame, R., Xingfa, G. U. 2001. Joint effects of pyrethrinoid insecticides and azole fungicides on honey bee thermoregulation. In: (Ed. Belzunces, L. P., Pélissier, C., Lewis, G. B.) Hazards of Pesticides to Bees. INRA, France, p. 297-298.

Benton, T. G., Vickery, J. A., Wilson, J. D. 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology and Evolution, 18 (4), 182-188.

Bertsch, A. 1984. Foraging in male bumblebees (*Bombus lucorum* L.): maximising energy or minimising water load? Oecologia, 62, 325-336.

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D, Settele, J., Kunin, W. E. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313 (5785), 351-354.

Blight, M. M., Mètayer, M., Pham-Delègue, M. H., Pickett, J. A., Marion-Poll, F., Wadhams, L. J. 1997. Identification of floral volatiles in-

volved in recognition of oilseed rape flowers, *Brassica napus* by honeybees, *Apis mellifera*. Journal of Chemical Ecology, 23 (7), 1715-1727.

Boardman, L., Terblanche, J. S., Hetz, S. K., Marais, E., Chown, S. L. 2012. Reactive oxygen species production and discontinuous gas exchange in insects. Proceedings of the Royal Society B – Biological Sciences, 279 (1730), 893-901.

Bommarco, R., Marini, L., Vaissière B. E. 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. Oecologia. <http://www.ncbi.nlm.nih.gov/pubmed/22311256> [accessed 03 04 2012]

Bortolotti, L., Montanari, R., Marcelino, J., Medrzycki, P., Maini, S., Porrini, C. 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bulletin of Insectology, 56 (1), 63-67.

Bradley, T. J. 2007. Control of the respiratory pattern in insects. Advances in Experimental Medicine and Biology, 618, 211–220.

Carreck, N. L., Williams, I. H., Little, D. J. 1997. The movement of honey bee colonies for crop pollination and honey production by beekeepers in Great Britain. Bee World, 78 (2), 67-77.

Cartar, R. V., Real, L. A. 1997. Habitat structure and animal movement: the behaviour of bumble bees in uniform and random spatial resource distributions. Oecologia, 112 (3), 430-434.

Cetin, H., Erler, F., Yanikoglu, A. 2006. Larvicidal activity of novaluron, a chitin synthesis inhibitor, against the housefly, *Musca domestica*. Journal of Insect Science, 6 (50), 1–4.

Chifflet, R., Klein, E. K., Lavigne, C., Le Féon, V., Ricroch, A. E., Lecomte, J., Vaissière, B. E. 2011. Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape. Journal of Applied Ecology, 48 (3), 689-696.

Choudhary, A., Sharma, D. C. 2008. Dynamics of pesticide residues in nectar and pollen of mustard (*Brassica juncea* (L.) Czern.) grown in Himachal Pradesh (India). Environmental Monitoring and Assessment, 144, 143–150.

Chown, S. L. 2002. Respiratory water loss in insects. Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology, 133 (3), 791-804.

Chown, S. L. 2011. Discontinuous gas exchange: new perspectives on evolutionary origins and ecological implications. Functional Ecology, 25 (6), 1163-1168.

Chown, S. L., Gaston, K. J. 1999. Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. Biological Reviews, 74 (1), 87-120.

Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B., Marais, E. 2006. Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. Physiological and Biochemical Zoology, 79 (2), 333-343.

Cook, S. M., Awmack, C. S., Murray D. A., Williams I. H. 2003. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecological Entomology, 28 (5), 622-627.

Cook, S. M., Denholm, I. 2008. Ecological approaches to the control of pollen beetles in oilseed rape. EPPO Bulletin, 38 (1), 110-113.

Corbet, S. A. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie, 34 (1), 1-10.

Corbet, S. A., Williams, I. H., Osborne, J. L. 1991. Bees and the pollination of crops and wild flowers in the European Community. Bee World, 72 (2), 47-59.

Corbett, J. R. 1974. The biochemical mode of action of pesticides. Academic Press, London, New York, 330 p.

Cox, C. 1996. Cypermethrin. Journal of Pesticide Reform, 16 (2), 15-20.

Cox, R. L., Wilson, W. T. 1984. Effects of cypermethrin on the behaviour of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Environmental Entomology, 13, 375-378.

Cresswell, J. E. 1999. The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). Journal of Ecology, 87 (4), 670-677.

Cresswell, J. E. 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology, 20 (1), 149-157.

Cresswell, J. E., Hagen, C., Woolnough, J. M. 2001. Attributes of individual flowers of *Brassica napus* L. are affected by defoliation but not by intraspecific competition. Annals of Botany, 88 (1), 111-117.

Cruse, H., Wehner, R. 2011. No need for a cognitive map: decentralized memory for insect navigation. PLoS Computational Biology, 7 (3), e1002009.

Currie, R. W., Pernal, S. F., Guzmán-Novoa, E. 2010. Honey bee colony losses in Canada. Journal of Apicultural Research, 49 (1), 104-106.

Davis, A. R., Sawhney, V. K., Fowke, L. C., Low, N. H. 1994. Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. Apidologie, 25 (6), 602-614.

Day, S., Beyer, R., Mercer, A., Ogden, S. 1990. The nutrient composition of honey bee-collected pollen in Otago, New Zealand. Journal of Apicultural Research, 29 (3), 138-146.

Decourtye, A., Devillers, J., Aupinel, P., Brun, F., Bagins, C., Fourrier, J., Gauthier, M. 2011. Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. Ecotoxicology, 20 (2), 429-437.

Decourtye, A., Devillers, J., Genecque, E., Le Menach, K., Budzinski, H., Cluzeau, S., Pham-Delègue, M. H. 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Archives of Environmental Contamination and Toxicology, 48 (2), 242-250.

Decourtye, A., Lacassie, E., Pham-Delègue, M. H. 2003. Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. Pest Management Science, 59 (3), 269-278.

Decourtye, A., Le Métayer, M., Pottiau, H., Tisseur, M., Odoux, J. F., Pham-Delègue, M. H. 1999. Impairment of olfactory learning performances of *Apis mellifera* L. by long term ingestion of imidacloprid. In: (Ed. Belzunces, L. P., Pélissier, C., Lewis, G. B.) Hazards of pesticides to bees. 7th International Symposium ICPBR Bee Protection Group. Avignon, France, INRA, p. 113-117.

Delaplane, K. S., Mayer, D. F. 2000. Crop pollination by bees. Washington, USA, 352 p.

Desneux, N., Decourtye, A., Delpuech, J.-M. 2007. The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology, 52 (1), 81-106.

Devaux, C., Klein, E. K., Lavigne, C., Sausse, C., Messéan, A. 2008. Environmental and landscape effects on cross-pollination rates observed at long distance among French oilseed rape *Brassica napus* commercial fields. Journal of Applied Ecology, 45 (3), 803-812.

Diekötter, T., Kadoya, T., Peter, F., Wolters, V., Jauker, F. 2010. Oilseed rape crops distort plant-pollinator interactions. Journal of Applied Ecology, 47 (1), 209-214.

Dingha, B. N., Appel, A. G., Eubanks, M. D. 2005. Discontinuous carbon dioxide release in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), and its effect on respiratory transpiration. Journal of Insect Physiology, 51 (7), 825–836.

Dobson, H. E. M., Peng, Y.-S. 1997. Digestion of pollen components by larvae of flower-specialist bee *Chelostoma florisomne* (Hymenoptera: Megachilidae). Journal of Insect Physiology, 43 (1), 89-100.

Duncan, F. D., Krasnov, B., McMaster, M. 2002. Novel case of a tenebrionid beetle using discontinuous gas exchange cycle when dehydrated. Physiological Entomology, 27 (1), 79-83.

Dyer, A. G., Rosa, M. G., Reser, D. H. 2008. Honeybees can recognise images of complex natural scenes for use as potential landmarks. Journal of Experimental Biology, 211 (Pt 8), 1180-1186.

Eisikowitch, D. 1981. Some aspects of pollination of oilseed rape (*Brassica napus* L.). Journal of Agricultural Science, 96 (2), 321-326.

Ekbom, B. 2010. Pests and their enemies in spring oilseed rape in Europe and challenges to integrated pest management. In: (Ed. Williams, I. H.) Biocontrol-based integrated management of oilseed rape pests. Springer Dordrecht Heidelberg, London, New York, p. 151-165.

Erler, F., Cetin, H., Saribasak, H., Serttas, A. 2010. Laboratory and field evaluations of some botanical pesticides against the cedar leaf moth, *Acleris undulana*. Journal of Pest Science, 83 (3), 265–272.

Faegri, K., Iversen, J. 1989. Textbook of pollen analysis. 4. ed. Chichester, UK, 328 p.

Faegri, K., van der Pijl, L. 1979. The principles of pollination ecology. 3. ed. Pergamon Press, Oxford, 291 p.

FAO. 2006. World reference base for soil resources 2006. Rome, 132 p.

Farina, W. M., Grüter, C., Acosta, L., Mc Cabe, S. 2007. Honeybees learn floral odors while receiving nectar from foragers within the hive. Naturwissenschaften, 94 (1), 55-60.

Ferguson, F. 1987. Long-term effects of systemic pesticides on honey bees. Australian Beekeeper, 89, 49-54.

Free, J. B. 1970. The flower constancy of bumble bees. Journal of Animal Ecology, 39, 395-402.

Free, J. B. 1993. Insect Pollination of Crops. 2. ed. Academic Press, London, UK, 684 p.

Free, J. B., Ferguson, A. W. 1980. Foraging of bees on oil-seed rape (*Brassica napus* L.) in relation to the stage of flowering of the crop and pest control. Journal of Agricultural Science, 94 (1), 151-154.

Gabriel, D., Tscharntke, T. 2007. Insect pollinated plants benefit from organic farming. Agriculture, Ecosystems and Environment, 118 (1-4), 43-48.

Gallai, N., Salles, J.-M., Settele, J., Vassière, B. E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics, 68 (3), 810-821.

Gegear, R. J., Thomson, J. D. 2004. Does the flower constancy of bumble bees reflect foraging economics? Ethology, 110 (10), 793-805.

Gels, J. E., Held, D. W., Potter, D. A. 2002. Hazards of insecticides to the bumble bee *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. Journal of Economic Entomology, 95 (4), 722-728.

Gerolt, P. 1976. The mode of action of insecticides: accelerated water loss and reduced respiration in insecticide-treated *Musca domestica* L. Pesticide Science, 7 (6), 604-620.

Gerolt, P. 1983. Insecticides: their route of entry, mechanisms of transport and mode of action. Biological Reviews, 58 (2), 233-274.

Gibbs, A. G., Johnson, R. A. 2004. The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. Journal of Experimental Biology, 207 (Pt 20), 3477-3482.

Giray, T., Kence, M., Oskay, D., Döke, M. A., Kence, A. 2010. Scientific note: colony losses survey in Turkey and causes of bee deaths. Apidologie, 41 (4), 451-453.

Goller, F., Esch, H. 1990. Comparative study of chill-coma temperatures and muscle potentials in insect flight muscles. Journal of Experimental Biology, 150, 221–231.

Goulson, D., Hanley, M. E., Darvill, B., Ellis, J. S., Knight, M. E. 2005. Causes of rarity in bumblebees. Biological Conservation, 122 (1), 1-8.

Grant, C. A., Bailey, L. D. 1993. Fertility management in canola production. Canadian Journal of Plant Science, 73, 651-670.

Gray, E. M., Chown, S. L. 2008. Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: A case study from a highly variable cockroach, *Perisphaeria sp*. Journal of Insect Physiology, 54 (1), 169-179.

Gross, H. J., Pahl, M., Si, A., Zhu, H., Tautz, J., Zhang, S. 2009. Number-based visual generalisation in the honeybee. PLoS ONE, 4 (1), e4263.

Guez, D., Suchail, S., Gauthier, M., Maleszka, R., Belzunces, L. P. 2001. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). Neurobiology of Learning and Memory, 76 (2), 183-191.

Gumbert, A., Kunze, J. 2001. Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. Biological Journal of the Linnean Society, 72 (3), 419-433.

Haneklaus, S., Paulsen, H. M., Gupta, A. K., Bloem, E., Schnug, E. 1999. Influence of sulfur fertilization on yield and quality of oilseed rape and mustard. In: Proceedings of 10th International Rapeseed Congress, Canberra, Australia (CD-ROM).

Harak, M., Lamprecht, I., Kuusik, A., Hiiesaar, K., Metspalu, L., Tartes, U. 1999. Calorimetric investigations of insect metabolism and development under the influence of a toxic plant extract. Thermochimica Acta, 333 (1), 39-48.

Hayter, K. E., Cresswell, J. E. 2006. The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: implications for landscape-scale gene dispersial. Journal of Applied Ecology, 43 (6), 1196-1202.

Herbert, E. W. 1992. Honey bee nutrition. In: (Ed. Graham, J. M.) The Hive and the Honey Bee. Dadant & Sons, Hamilton, Illinois, USA.

Hetz, S. K., Bradley, T. J. 2005. Insects breathe discontinuously to avoid oxygen toxicity. Nature, 433, 516-519.

Hetz, S. K., Psota, E., Wasserthal, L. T. 1999. Roles of aorta, ostia and

tracheae in heartbeat and respiratory gas exchange in pupae of *Troides rhadamantus* Staudinger 1888 and *Ornithoptera priamus* L. 1758 (Lepidoptera, Papilionidae). International Journal of Insect Morphology and Embryology, 28 (1-2), 131-144.

Hetz, S. K., Wasserthal, L. T., Hermann, S., Kaden, H., Oelssner, W. 1994. Direct oxygen measurements in the tracheal system of lepidopterous pupae using miniaturized amperometric sensors. Bioelectrochemistry and Bioenergetics, 33 (2), 165-170.

Hoffmann, A. A., Parsons, P. A. 1989. Selection for increased desiccation resistance in *Drosophila melanogaster*: Additive genetic control and correlated responses for other stresses. Genetics, 122 (4), 837-845.

Hokkanen, H. 2000. The making of a pest: recruitment of *Meligethes aeneus* onto oilseed Brassicas. Entomologia Experimentalis et Applicata, 95 (2), 141-149.

Holmes, M. R. J. 1980. Nutrition of the oilseed rape crop. Applied science publishers LTD London, 158 p.

Holzschuh, A., Dormann, C. F., Tscharntke, T., Steffan-Dewenter, I. 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. Proceedings of the Royal Society B – Biological Sciences, 278, 3444-3451.

Hrassnigg, N., Crailsheim, K. 1998. The influence of brood on the pollen consumption of worker bees (*Apis mellifera* L.). Journal of Insect Physiology, 44 (5-6), 393-404.

Isayama, S., Saito, S., Kuroda, K., Umeda, K., Kasamatsu, K. 2005. Pyridalyl, a novel insecticide: potency and insecticidal selectivity. Archives of Insect Biochemistry and Physiology, 58 (4), 226–233.

Jagers op Akkerhuis, G. A. J. M., Kjær, C., Damgaard, C., Elmegaard, N. 1999a. Temperature-dependent, time-dose-effect model for pesticide effects on growing, herbivorous arthropods: bioassays with dimethoate and cypermethrin. Environmental Toxicology and Chemistry, 18 (10), 2370-2378.

Jagers op Akkerhuis, G. A. J. M., Seidelin, N., Kjaer, C. 1999b. Are we analysing knockdown in the right way? How independence of the knockdown-recovery process from mortality may affect measures for behavioural effects in pesticide bioassays. Pesticide Science, 55 (1), 62-68.

Jagers op Akkerhuis, G. A. J. M., Westerhof, R., Straalen, N. M. van,

Koeman, J. H. 1995. Water balance, respiration and immobilisation in relation to deltamethrin poisoning and physical conditions in the epigeal spider *Oedothorax apicatus*. Pesticide Science, 44 (2), 123-130.

Jaycox, E. 1964. Effect on honey bees of nectar from systemic insecticide treated plants. Journal of Economic Entomology, 57 (1), 31-35.

Johansen, C. A., Mayer, D. F. 1990. Pollinator protection: A bee and pesticide handbook. Wicwas Press, Cheshire, Connecticut. 212 p.

Jõgar, K., Kuusik, A., Metspalu, L., Hiiesaar, K., Grishakova, M., Luik, A. 2008. Effects of Neem EC on gas exchange, tracheal ventilation, and water loss in diapausing pupae of *Pieris brassicae*. Entomologia Experimentalis et Applicata, 126 (2), 165-173.

Jõgar, K., Kuusik, A., Metspalu, L., Hiiesaar, K., Luik, A., Grishakova, M. 2006. Results of treatments with natural insecticidal substances on the development and physiological state of insects. Agronomy Research, 4, 203-210.

Kaarli, K. 2004. Õlikultuuride kasvataja käsiraamat. Eesti Vabariigi Põllumajandusministeerium, Eesti Maaviljeluse Instituut, 132 lk. (in Estonian)

Karise, R., Kuusik, A., Mänd, M., Metspalu, L., Williams, I. H., Hiiesaar, K., Luik, A., Muljar, R., Liiv, K. 2010. Gas exchange patterns of bumble bee foragers before and after exposing to lowered temperature. Journal of Insect Physiology, 56 (5), 529-535.

Karise, R., Mänd, M., Viik, E., Martin, A.-J., Lääniste, P. 2004. Flower visitors on spring oilseed rape in different cropping systems. Latvian Journal of Agronomy, 7, 6-11.

Kasina, J. M., Mburu, J., Kraemer, M., Holm-Mueller, K. 2009. Economic benefit of crop pollination by bees: a case of Kakamega smallholder farming in Western Kenya. Journal of Economic Entomology, 102 (2), 467-473.

Kestler, P. 1971. Die Diskontinuierliche Ventilation bei *Periplaneta americana* L. und Anderen Insekten. Dissertation, Julius-Maximilians-Universität Würzburg, 232 p.

Kestler, P. 1985. Respiration and respiratory water loss. In: (Ed. Hoffmann K. H.) Environmental physiology and biochemistry of insects. Springer Verlag, Berlin, Heidelberg, p. 137–189.

Kestler, P. 1991. Cyclic CO_2 release as a physiological stress indicator in insects. Comparative Biochemistry and Physiology C – Comparative

Pharmacology, 100 (1-2), 207-211.

Klein, A.-M., Steffan-Dewenter, I., Tscharntke, T. 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proceedings of the Royal Society B – Biological Sciences, 270 (1518), 955-961.

Klein, A.-M., Vassière, 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 (1608), 303-313.

Klowden, M. J. 2002. Physiological systems in insects. Academic Press, San Diego, USA., 415 p.

Koltowski, Z. 2001. Beekeeping value and pollination requirements of double-improved cultivars of spring rapeseed (*Brassica napus* L. var. *oleifera* Metzger). Journal of Apicultural Science, 45, 69-84.

Koskor, E., Muljar, R., Drenkhan, K., Karise, R., Bender, A., Viik, E., Luik, A., Mänd, M. 2009. The chronic effect of the botanical insecticide Neem EC on the pollen forage of the bumble bee *Bombus terrestris* L. Agronomy Research, 7 (Special issue I), 341–346.

Kotowski, Z. 2001. Results of the investigations into nectar secretion and pollen production of new cultivars of rape (*Brassica napus* L.). Acta Horticulturae, 561, 127-129.

Kovac, H., Stabentheiner, A., Hetz, S. K., Petz, M., Crailsheim, K. 2007. Respiration of resting honeybees. Journal of Insect Physiology, 53 (12), 1250-1261.

Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P., Thorp, R. W. 2004. The area requirements on an ecosystem service: crop pollination by native bee communities in California. Ecology Letters, 7, 1109-1119.

Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G., Given, K. 2012. Multiple routs of pesticide exposure for honey bees living near agricultural fields. PLoS ONE, 7 (1), e29268.

Kuusik, A., Martin, A.-J., Mänd, M., Hiiesaar, K., Metspalu, L., Tartes, U. 2002. Interrelations of gas exchange cycles, body movements and heartbeats in the foragers of bumblebee *Bombus terrestris* (Hymenoptera: Apidae) at low temperatures. European Journal of Entomology, 99 (2), 209–214.

Kärblane, H. 1996. Taimede toitumise ja väetamise käsiraamat. Eesti Vabariigi Põllumajandusministeerium, Tallinn, 288 lk. (in Estonian)

Köster, T., Vask, K., Koorberg, P., Selge, I., Viik, E. 2009. Do we need broad and shallow agri-environment schemes? – Outcomes of ex-post evaluation of Estonian Rural Development Plan 2004-2006. Rural In: Rural Development 2009. The Fourth International Scientific Conference. Proceedings I. Lithuania: Lithuanian University of Agriculture, p. 219-224.

Laloi, D., Bailez, O., Blight, M. M., Roger, B., Pham-Delègue, M.-H., Wadhams, L. J. 2000. Recognition of complex odors by restrained and free-flying honeybees, *Apis mellifera*. Journal of Chemical Ecology, 26 (10), 2307-2319.

Lamprecht, I., Seymour, R. S., White, C. R., Matthews, P. G. D., Wadsö, L. 2009. Discontinuous ventilation in the rhinoceros beetle *Oryctes nasicornis*: Direct and indirect calorimetry. Journal of Thermal Analysis and Calorimetry, 95 (3) 743-747.

Lancashire, P. D., Bleiholder, H., van den Boom, T., Langelüddeke, P., Stauss, R., Weber, E., Witzenberger, A. 1991. A uniform decimal code for growth stages of crops and weeds. Annals of Applied Biology, 119 (3), 561–601.

Langridge, D. F., Goodman, R. D. 1982. Honeybee pollination of oilseed rape, cultivar Midas. Australian Journal of Experimental Agriculture and Animal Husbandry, 22 (115), 124-126.

Laska, M., Liesen, A., Teubner, P. 1999. Enantioselectivity of odor perception in squirrel monkeys and humans. American Journal of Physiology, 277 (4), 1098-1103.

Laurino, D., Porporato, M., Patetta, A., Manino, A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory test. Bulletin of Insectology, 64 (1), 107-113.

Lehmann, M., Gustav, D., Galizia, C. G. 2011. The early bee catches the flower – circadian rythmicity influences learning performance in honey bees, *Apis mellifera*. Behavioural Ecology and Sociobiology, 65 (2), 205-215.

Leonard, A. S., Dornhaus, A., Papaj, D. R. 2011. Flowers help bees cope with uncertainty: signal detection and the function of floral complexity. Journal of Experimental Biology, 214 (Pt 1), 113-121.

Levy, R. I., Schneiderman, H. A. 1966. Discontinuous respiration in

insects. II. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. Journal of Insect Physiology, 12 (1), 83-104.

Lighton, J. R. B. 1994. Discontinuous ventilation in terrestrial insects. Physiological Zoology, 67 (1), 142-162.

Lighton, J. R. B. 1996. Discontinuous gas exchange in insects. Annual Review of Entomology, 41, 309-324.

Lighton, J. R. B. 1998. Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. American Zoologist, 38 (3), 483-491.

Lighton, J. R. B. 2007. Respiratory biology: why insects evolved discontinuous gas exchange. Current Biology, 17 (16), 645-647.

Lighton, J. R. B., Lovegrove, B. G. L. 1990. A temperature-induced switch from diffusive to convective ventilation in the honeybee. Journal of Experimental Biology, 154, 509-516.

Lighton, J. R. B., Schilman, P. E., Holway, D. A. 2004. The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. Journal of Experimental Biology, 207 (Pt 25), 4463-4471.

Maini, S., Medrzycki, P., Porrini, C. 2010. The puzzle of honey bee losses: a brief review. Bulletin of Insectology, 63 (1), 153-160.

Marais, E., Chown, S. L. 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. Journal of Experimental Biology, 206 (Pt 24), 4565-4574.

Marais, E., Klok, C. J., Terblanche, J. S., Chown, S. L. 2005. Insect gas exchange patterns: a phylogenetic perspective. Journal of Experimental Biology, 208 (Pt 23), 4495-4507.

Martin, A.-J., Kuusik, A., Mänd, M., Metspalu, L., Tartes, U. 2004. Respiratory patterns in nurses of the red wood ant *Formica polyctena* (Hymenoptera, Formicidae). Insectes Sociaux, 51 (1), 62–66.

Matthews, P. G. D., White, C. R. 2011. Discontinuous gas exchange in insects: Is it all in their heads? American Naturalist, 177 (1), 130-134.

Matthews, P. G. D., White, C. R. 2012. Discontinuous gas exchange, water loss, and metabolism in *Protaetia cretica* (Cetoniinae, Scarabaeidae). Physiological and Biochemical Zoology, 85 (2) 174-182.

Mayer, D. F., Lunden, J. D. 1999. Field and laboratory tests of the effects of fipronil on adult female bees of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*. Journal of Apicultural Research, 38 (3-4), 191-197.

Menzel, R., Giurfa, M. 2001. Cognitive architecture of a mini-brain: the honeybee. Trends in Cognitive Sciences, 5 (2), 62-71.

Menzel, R., Greggers, U., Hammer, M. 1993. Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In: (Ed. Papaj, D. R., Lewis, A. C.) Insect learning. Chapman Hall, New-York, p. 79-125.

Mesquida, J., Marilleau, R., Pham-Delegue, M.-H., Renard, M. 1988. A study of rapeseed (*Brassica napus* L. var. *olefeira* Metzger) flower nectar secretion. Apidologie, 19 (3), 307-318.

Meyerhoff, G. 1958. The foraging behaviour of honeybee on rape. Leipzig Bienenztg, 72, 164-165. (in German)

Miranda, J. E., Navickiene, H. M. D., Nogueira-Couto, R. H., De Bortoli, S. A., Kato, M. J., Bolzani, V. da S., Furlan, M. 2003. Susceptibility of *Apis mellifera* (Hymenoptera: Apidae) to pellitorine, an amide isolated from *Piper tuberculatum* (Piperaceae). Apidologie, 34 (4), 409-415.

Moerbitz, C., Hetz, S. K. 2010. Tradeoffs between metabolic rate and spiracular conductance in discontinuous gas exchange of *Samia cynthia* (Lepidoptera, Saturniidae). Journal of Insect Physiology, 56 (5), 536-542.

Mohr, N. A., Jay, S. C. 1990. Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. Journal of Apicultural Research, 29 (2), 95-100.

Morandin, L. A., Winston, M. L. 2005. Wild bee abundance and seed production in conventional, organic, and genetically modified canola. Ecological Applications, 15 (3), 871-881.

Moritz, R. F. A., de Miranda, J., Fres, I., Le Conte, Y., Neumann, P., Paxon, R. J. 2010. Research strategies to improve honeybee health in Europe. Apidologie, 41 (3), 227-242.

Muranjan, P. N., Gandhale, D. N., Chaudhari, C. S., Patil, B. D., Pokharkar, D. S., Naik, R. L. 2006. Toxocity of ready-mix formulations of pyrethroids and ad-mixed insecticides on forager honey bee,

Apis cerana indica Fabricius. Annals of Plant Protection Sciences, 14 (1), 90-93.

Mänd, M., Kuusik, A., Martin, A.-J., Williams, I. H., Luik, A., Karise, R., Metspalu, L., Hiiesaar, K. 2005. Discontinuous gas exchange cycles and active ventilation in pupae of the bumblebee *Bombus terrestris*. Apidologie, 36 (4), 561-570.

Mänd, M., Kuusik, A., Martin, A.-J., Williams, I. H., Luik, A., Karise, R., Metspalu, L., Hiiesaar, K. 2006. Regular periods of abdominal contractions recorded in the larvae of the bumblebee, *Bombus terrestris* L. (Hymenoptera: Apidae). European Journal of Entomology, 103, 319-322.

Mänd, M., Mänd, R., Williams, I. H. 2002. Bumblebees in the agricultural landscape of Estonia. Agriculture, Ecosystems and Environment, 89 (1-2), 69-76.

Nicolson, S. W. 2009. Water homeostasis in bees, with the emphasis on sociality. Journal of Experimental Biology, 212, 429-434.

Nieh, J. C., Leon, A., Cameron, S., Vandame, R. 2006. Hot bumble bees at good food: thoracic temperature of feeding *Bombus wilmattae* foragers is tuned to sugar concentration. Journal of Experimental Biology, 209 (Pt 21), 4185-4192.

Orlovius, K. 2003. Fertilizing for high yield and quality oilseed rape. International Potash Institute, 130 p.

Osborne, J. L., Clark, S. J., Morris, R. J., Williams, I. H., Riley, J. R., Smith, A. D., Reynolds, D. R., Edwards, A. S. 1999. A landscape scale study of bumble bee foraging range and constancy, using harmonic radar. Journal of Applied Ecology, 36 (4), 519-533.

Osborne, J. L., Williams, I.H., Corbet, S. A. 1991. Bees, pollination and habitat change in the European Community. Bee World, 72 (3), 99-116.

Pahl, M., Zhu, H., Tautz, J., Zhang, S. 2011. Large scale homing in honeybees. PLoS ONE, 6 (5), e19669.

Pareja, L., Colazzo, M., Pérez-Parada, A., Niell, S., Carrasco-Letelier, L., Besil, N., Cesio, M. V., Heinzen, H. 2011. Detection of pesticides in active and depopulated beehives in Uruguay. International Journal of Environmental Research and Public Health, 8 (10), 3844-3858.

Pauw, A., Hawkins, J. A. 2011. Reconstruction of historical pollination

rates reveals linked declines of pollinators and plants. Oikos, 120 (3), 344-349.

Pettersson, M. W., Sjödin, E. 2000. Effects of experimental plant density reductions on plant choice and foraging behaviour of bees (Hymenoptera: Apoidea). Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, 50 (1), 40-46.

Pettis, J. S., Delaplane, K. S. 2010. Coordinated responses to honey bee decline in the USA. Apidologie, 41 (3), 256-263.

Pettis, J. S., van Engelsdorp, D., Johnson, J., Dively, G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema.* Naturwissenschaften, 99 (2), 153-158.

Pham-Delègue, M. H., Blight, M. M., Kerguelen, V., Le Métayer, M., Marion-Poll, F., Sandoz, J. C., Wadhams, L. J. 1997. Discrimination of oilseed rape volatiles by the honeybee: combined chemical and biological approaches. Entomologia Experimentalis et Applicata, 83 (1), 87-92.

Pierre J., Mesquida J., Marilleau R., Pham-Delègue, M. H., Renard, M. 1999. Nectar secretion in winter oilseed rape, *Brassica napus* – quantitative and qualitative variability among 71 genotypes. Plant Breeding, 118 (6), 471-476.

Pohtio, I., Teräs, I. 1995. Bumblebee visits to different color morphs of the Washington lupine, *Lupinus polyphyllus*. Entomologica Fennica, 5, 139-151.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W. E. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution, 25 (6), 345-353.

Quinlan, M. C., Lighton, J. R. B. L. 1999. Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). Physiological Entomology, 24 (4), 293-302.

Quinlan, M. C., Hadley, N. F. 1993. Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. Physiological Zoology, 66 (4), 628 -642.

Radchenko, T. G. 1964. The influence of pollination on the crop and the quality of the seed of winter rape. Bdzhil'nitstvo, 1, 68-74. (in Ukrainian)

Raine, N. E., Chittka, L. 2008. The correlation of learning speed and

natural foraging success in bumble-bees. Proceedings of the Royal Society B – Biological Sciences, 275 (1636), 803-808.

Ramirez-Romero, R., Chaufaux, J., Pham-Delègue, M.-H. 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. Apidologie, 36 (4), 601-611.

Rasheed, S. A., Harder, L. D. 1997. Economic motivation for plant species preferences of pollen-collecting bumble bees. Ecological Entomology, 22 (2), 209-219.

Rathcke, B. J. 1992. Nectar distributions, pollinator behaviour, and plant reproductive success. In: (Ed. Hunter, M., Ohgushi, T., Price, P.). The effect of resource distribution on animal plant interactions. Academic Press, London, UK, p. 113-138.

Rathke, G.-W., Behrens, T., Diepenbrock, W. 2006. Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): a review. Agriculture, Ecosystems and Environment, 117 (2-3), 80-108.

Richardson, D. M. 2008. Summary of findings from a participant country pollen beetle questionnaire. EPPO Bulletin, 38 (1), 68-72.

Rieth, J. P., Levin, M. D. 1988. The repellent effect of two pyrethroid insecticides on the honey bee. Physiological Entomology, 13 (2), 213-218.

Rosa, A. S., Blochtein, B., Ferreira, N. R., Witter, S. 2010. *Apis mellifera* (Hymenoptera: Apidae) as a potential *Brassica napus* pollinator (cv. Hyola 432) (Brassicaceae), in Southern Brazil. Brazilian Journal of Biology, 70 (4), 1075-1081.

Rosa, A. S., Blochtein, B., Lima, D. K. 2011. Honey bee contribution to canola pollination in Southern Brazil. Scientia Agricola, 68 (2), 255-259.

Roulston, T. H., Cane, J. H. 2000. Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution, 222 (1-4), 187-209.

Saba F. 1971. A simple test method for evaluating response to toxicants in mite populations. Journal of Economic Entomology, 64 (1), p 321.

Sabbahi, R., de Oliveira, D., Marceau, J. 2005. Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Crucif-

era: Brassicaceae). Journal of Economic Entomology, 98 (2), 367-372.

Sandoz, J. C. 2011. Behavioral and neurophysiological study of olfactory perception and learning in honeybees. Frontiers in Systems Neuroscience, 5 (98).

Sausse, C., Colbach, N., Young, M. W., Squire, G. R. 2012. How to manage the impact of gene flow on oilseed rape grain quality? Simulation case studies of three contrasted landscapes. European Journal of Agronomy, 38, 32-42.

Schimpf, N. G., Matthews, P. G., Wilson, R. S., White, C. R. 2009. Cockroaches breathe discontinuously to reduce respiratory water loss. Journal of Experimental Biology, 212, 2773-2780.

Schneider, C. W., Tautz, J., Grünewald, B., Fuchs, S. 2012. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behaviour of *Apis mellifera*. PLoS ONE, 7 (1), e30023.

Sepp, K., Mikk, M., Mänd, M., Truu, J. 2004. Bumblebee communities as an indicator for landscape monitoring in the agri-environmental programme. Landscape and Urban Planning, 67 (1-4), 173-183.

Shires, S. W., Le Blanc, J., Murray, A., Forbes, S., Debray, P. 1984. A field trial to assess the effects of a new pyrethroid insecticide, WL85871, on foraging honeybees in oilseed rape. Journal of Apicultural Research, 23 (4), 217-226.

Sibul, I., Kuusik, A., Voolma, K. 2004. Monitoring of gas exchange cycles and ventilatory movements in the pine weevil *Hylobius abietis*: respiratory failures evoked by a botanical insecticide. Entomologia Experimentalis et Applicata, 110 (2), 173-179.

Sildauskas, G., Bernotas, S. 2003. Some factors affecting seed yield of spring oilseed rape (*Brassica napus* L.). Agronomy Research, 1 (2), 229-243.

Slama, K. 1999. Active regulation of insect respiration. Annals of the Entomological Society of America, 92 (6), 916-929.

Slama, K., Sobotnik, J., Hanus, R. 2007. Respiratory concerts revealed by scanning microrespirography in a termite *Prorhinotermes simplex* (Isoptera: Rhinotermitidae). Journal of Insect Physiology, 53 (4), 295-311.

Smeets, P., Duchateau, M. J. 2003. Longevity of *Bombus terrestris* work-

ers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. Apidologie, 34 (4), 333-337.

Szulc, P. M., Drozdowska, L., Kachlicki, P. 2003. Effect of sulphur on the yield and content of glucosionolates in spring oilseed rape seeds. Electronic Journal of Polish Agricultural Universities, 6 (2).<http://www.ejpau.media.pl/volume6/issue2/agronomy/art-01.html > [accessed 02 04 2012]

Steffan-Dewenter, I. 2003. Seed set of male-sterile and male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density. Apidologie, 34 (3), 227-235.

Stoate, C., Báldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., de Snoo, G. R., Rakosy, L., Ramwell, C. 2009. Ecological impacts of early 21st century agricultural change in Europe – A review. Journal of Environmental Management, 91 (1), 22-46.

Stokstad, E. 2006. Pollinator diversity declining in Europe. Science, 313 (5785), 286 p.

Stokstad, E. 2012. Field research on bees raises concern about low-dose pesticides. Science, 30: 335 (6076), 1555.

Stoner, A., Wilson, W. T., Harvey, J. 1985. Acephate (Orthene): effects on honey bee queen, brood and worker survival. American Bee Journal, 125 (6), 448-450.

Sur, R., Stork, A. 2003. Uptake, translocation and metabolism of imidacloprid in plants. Bulletin of Insectology, 56 (1), 35-40.

Zafeiridou, G., Theophilidis, G. 2006. A simple method for monitoring the respiratory rhythm in intact insects and assessing the neurotoxicity of insecticides. Pesticide Biochemistry and Physiology, 86 (3), 211-217.

Zimmer, C. T., Nauen, R. 2011. Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. Pest Management Science, 67 (5), 599-608.

Tasei, J.-N. 2001. Effects of insect growth regulators on honey bees and non-*Apis* bees. Apidologie, 32 (6), 527-545.

Terblanche, J. S., Marais, E., Hetz, S. K., Chown, S. L. 2008. Control of discontinuous gas exchange in *Samia cynthia*: effects of atmospheric oxygen, carbon dioxide and moisture. Journal of Experimental Biol-

ogy, 211 (Pt 20), 3272-3280.

Thompson, H. M. 2001. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). Apidologie, 32 (4), 305-321.

Thompson, H. M. 2003. Behavioural effects of pesticides in bees – their potential for use in risk assessment. Ecotoxicology, 12 (1-4), 317-330.

Thompson, H. M., Hunt, L. V. 1999. Extrapolating from honeybees to bumblebees in pesticide risk assessment. Ecotoxicology, 8 (3), 147-166.

Thompson, H. M., Maus, C. 2007. The relevance of sublethal effects in honey bee testing for pesticide risk assessment. Pest Management Science, 63 (11), 1058-1061.

Thompson, H. M., Wilkins, S. 2003. Assessment of the synergy and repellency of pyrethroid/fungicide mixtures. Bulletin of Insectology, 56 (1), 131-134.

Treu, R., Emberlin, J. 2000. Pollen dispersal in the crops Maize (*Zea mays*), oil seed rape (*Brassica napus* ssp. *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*). A report for the Soil Association from the National Pollen Research Unit. Worcester, UK, 57 p.

Vandame, R., Meled, M., Colin, M. E., Belzunces, L. P. 1995. Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. Environmental Toxicology and Chemistry, 14 (5), 855-860.

Van der Steen, J. J. M. 2001. Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. Apidologie, 32 (5), 399–406.

Varis, A.-L. 1995. Abundance, species composition and daily pattern of bees visiting field bean, goat's rue and turnip rape in southern Finland. Agricultural Science in Finland, 4, 473-478.

Veličkа, R. 2003. Rape: summary of monograph, presented for hibernation conference. Lithuanian University of Agriculture, Kaunas, 78 p.

Veromann, E., Kevväi, R., Luik, A., Williams, I. H. 2008. Do cropping system and insecticide use in spring oilseed rape affect the abundance of pollen beetles (*Meligethes aeneus* Fab.) on the crop? International Journal of Pest Management, 54 (1), 1-4.

Veromann, E., Luik, A., Kevväi, R. 2006a. Oilseed rape pests and their parasitoids in Estonia. Bulletin IOBC/wprs, Integrated Control in Oilseed Crops, 29 (7), 165-172.

Veromann, E., Luik, A., Metspalu, L., Williams, I. H. 2006b. Key pests and their parasitoids on spring and winter oilseed rape in Estonia. Entomologica Fennica, 17, 400-404.

Veromann, E., Tarang, T., Kevväi, R., Luik, A. 2006c. Insect pests and their natural enemies on spring oilseed rape in Estonia: impact of cropping systems. Agricultural and Food Science, 15 (1), 61-72.

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.-L., Texier, C., Biron, D. G., Blot, N., El Alaoui, H., Belzunces, L. P., Delbac, F. 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS One, 6 (6), e21550.

Vogt, J. T., Appel, A. G. 2000. Discontinuous gas exchange in the fire ant, *Solenopsos invicta* Buren: caste differences and temperature effects. Journal of Insect Physiology, 46 (4), 403-416.

Waser, N. M., Price, M. V. 1983. Pollinator behaviour and natural selection for flower colour in *Delphinium nelsonii*. Nature, 302, 422-424.

Westphal, C., Steffan-Dewenter, I., Tscharntke, T. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. Ecology Letters, 6, 961-965.

Westphal, C., Steffan-Dewenter, I., Tscharntke, T. 2009. Mass flowering oilseed rape improves early colony growth but not sexual reproduction of bumblebees. Journal of Applied Ecology, 46, 187-193.

White, C. R., Blackburn, T. M., Terblanche, J. S., Marais, E., Gibernau, M., Chown, S. L. 2007. Evolutionary responses of discontinuous gas exchange in insects. Proceedings of the National Academy of Science USA, 104 (20), 8357-8361.

Williams, C. M., Pelini, S. L., Hellmann, J. J., Sinclair, B. J. 2010. Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. Biology Letters, 6 (2), 274-277.

Williams, I. H. 1980. Oilseed rape and beekeeping, particular in Britain. Bee World, 61 (4), 141-153.

Williams, I. H. 1985. The pollination of swede rape (*Brassica napus* L.).

Bee World, 66 (1), 16-22.

Williams, I. H. 1994. The dependence of crop production within the European Union on pollination by honeybees. Agricultural Science Review, 6, 229 -257.

Williams, I. H. 1996. Aspects of bee diversity and crop pollination in the European Union. In: (Ed. Matheson, A., Buchmann, S. L., O'Toole, C., Westrich, C., Williams, I. H.). Conservation of Bees. Academic Press for IBRA and the Linnean Society of London, UK, p. 63-80.

Williams, I. H. 2004. Advances in insect pest management of oilseed rape in Europe. In (Ed. Horowitz, A. R., Ishaaya, I.) Insect pest management – field and protected crops. Springer-Verlag: Heidelberg, Germany, p. 181-208.

Williams, I. H., Carreck, N., Little, D. J. 1993. Nectar sources for honey bees and the movement of honey bee colonies for crop pollination and honey production in England. Bee World, 74 (4), 160-175.

Williams, I. H., Cook, V. A. 1982. The beekeeping potential of oilseed rape. British Bee Journal, 110, 68-70.

Williams, I. H., Corbet, S. A., Osborne, J. L. 1991. Beekeeping, wild bees and pollination in the European Community. Bee World, 72 (3), 170-180.

Williams, I. H, Martin, A. P., White, R. P. 1987. The effect of insect pollination on plant development and seed production in winter oilseed rape (*Brassica napus* L.). Journal of Agricultural Science, 109 (1), 135-139.

Williams, P. H. 1986. Environmental change and the distributions of British bumble bees (*Bombus* Latr.). Bee World, 67, 50-61.

Willmer, P. G. 1986. Foraging patterns and water balance: problems of optimisation for a xerophilic bee, *Chalicodoma sicula*. Journal of Animal Ecology, 55, 941-962.

Willmer, P., Stone, G. 1997. Temperature and water relations in desert bees. Journal of Thermal Biology, 22 (6), 453-465.

Winfree, R. 2010. The conservation and restoration of wild bees. Annals of the New York Academy of Sciences, 1195, 169-197.

Winston, M. L. 1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, Massachusetts, USA, 281 p.

Wobschall, A., Hetz, S. K. 2004. Oxygen uptake by convection and diffusion in diapausing moth pupae (*Attacus atlas*). International Congress Series, 1275, 157-164.

Woodman, J. D., Haritos, V. S., Cooper, P. D. 2008. Effects of phosphine on the neural regulation of gas exchange in *Periplaneta americana*. Comparative Biochemistry and Physiology C: Toxicology and Pharmacology, 147 (3), 271-277.

Woods, H. A. 2011. Breathing, bugs, and brains: conceptual unification? Functional Ecology, 25 (6), 1161–1162.

Wu, J. Y., Anelli, C. M., Sheppard, W. S. 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. PLoS ONE, 6 (2), e14720.

Öckinger, E., Smith, H. G. 2007. Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. Journal of Applied Ecology, 44 (1), 50-59.

Yang, E. C., Chuang, Y. C., Chen, Y. L., Chang, L. H. 2008. Abnormal foraging behaviour induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). Journal of Economic Entomology, 101 (6), 1743-1748.

SUMMARY IN ESTONIAN

Väetamise ja pestitsiidide kasutamise mõju mesilaselaadsetele (Apoidea) suvirapsil

Suviraps (*Brassica napus* L. var. *oleifera* subvar. *annua*) on oluline õlikultuur, mille kasvupind on Põhja-Euroopas, k.a Eestis viimasel paaril aastakümnel oluliselt suurenenud. Kiirekasvulise kultuurina vajab raps palju toitaineid, mistõttu korralikke saake saadakse piisaval väetamisel. Raps on peamiselt isetolmlev, kuid risttolmlemisel paraneb seemnete kvaliteet ja kvantiteet. Seega mõjutavad mesilased kui rapsi peamised tolmeldajad ka rapsikasvatuse majanduslikke näitajaid, mistõttu on otstarbekas soodustada nende kohalolekut rapsipõldudel. Viimasel ajal on aga nii Euroopa, Ameerika kui ka Aasia põllumajandusmaastikes täheldatud tolmeldajate arvukuse drastilist langust, mille peapõhjuseks peetakse intensiivistunud põllumajandusest tulenevat maakasutuse muutust ja suurenenud pestitsiidide kasutust.

Rapsi kasvupinna suurenemine on loonud soodsad tingimused ka rapsi kahjuritele, keda tõrjutakse keemiliste insektitsiididega. Samas on raps õite kõrge nektaritootlikkuse ning selle suure suhkrusisalduse tõttu väga atraktiivne toidutaim mesilastele, mistõttu on neil suur oht sattuda kontakti taimekaitsevahenditega. Selle vältimiseks on hakatud tootma mesilastele repellentseid pestitsiide, kuid nende eemalepeletav toime põllutingimustes on osutunud problemaatiliseks. Üldiselt piirdub uute pestitsiidide väljatöötamisel nende mõju uurimine kasulikele putukatele peamiselt toksilisuse määramistega. Kuid putukaile mõjuvad ka pestitsiidide subletaalsed doosid, mis võivad muuta näiteks mesilaste käitumist ning avaldada mõju pere eluvõimele. Problemaatiline on ka meemesilastega kui peamiste mudelorganismidega läbiviidud katsetulemuste automaatne ülekandmine looduslikele mesilaselaadsetele tolmeldajatele, kuigi nende käitumismustrites on olulisi erinevusi. Pestitsiidide subletaalsed ning järeltoimed ei pruugi avalduda alati käitumises. Selleks, et paremini mõista muutusi, mida kemikaalid organismis põhjustavad, on käitumuslikke uuringuid vaja toetada füsioloogiliste katsetega.

Lähtudes nendest probleemidest oli käesoleva doktoritöö eesmärkideks selgitada: 1) kas lehekaudne lisaväetamine mikroväetistega mõjutab suvirapsi õite tihedust ning nektari- ja õietolmu produktsiooni ning

seeläbi ka õisi külastavate mesilaselaadsete (Apoidea) – meemesilaste, kimalaste ja erakmesilaste – arvukust? (**II**), 2) kas meemesilased (*Apis mellifera* L.) väldivad oma toiduvalikus mesilastele repellentse insektitsiidiga Fastac 50 EC pritsitud õisi? (**III**), 3) kas madalad Fastac 50 EC kontsentratsioonid mõjutavad karukimalaste (*Bombus terrestris* L.) hingamistsükleid ja hingamisel tekkivat veekadu (**IV**)?

Antud uurimuse tulemusena leiti, et suvirapsi õisi külastavate meemesilaste, kimalaste ja erakmesilaste arvukus korreleerus positiivselt õite tihedusega (**II**). Mesilaste arvukus korreleerus mõõdukalt ka nektariproduktsiooniga ning 2005. aastal nõrgalt õietolmu produktsiooniga. Rapsi külvieelne väetamine kompleksväetisega suurendas nii õite arvu kui ka nektari- ja õietolmu produktsiooni ning sellega suurenes mesilaste toiduressurss. Mikroväetistega lehekaudne lisaväetamine ei mõjutanud õite arvu ega õietolmu produktsiooni, kuid mõjutas oluliselt nektaritoodangut, kusjuures mangaani lisamine mõjus võrreldes teiste lehekaudselt lisatud mikroväetistega viimasele pärssivalt. Antud uurimuse tulemustest järeldub: selleks, et tagada kõrgemat saaki ja teisigi risttolmeldamisest tulenevaid paremusi on tolmeldajate arvukuse soodustamiseks oluline väetada suvirapsi kompleksväetistega. Lehekaudne mikroväetistega lisaväetamine antud uurimuses tolmeldajate arvukust oluliselt ei mõjutanud.

Insektitsiidi Fastac 50 EC (toimeaine alfa-tsüpermetriin 50 g l^{-1}) repellentsuse testimine näitas, et meemesilaste arvukus antud insektitsiidiga töödeldud ja töötlemata rapsitaimedel ei erinenud. Sellest järeldub, et vastupidiselt tootekirjeldusele, ei peletanud Fastac 50 EC põllutingimustes meemesilasi (**III**). Antud insektisiidi repellentset toimet ei tuvastatud ka 24 tundi pärast pritsimist, kuigi ametlikel andmetel peletab Fastac 50 EC meemesilasi pritsitud alalt 48 tunni jooksul. Katsed näitasid, et meemesilastele oli kõige olulisemaks signaaliks õite tihedus, mis kaalus üle insektitsiidi peletava mõju. Kuivõrd pestitsiidiga töödeldud rapsipõldudel on kahjurite hukkumise tõttu õisi rohkem, siis on raps mesilastele atraktiivsem ning ühtlasi ka ohtlikum.

Füsioloogilised uuringud näitasid, et insektitsiidi Fastac 50 EC subletaalsed doosid mõjutasid oluliselt kimalaste hingamisrütme, ainevahetust ja lihaste aktiivsust (**IV**). Kimalaste töötlemine insektitsiidi lahusega, mis oli 10 korda lahjem (0.004% Fastac 50 EC lahus, alfa-tsüpermetriini 2 ppm) kui on Eestis tegelik registreeritud pritsimisnorm (20 ppm), vähendas 8ºC juures (mil kimalased on rahulikud ja hingavad

enamasti katkendlikult) määramisel oluliselt CO_2 väljalasete arvu ning keskmist ainevahetuse taset. Viimast vähendas oluliselt ka 20 korda lahjema lahusega (0.002% Fastac 50 EC lahus, alfa-tsüpermetriini 1 ppm) töötlemine. Kui sama katse viidi läbi 18ºC juures (sel temperatuuril käiakse tavaliselt ka põllul toitu kogumas), siis esimeses variandis (0,004% Fastac 50 EC), kadusid rütmilised CO_2 väljalasked ning lihaste aktiivsus. Teises variandis (0.002% Fastac 50 EC) ei kadunud regulaarsed CO_2 väljalasked ega ilmnenud häired lihaste töös, kuid katkendlik hingamine asendus tsüklilise hingamisega, mille puhul CO_2 väljalaske tase enam nullini ei jõua ehk hingamisel 'suletud faas' puudub. Mõlemad Fastac 50 EC kontsentratsioonid mõjutasid 18ºC juures oluliselt ka keskmist ainevahetuse taset. Fastac EC 0.004% lahusega töötlemise järelmõjuna vähenes oluliselt kimalaste eluiga. Samas ei tuvastatud selle insektitsiidi olulist mõju veekaole. Seega ei leitud kinnitust teooriale, et katkendlik hingamine toimib kui vett säästev mehhanism.

Käesoleva uurimustöö tulemustest järeldub, et mesilastele kui suvirapsi peamistele tolmeldajatele piisava toiduvaru tagamiseks on suvirapsi vaja väetada kompleksväetisega. Pestitsiide tootvatele ettevõtetele on küll kohustuslik lisada kõigile oma toodetele info letaalsete dooside kohta, kuid kahjuks ei peegelda laboris ja väikesemahulistes põllukatsetes tehtud uurimused alati tegelikku olukorda põllutingimustes. Siin võivad mesilaste valikuid mõjutada mitmed neile olulised faktorid, nt õite tihedus, mis oli meie uurimuses nende käitumisel peamiseks signaaliks. Lisaks mõjutavad insektitsiidide subletaalsed doosid tolmeldajate füsioloogilist seisundit, mis võib olla nende arvukuse globaalse vähenemise üheks võimalikuks põhjuseks. Tolmeldajad on evolutsioonis kohastunud ära tundma erinevaid signaale ning vastavalt nendele käituma. Kuna pestitsiidide kasutamine on selles protsessis küllaltki uus nähtus, ei ole tolmeldajad nendega veel kohastunud ja riske ära tundma õppinud.

Pestitsiide ei tohiks kasutada rutiinselt ja lihtsalt profülaktika mõttes, vaid vajadust nende järele tuleks põllul eelnevalt seirata. Tolmeldajate kaitsmist pestitsiidide negatiivsete mõjude eest tuleks toetada ka läbi poliitikate, nt läbi Euroopa Liidu ühise põllumajanduspoliitika rakendatava põllumajandusliku keskkonnatoetuse meetme. Lisaks pestitsiidide kasutamise vähendamisele tuleks läbi viia täiendavaid uurimusi pestitsiidide toksilisusest, järeltoimetest ning nõrkade dooside mõjust elusorganismidele ning välja töötada keskkonnasõbralikumaid kasvatustehnoloogiaid (nt entomovektor-tehnoloogia ja biopestitsiidid).

ACKNOWLEDGEMENTS

This study was carried out at the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences. My deepest gratitude to my main supervisor Marika Mänd, for her advise and comments throughout the preparation of the manuscript and for the encouragement, guidance and patience she demonstrated during my studies. I am very grateful to Anne Luik, my co-supervisor, for being always supportive and encouraging.

I sincerely thank Reet Karise for her support, valuable comments and suggestions during the study period and throughout the preparation of the manuscript. She has given me constant support in so many various ways.

I am very thankful to Ingrid H. Williams and Luule Metspalu for linguistic help.

I would like to thank Madis Ajaots and Peeter Lääniste for preparing the fields, and my colleagues in the institute for their support with advice and field work.

I would like to thank my colleagues in Agricultural Research Centre, especially Tiina Köster, Katrin Vask, Pille Koorberg and Marje Särekanno for their patience and advice.

I warmly thank my family and friends for their support, tolerance and understanding during the completion of this thesis.

I am very grateful to Luule Metspalu and Ivar Sibul for peer-reviewing the manuscript of my thesis.

This research was funded by the Estonian Science Foundation (Grants No. 7391, 8895, 5737, 6722, 9450), Target Financing SF170057s09 and the Estonian Ministry of Agriculture.

I

PUBLICATIONS

Mänd, M., Williams, I. H., **Viik, E.**, Karise, R. 2010

OILSEED RAPE, BEES AND
INTEGRATED PEST MANAGEMENT

In: (Ed. Williams, I.H.) Biocontrol-based integrated management of oilseed rape pests. Springer Dordrecht Heidelberg, London, New York, p. 357-379

Chapter 14
Oilseed Rape, Bees and Integrated Pest Management

Marika Mänd, Ingrid H. Williams, Eneli Viik, and Reet Karise

Abstract As a major mass-flowering crop producing an abundance of nectar and pollen, oilseed rape is very attractive to honey bees, bumblebees and solitary bees. It provides a food resource of considerable value in sustaining bee populations in agroecosystems at a time when bees are in decline. Although the flowers are self-fertile, they are entomophilous, and pollination studies, both in the glasshouse and in the field, suggest that bee foraging activities on the crop have many beneficial effects for the grower, including improving both the quantity and quality of the seed produced. However, bees foraging on the crop are vulnerable to the effects of insecticides, mostly pyrethroids applied to the crop, particularly when these are applied during flowering to control inflorescence pests. Effects may be lethal or sub-lethal; the latter have been little studied but there is growing evidence that insecticides affect many aspects of bee behaviour and physiology, such as division of labour, foraging and orientation, reproduction and respiration. Husbandry practices on the crop must therefore seek to minimise the use of insecticides on the crop, particularly during flowering, in order to sustain and not diminish bee populations foraging on the crop. Bees may even have a role in integrated pest management strategies incorporating biocontrol through their capacity to vector entomopathogenic fungal spores to the flowering canopy of oilseed rape to kill inflorescence pests.

14.1 Introduction

Oilseed rape (*Brassica napus* L.) is an oil crop of increasing importance worldwide. It is the major oilseed crop grown in northern and central Europe with over 5 million ha grown and a production of over 15 million tonnes in 2006 (Eurostat 2009, see also Williams Chapter 1 this volume). The flowers of oilseed rape yield abundant nectar and pollen and are very attractive to bees, which consequently are often abundant on flowering rape crops. The growing of mass-flowering oilseed rape

M. Mänd (✉)
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, 51014, Estonia
e-mail: marika.mand@emu.ee

I.H. Williams (ed.), *Biocontrol-Based Integrated Management of Oilseed Rape Pests*,
DOI 10.1007/978-90-481-3983-5_14,

crops thus greatly enhances nectar and pollen resource availability in agricultural areas and, when appropriately managed, have potential to promote the abundance as well as the fitness of bee populations (Westphal et al. 2009). Many beekeepers move their honey bee colonies to crops of oilseed rape during flowering; honey is therefore an important by-product of the crop (Williams 1980, Williams and Cook 1982, Williams et al. 1993).

Although commonly considered to be a self-pollinating species, oilseed rape has entomophilous flowers capable of both self- and cross-pollination and there is substantial evidence that seed quantity and quality can be improved by the foraging activities of bees on the crop.

On the other hand, bees foraging on the crop are vulnerable to the toxic effects of pesticides applied to the crop and this may contribute to the decline of wild bees as well as honey bees (Corbet et al. 1991, Miranda et al. 2003). Frequent applications of broad-spectrum, non-selective insecticide compounds, mainly synthetic pyrethroids, are commonly applied to rape crops throughout Europe each year for the control of economically-important insect pests in autumn, spring and summer (see Williams et al. Chapter 1, Ulber et al. Chapter 13, this volume); some applications are made during flowering when bees may be foraging on the crop and are particularly vulnerable to their toxic effects. Further, insecticides are often applied in tank-mixes with fungicides; this may change the effects of both products on non-target organisms; the toxicity of the insecticide may be greater when applied in a tank-mix (Muranjan et al. 2006). Despite research data indicating severe mortality effects on beneficial insects, less attention has been paid to sub-lethal effects (Gels et al. 2002, Thompson 2003). There is increasing concern amongst beekeepers that sub-lethal doses of pesticides may have a significant impact on the behaviour of honey bees (Pajot 2001) and there is growing evidence that they also affect their physiology.

The intensification of agriculture has lead to a rapid decline in the species-richness of farmland (Benton et al. 2003). General and widespread shortage of bee-pollinators is predicted in agricultural areas of America (Kremen et al. 2004), Asia (Klein et al. 2003) and Europe (Williams et al. 1991, Williams 1996). Bees are important pollinators not only of agricultural ecosystems but of almost all terrestrial ecosystems because they provide a vitally important ecosystem service as pollinators for a wide range of agricultural, horticultural and wild plants (Corbet et al. 1991, Williams 1994, 1996, Klein et al. 2007). The decline of bee populations is therefore currently giving cause for great concern (Williams 1996, Biesmeijer et al. 2006, Gabriel and Tscharntke 2007).

Several factors have been suggested as possible contributors to this decline, including changes in climate and the effects of predators and parasites (Williams 1986). However, the principal factor is likely to have been the loss and degradation of habitats and of critical food resources due to changes in land-use and agricultural practice (Osborne et al. 1991, Williams et al. 1993, Mänd et al. 2002, Sepp et al. 2004, Goulson et al. 2005, Öckinger and Smith 2007). The supply of nectar and pollen is now often insufficient in European agricultural landscapes to support healthy bee populations (Goulson et al. 2005, Öckinger and Smith 2007). Oilseed rape, as a mass flowering crop, provides highly rewarding resources of both nectar

and pollen for bees and therefore promotes colony growth and bee abundance (Westphal et al. 2003, 2009). Thus it is vital that husbandry of the oilseed rape crop helps to sustain and not to diminish bee populations.

It is essential therefore to consider bee populations, their interactions with the oilseed rape crop as well as their importance to the wider environment when developing pest management strategies for the crop. Alternatives to chemical insecticides for pest management are needed to reduce pesticide applications to the crop and thereby minimize the pressure on beneficial insects such as bees and parasitoids (see also Ulber et al. Chapter 13 this volume). Due to their morphological and behavioural characteristics, bees may even be used to aid pest management on the crop. Their hairy bodies are adapted for carrying pollen grains but they can also be used to vector antagonistic micro-organisms, such as entomopathogenic fungi for the control of inflorescence pests. Development of bee-mediated biological control vector-technology has great potential in integrated pest management strategies for crop protection (Williams 2004, Williams et al. 2005).

This review analyses the importance of oilseed rape as a food resource for bees, describes its pollination requirements, discusses the vulnerability of bees to pesticides applied to the crop and examines the potential for use of bees as entomovectors within integrated pest management strategies for the control of inflorescence pests of oilseed rape.

14.2 Oilseed Rape as a Source of Forage for Bees

14.2.1 The Flower

Oilseed rape is a typical cruciferous plant with yellow (or in some cultivars, white) flowers arranged in elongated terminal racemes. Each flower has four sepals, four petals and, usually, six stamens, four of which are longer and two shorter than the style. The flower bears four partly-hidden nectar glands (nectaries) at the base of the six stamens, two at the inner bases of the short stamens and two outside the ring of stamens (Hasler and Maurizio 1950, Eisikowitch 1981).

The flowers may open at any time of the day, but usually begin to open early in the morning and most are fully open by 9.00 h. They remain open for up to 3 days, closing slightly at night, but opening fully again the next morning; winter rape flowers are open for 1–3 days, whereas flowers of spring rape open for 1–2 days. Flowering extends from 22 to 45 days (Radchenko 1964, Free 1993, Delaplane and Mayer 2000) depending on weather conditions. When the weather is cold and damp, the flowers are open for longer time than in warmer and drier weather (Williams 1985).

14.2.2 Nectar Production

Oilseed rape flowers yield abundant nectar. Nectar volume can vary greatly from 0.2 µl per flower up to 6 µl per flower (Free 1993, Davis et al. 1994, Pierre et al. 1999). Pierre et al. (1999) tested 71 cultivars of winter oilseed rape for floral nectar volume and found that on average a flower secretes about 2 µl. Nectar volume per

flower may be affected by genotype (Pierre et al. 1999), cultivar (Davis et al. 1994), flower age (Williams 1980) and local environmental conditions (Williams 1985, Rathcke 1992). Nectar production has been reported to decrease towards the end of the day (Radchenko 1964), and to be greater in the morning and early afternoon than midday (Williams 1985). The flowers are able to replenish the level of nectar completely within 30 min of being emptied which makes them very attractive to bees. Nectar production even increases if bee density is high, and flowers are visited more than three times per day (Williams 1985). In a given genotype, nectar secretion can fluctuate from one- to three-fold depending on the time of day (Williams 1985, Pierre et al. 1999).

Nectar production in the two types of nectaries varies within a single flower. Inner nectaries begin to secrete nectar before the flowers are fully open and produce much more nectar than the two outer nectaries (Hasler and Maurizio 1950, Eisikowitch 1981), whereas, the outer nectaries are more accessible to pollinators than the inner ones, particularly towards the end of flowering (Davis et al. 1994, Pierre et al. 1999).

Due to the significant heterosis for seed yield, in addition to the conventional cultivars, hybrid cultivars of oilseed rape were evolved (Riaz et al. 2001). Hybrid composites consisting of a male-sterile component and a male-fertile component have been widely used in the EU. However, the male-sterile lines did not secrete enough nectar for pollinators. Pierre et al. (1999) demonstrated a clear difference in nectar production between male-sterile lines and their isogenic male-fertile counterparts. Mesquida and Renard (1979) showed that 68% of male-sterile flowers had only two of the four nectaries present, 20% had only one nectary and 12% had none. The remaining nectaries of male sterile flowers were small, with the consequence that male-sterile flowers secreted ten times less nectar than male-fertile ones. Under different environmental conditions, five male-sterile cybrid (hybrid composite) lines of 'Darmor' produced from 50% up to 90% less nectar than male-fertile lines (Mesquida et al. 1991). By contrast, Pierre et al. (1999) showed that nectar production of some of the male-sterile lines, compared with male-fertile genotypes, was generally not all that low. For example, male sterile 'Fu58 Darmor' produced 2.83 µl of nectar per flower which was greater than the average amount of nectar produced by male-fertile genotypes.

More recently, composite hybrid cultivars have been replaced with restored hybrid cultivars (Pinochet and Bertrand 2000). Unlike the male-sterile lines of composite hybrid cultivars, the nectar quantities produced by restored lines are similar to those produced by male-fertile oilseed rape cultivars (Pierre et al. 1999). However, for the breeding of restored hybrid cultivars and seed production for commercial growing, the combination of male-sterile and male-fertile lines is still necessary (Steffan-Dewenter 2003).

The nectar of oilseed rape flowers contains carbohydrates, such as sucrose, glucose, fructose and ribose (Hasler and Maurizio 1950, Pierre et al. 1999). The sugar concentration in the nectar is highest at the beginning of the flowering period (30.24 g/100 ml) and decreases towards the end (10.64 g/100 ml) (Pernal and Currie 1998). The same temporal trend was observed in different cultivars (Pierre et al. 1999). Similarly, during the life of a flower, sugar concentration of the nectar is

greatest when the flower opens, and lowest before it withers (Radchenko 1964). Nectar production is greatest at the beginning of the day, while the sugar concentration increases toward the end of the day (Meyerhoff 1958, Radchenko 1964). Most (95%) of the total nectar carbohydrate per flower is secreted by the inner pair of glands, because the inner nectaries are directly supplied with phloem alone, whereas the outer glands, which are poor nectar yielders, lack any vascular supply or are barely innervated by phloem (Davis et al. 1994).

Climatic factors influence nectar sugar concentration of many plants, including that of oilseed rape (Corbet et al. 1979); these include temperature, rainfall, relative humidity of air and sunshine, as well as edaphic factors (Mesquida et al. 1991). For example, at high relative humidity (80–90%) nectar from the inner and outer nectaries has the same sugar concentration (22–33%), but at a lower range of relative humidity the outer nectaries, which are relatively exposed, have a higher sugar concentration (Eisikowitch 1981).

14.2.3 Pollen Production

Oilseed rape flowers produce a lot of pollen. For example, the number of pollen grains produced per flower of the spring oilseed rape cultivar 'Drakkar' averaged 125×10^3 (Pertl et al. 2002). Pollen contains proteins, lipids, carbohydrates, starch, sterols, vitamins, and minerals (Herbert 1992, Day et al. 1990). All are important nutrients for brood rearing and development of young worker bees, particularly the protein content (Winston 1987, Hrassnigg and Crailsheim 1998). Pollens from different plant species differ in amino acid composition, concentration or both, and pollens with high proportions of essential amino acids are assumed to be of greater nutritional value. Oilseed rape pollen is rich in the amino acids most essential for bees, i.e., leucine, valine and isoleucine (Cook et al. 2003).

14.3 Pollination Requirements of Oilseed Rape

The flowers of oilseed rape are self-fertile (autogamous). Before the corolla fully expands, the four long stamens dehisce and release pollen outward the flower. Anthers on the two short stamens release pollen below the stigma which lengthens during flowering to reach the height of the anthers of the long stamens. When the flower is old, the long stamens bend towards the flower centre so that they become directed towards the stigma, and self-pollination can occur. Thus the morphology and behaviour of the oilseed rape flower encourage cross-pollination at first, but self-pollination later (Eisikowitch 1981, Williams 1985, Free 1993, Bell and Cresswell 1998, Delaplane and Mayer 2000).

Pollination studies (Williams 1978, 1984, Williams et al. 1986, 1987) have shown that oilseed rape cultivars set equally well whether self- or cross-pollinated; cultivars differed in the proportion of seed set from cross-pollination (up to 40%) (Williams 1985). However, in the case of cross-pollination more pollen can reach the stigmas, particularly pollen from the short stamens (Free 1993). Cross-pollination with

pollen from short stamens is significantly superior to that from long stamens, and gives a 14% greater weight of seed per pod (Free 1993, Steffan-Dewenter 2003). Moreover, in a normal population there are individual plants which are self-sterile or prefer foreign pollen (Rives 1957, Williams et al. 1987, Williams and Simpkins 1989, Becker et al. 1992, Free 1993).

Overall, most authors agree that pollen vectored by wind, insects or gravity is necessary for seed production in oilseed rape (Williams 1978, Eisikowitch 1981, Free 1993, Westcott and Nelson 2001). However, the proportion of pollen vectored by wind and insects and over what distance, is still debated (Timmons et al. 1995, Ramsay et al. 2003, Devaux et al. 2008).

Plants grown in the still air of a glasshouse have poor seed set (Eisikowitch 1981, Mesquida and Renard 1982, Mesquida et al. 1988); shaking plants to simulate movement by wind improves seed set (Williams et al. 1986). Pollination studies in the field have shown that plots exposed to wind but caged to exclude insects often yield at least as well as open-pollinated plots (Williams 1978, 1984, Williams et al. 1987). Wind has been even suggested to be a primary pollen vector of oilseed rape (Timmons et al. 1995, Wilkinson et al. 2003). Under field conditions, the movement of plants by wind could increase the self-pollination of cultivars that auto-pollinate poorly. Pollen grains may be carried over long distance: from 400 m up to 3,000 m (Scheffler et al. 1995, Hall et al. 2000, Rieger et al. 2002, Beckie et al. 2003, Devaux et al. 2008). Thus, wind not only causes self-pollination of flowers by moving them, but also causes cross-pollination by transporting considerable quantities of pollen. But Rieger et al. (2002) have questioned the efficacy of wind and others have shown that wind alone is insufficient to attain maximum seed set (Williams 1978, Eisikowitch 1981, Free 1993, Cresswell et al. 2002, 2004, Ramsay et al. 2003). Oilseed rape has entomophilic pollen grains, which cannot be transferred by wind alone; anthers when flicked by insects or artificially under dry conditions behave like catapults raising a cloud of pollen grains (Eisikowitch 1981). So, additional pollination by insects may be necessary.

14.4 Bees as Pollinators of Oilseed Rape

Oilseed rape is visited by honey bees, bumblebees and solitary bees, including species of *Andrena*, *Halictus* and *Megachile*. Honey bees are usually the most abundant visitors. Rape flowers produce such abundant nectar and at a time when there are few other cultivated food plants available for them, that honey bees visit rape crops from a distance of 3.5–4 km from their hives and neglect fruit trees in favour of rape (Free 1993). Furthermore, many beekeepers move their colonies onto or near to oilseed rape crops to benefit from the nectar and pollen it produces (Williams 1980, Williams and Cook 1982, Williams et al. 1993, Carreck et al. 1997). Although many species of bumblebee and solitary bee may visit a crop, their proportion is often quite low (Free 1993, Varis 1995, Karise et al. 2004). All bee species successfully transfer rape pollen from anthers to stigmas.

Earlier studies have shown that insect pollination of oilseed rape can lead to higher seed set and yield (Williams 1978, Williams and Simpkins 1989, Westcott and Nelson 2001). According to Free and Nuttall (1968), plants caged with bees produced 13% more seed than plants caged without bees. Recorded benefit from bee pollination ranges from 13 to 64% more seeds per pod (Williams 1985). But there are still some questions about the degree of benefit to seed production from insect pollinators. Positive effects are dependent on cultivar, environmental growing conditions, and the compensatory capacity of the crop (Williams and Free 1979, Williams et al. 1987) and include shortening of the flowering period, reduction of raceme production, acceleration of ripening (Mesquida and Renard 1981, Williams 1984, Mesquida et al. 1988), and increases in seed germination rate (Frediani et al. 1987, Kevan and Eisikowitch 1990) and seed oil content (Radchenko 1964, Mishra and Kaushic 1992).

The influence of honey bees on oilseed rape flowering may be explained by the fact that flowers are visited early in their development. Such early visiting is immediately followed by deposition of abundant pollen on the receptive stigmas. Consequently flowers pollinated in this way wither more quickly. Flower life is strongly reduced, flowering is shorter, and is more uniform and coordinated than for plants that are not insect pollinated (Mesquida et al. 1988).

Mesquida and Renard (1979) found that bee pollination slightly increased the final yield of the male-sterile plants, but significantly increased the yield of the male-fertile plants. Sabbahi et al. (2005) showed an improvement in rape seed yield of 46% in the presence of three honey bee hives per hectare, compared with the absence of hives. This suggests that supplemental pollination may increase set of early flowers, evenness of ripening, and ease of harvest (Williams 1978), therefore the plant would produce fewer flowers (Free 1993), and the flowering period and vegetative growth would shorten (Mesquida et al. 1988, Free 1993). It increases the number of seeds per pod, the number of seeds per plant (Steffan-Dewenter 2003), the evenness of ripening, thus reducing seed loss at harvesting (Free 1993). Altogether the seed yield of oilseed rape could be higher by up to 25–46% (Delaplane and Mayer 2000, Sabbahi et al. 2005).

14.5 Toxicity to Bees of Insecticides Applied to Oilseed Rape

Bees are especially vulnerable to the toxic effects of insecticides applied during flowering when they are foraging on the crop. They may be exposed through direct contact with spray droplets, through chemical residues left on the plant surface, and through feeding on contaminated nectar or pollen, either as adults or larvae. Effects may be lethal or sub-lethal; most studies have assessed lethal effects, while only a few have addressed sub-lethal effects. The effects of pesticides on non-target organisms have been studied extensively. It is obligatory for chemical companies to provide mortality data for their products for all larger organism groups. But, despite research data indicating the severe mortality rate on bees, less attention has been paid to the sub-lethal effects. In recent years, this has been an increasing area

of study and a subject of discussion between scientists and regulatory authorities (Thompson and Maus 2007).

In addition to deficient information of the sub-lethal effects of insecticides, there exists the problem of extrapolating data from honey bees to bumblebee and other pollinating bees. Pesticide risk assessments for honey bees are based on hazard ratios which rely on application rates and toxicity data that are unlikely to be appropriate for bumblebees. The latter are active at different times and on different crop species and, therefore, are likely to have different exposure profiles. Unlike honey bees, deaths of bumblebees due to pesticides are unlikely to be reported, since the bees are not kept domestically and die in small numbers (Thompson and Hunt 1999). The information on pesticide toxicity on non-*Apis* bees is scarce, and limited to species managed for crop pollination (Ladurner et al. 2003).

14.5.1 Lethal Effects

In conventional farming, application of many insecticides (e.g., pyrethroids) considered to be safe for honey bees, is permitted to the oilseed rape crop while it is in flower. Despite this, 57 out of 117 honey bee poisoning incidents in UK during 1994–2003 resulted from spray applications to flowering crops; 17 of these incidents were through approved use of the products (Barnett et al. 2007). Pyrethroids, most often sprayed on flowering oilseed crops, have been reported to be repellent to honey bees (Thompson 2001), although this is still in question in the field situation. Karise et al. (2007) found no repellency of alpha-cypermethrin to honey bees on oilseed rape under field conditions but found that flower visitation depended on the density of flowers present. If any repellency does occur with respect to this insecticide, the attractiveness of the flower resource is likely to override it.

In organic farming, pesticides are also needed and many botanical insecticides are permitted for use in controlling pests. The main ingredient of Neem extracts, azadirachtin, is considered to be safe for honey bees (Zehnder et al. 2007), but has been found to cause changes in the foraging behaviour in bumblebees (Karise et al. 2006). Pyrethrins are toxic to bees; quassia and rotenone do not harm bees (Zehnder et al. 2007). The toxicity of botanical compounds to bees tends to be lower than that of synthetic compounds because their degradation time is shorter and timing of application helps to minimize harmful effects on beneficial insects (Kühne 2008).

14.5.2 Sub-lethal Effects

Studying the sub-lethal effects of pesticides is complicated due to difficulties in measuring the effects. Results obtained in the laboratory may not match with those obtained in the field (Thompson and Maus 2007). Under certain circumstances, sub-lethal effects may cause more harm than lethal doses since they affect the survival of the brood and colony. Systemic compounds have been considered safe for pollinators when not applied to the flowers. However, the residues of the compounds still contaminate nectar and pollen in sub-lethal doses via both active and passive

transport (Thompson 2001, Cutler and Scott-Dupree 2007). Contamination may occur after application of the compounds to other parts of plants (Ferguson 1987), to the soil (Jaycox 1964) or on seeds (Dikshit et al. 2002, Sur and Stork 2003). Contaminated nectar and pollen poses a potential danger not only to forager bees but also to bees in the hive and to brood. The toxicity of pesticides to brood has been investigated far less than toxicity for adults (Alix and Vergnet 2007).

14.5.2.1 Effects on Division of Labour

Division of labour plays an important role in colonies of social insects. Workers have specific, often age-dependent tasks. Treatment of honey bees with juvenile hormone analogues (synthetic hormone-like compounds used as insecticides), results in a decreasing ability of young emerging bees to feed larvae, due to the early degeneration of the hypopharyngeal glands and precocious foraging ability (Tasei 2001). Changes in the division of labour of honey bees, such as decreased house cleaning abilities, delayed onset and duration of foraging and handling of nectar, have also been recorded (reviewed by Thompson 2003). These changes affect both honey yield and the overwintering of colonies (Thompson et al. 2005).

14.5.2.2 Effects on Foraging and Orientation

Foraging depends on the bee's ability to discriminate odours, to learn, to communicate, and to orientate within its environment; altering these systems may result in a decrease in foraging. The bees' orientation and communication ability have been found to be affected by sub-lethal doses of organophosphorus insecticides (Schricker and Stephen 1970), synthetic pyrethroids (Cox and Wilson 1984, Vandame et al. 1995) and neonicotinoids (Bortolotti et al. 2003, Yang et al. 2008). Pyrethroids and neonicotinoids have also been shown to affect both foraging activity (Thompson 2003) and learning capacities (Decourtye et al. 1999, 2003, Guez et al. 2001, Ramirez-Romero et al. 2005). Pyrethroids may also affect thermoregulation (Jagers op Akkerhuis et al. 1999b, Belzunces et al. 2001); in cooler climates, this can lead to decreased flying ability. The decrease in foraging and in returning foragers reduces brood production (Thompson 2003), which in turn may weaken a colony's potential to survive the winter.

14.5.2.3 Effects on Reproduction

All classes of insecticides affect the reproductive behaviour of bees (reviewed by Thompson 2003). Reduction of brood may have more damaging consequences for honey bees than simply the moderate loss of foragers (Haynes 1988, Thompson et al. 2007). Thompson et al. (2005) have reported 40–95% egg mortality over 2 weeks after diflubenzuron application and 45–60% egg mortality over 2 weeks after fenoxycarb application. The insect growth regulator fenoxycarb has caused the death of almost all larvae or developing malformed pupae (Van der Steen and de Ruijter 1990, Aupinel et al. 2007).

Besides killing brood, insecticides can cause changes in the development of the larvae. Contamination of the food by insect growth regulators (Tasei 2001) can increase development time and cause malformations. In solitary bees, pyrethroids (Tasei et al. 1988) and in honey bees, pyrethroids (Tasei et al. 1988) and neonicotinoids (Schmuck et al. 2001) have been found to affect their fecundity. Some organophosphates, pyrethroids and neonicotinoids have affected the honey bee queen's status or have interfered with a colony's ability to requeen itself (Stoner et al. 1985, Thompson et al. 2005). Organophosphates have decreased the longevity of honey bees (Johansen and Mayer 1990). Neonicotinoids (Tasei et al. 2000) and organophosphates (Johansen and Mayer 1990) have decreased brood production in the bumblebee.

14.5.2.4 Effects on Respiration

Better understanding of the effects of insecticides in the field benefits from insight into their effects on different physiological functions, for example, on respiration. In the case of bees, it is difficult to examine the effects of insecticides on respiration patterns because there is little data on their normal respiration patterns. However, this has been an area of increasing interest during the past decade.

Since water is a key element in every living organism, most insects have probably evolved mechanisms to prevent excessive water loss (Klowden 2002). Resting insects often exhibit discontinuous gas exchange cycles (DGC), a function of which may be the reduction of respiratory water loss (Levy and Schneiderman 1966, Lighton 1994) through the large inner surface of the tracheal system.

According to Lighton (1994, 1996), in the state of discontinuous gas exchange, the spiracles are closed most of the time. At low oxygen rates inside the trachea the spiracular valves flutter, allowing oxygen to enter the tracheal system. As larger amounts of carbon dioxide accumulate in the tracheae and haemolymph (Wobschall and Hetz 2004), the spiracles open and allow the gas to escape. Thus, as compared with continuous respiration, loss of carbon dioxide along with evaporated water occurs only discontinuously during the brief open phases of the spiracles. There are different views about the origin of DGC, as reviewed by Chown (2002) and Chown et al. (2006). There are also hypotheses that DGC serves as an adaptation for coping with hypercapnia and/or hypoxia in soil-living insects (Lighton 1998, Vogt and Appel 2000, Lighton et al. 2004) or protection against the oxidative damage during the periods with low metabolic cost (Hetz and Bradley 2005).

The existence and the precise pattern of DGC depend on the species (Lighton 1994, 1996, Slama 1999, Chown et al. 2006), individual characteristics (Marais and Chown 2003, Gibbs and Johnson 2004, Karise et al. 2010), life stage of the individuals (Beekman and van Stratum 1999, Mänd et al. 2005, 2006) and environmental conditions like temperature (Lighton and Lovegrove 1990, Lighton 1996, Vogt and Appel 2000, Kovac et al. 2007), relative humidity (Duncan et al. 2002, Lighton 2007, Slama et al. 2007) and the amount of oxygen or carbon dioxide in the air (Lighton 1998, Vogt and Appel 2000, Lighton et al. 2004).

DGC patterns have been used to characterize the physiological state of an insect, as several stress factors, including chemical ones, can affect them (Kestler 1991). Although knowledge about the sub-lethal effects of pesticides on insect physiology is scarce, it is known that treatments of arthropods with pyrethroids cause neurotoxic effects in parts of the nervous system, including the central nervous system and sensory, motor or neurosecretory neurons (Corbett 1974, Jagers op Akkerhuis et al. 1995). Because the closing and opening of spiracular valves is controlled by the nervous system, the neurotoxic effects may also include interference by DGC. In pupae of cabbage butterfly *Pieris brassicae*, after the treatment with original pyrethrum, the DGCs disappeared and metamorphosis was disrupted (Harak et al. 1999, Jõgar et al. 2008).

Pyrethroids, as well as many other insecticides, can induce increased water loss in arthropods (Gerolt 1976, 1983), due to production of diuretic hormones (Jagers op Akkerhuis et al. 1999a). This process could be reversible if the insect could replenish its water reserves. Since the pyrethroids often affect motion as well, causing the knockdown effect, death may come through desiccation (Jagers op Akkerhuis et al. 1995, 1999a, Thompson 2003).

14.6 Bees as Vectors of Entomopathogenic Fungi for Pest Control on Oilseed Rape

Bees are covered in an abundance of branched body hair, specially adapted to trap and transport pollen grains from flowers back to the colony or nest site to feed to brood (Free and Williams 1972). These hairs can also trap and transport the spores of bacteria and fungi (Batra et al. 1973, Sandu and Waraich 1985). This ability has been utilized in the development of biocontrol strategies to control various plant pests and diseases on a variety of crops. For example, Thomson et al. (1990) showed that honey bees could be used to carry spores of the bacteria *Pseudomonas fluorescens* (Trevisan) and *Erwinia herbicola* (Brown) to the flowers of apple to control fireblight disease caused by the bacterium *Erwinia amylovora* (Burrill). Similarly, honey bees have vectored spores of the fungus *Gliocladium roseum* (Bainier) to strawberry (Peng et al. 1992) and to raspberry (Yu and Sutton 1997) flowers to control growth of the grey mould fungus *Botrytis cinerea* Pers. More recently, the bumblebee, *Bombus impatiens* (Cresson) has been used in the glasshouse, to transport spores of the entomopathogenic fungus *Beauveria bassiana* (Balsamop-Crivelli) Vuillemin to sweet pepper flowers to control two insect pests, the plant bug, *Lygus lineolaris* (Palisot de Beauvois) and the thrips, *Frankliniella occidentalis* (Pergande) (Al-mazra'awi et al. 2006).

Bees have similarly been shown able to deliver entomopathogenic fungal spores to oilseed rape flowers to infect and kill insect pests living within the flowering canopy of the oilseed rape crop (Butt et al. 1998, Carreck et al. 2006). The pollen beetle, *Meligethes aeneus* (Fabricius) and the cabbage seed weevil, *Ceutorhynchus assimilis* (Paykull) are major inflorescence pests of oilseed rape throughout Europe (Williams Chapter 1 this volume); the latter is also a major pest in North America

(Dosdall and Mason Chapter 6 this volume). The pollen beetle feeds, as an adult, on pollen in the buds and flowers of the crop, and lays its eggs in the buds. Its larvae also feed on pollen in the buds and flowers, usually lying alongside the filaments of the stamens. The larvae are mobile, moving up the flowering inflorescence to younger flowers as they grow (Williams and Free 1978). On maturity, second instar larvae drop to the ground to pupate in the soil. The cabbage seed weevil also feeds on pollen in the flowers as well as on young buds, shoots and pods. The females lay their eggs singly in young pods on the flowering racemes. The seed weevil larva feeds within the pod on the growing seeds and on maturity, bores an exit hole through the pod wall and drops to the soil to pupate (Williams and Free 1978).

Honey bees foraging from hives, fitted with inoculum dispensers at their entrances (Fig. 14.1), have been shown to effectively deliver conidia of the entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, to the flowers of oilseed rape plots enclosed in field cages (Fig. 14.2, Butt et al. 1998, Carreck et al. 2006). Pollen beetles and seed weevils, sampled from the treated plots, both picked up lethal doses of the conidia from the flowers. When incubated in the laboratory, the fungus caused infection and mortality of both adult and larval pollen beetles, as well as of adult seed weevils (Figs. 14.3 and 14.4). Pod infestation by seed weevil larvae was too low to determine whether they were also infected by the fungus. After death, the bodies of many of the pest insects showed external conidiation of the fungus, confirming infection by *M. anisopliae* (Fig. 14.5).

Conidia of *M. anisopliae* disseminated initially from an inoculum source to rape flowers by honey bees in this way, would probably be further disseminated horizontally within the crop canopy by other insects, such as by bumblebees, foraging on the flowers. The inoculum would also be disseminated to the soil below the crop, as inoculated flowers shed their petals. How long the conidia can survive on petals is not known, but they occur naturally, albeit at a low level, and persist well in soil (Vanninen et al. 2000, Hokkanen et al. 2003). Laboratory and pot experiments have

Fig. 14.1 Honey bee hive fitted at its entrance with an inoculum dispenser containing the entomopathogenic fungus *Metarhizium anisopliae* for dissemination by the bees to the flowering canopy of oilseed rape (Photo: Ingrid Williams)

Fig. 14.2 Oilseed rape plots enclosed in field cages for the study of bee-mediated dissemination of the entomopathogenic fungus *Metarhizium anisopliae* (Photo: Ingrid Williams)

shown that mature larvae of the pollen beetle are susceptible to the fungus, not only when directly exposed to an inoculum, but also when the inoculum is applied to soil before the insects pupate in it (Husberg and Hokkanen 2000). However, in the semi-field experiments, described above, Carreck et al. (2006) found no effect on the numbers of new generation pollen beetle and seed weevil adults that emerged from pupation, following dissemination of inoculum by honey bees to the flowering crop canopy.

The effects of *M. anisopliae* on bees need further investigation as extrapolating risk from laboratory tests to bees in the field may be misleading (Alves et al. 1996). Butt et al. (1994) showed, in laboratory studies, that the honey bee was susceptible to *M. anisopliae* V245; when inoculated and then incubated at 30°C, the mean LT_{50} was 8.5 days. However, they also showed, in the laboratory, that isolates vary in

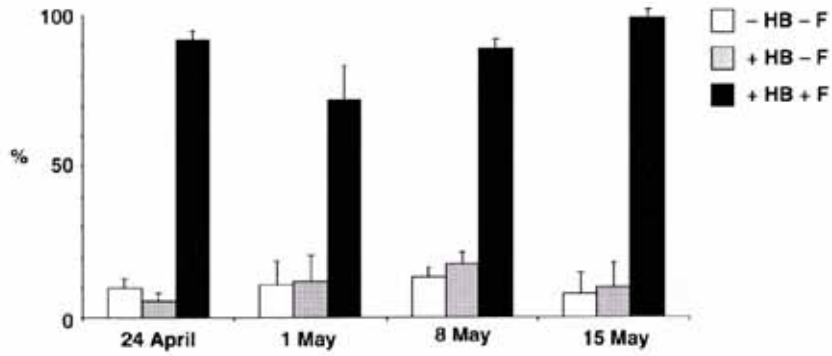


Fig 14.3 Percentage mortality of adult pollen beetles (*Meligethes aeneus*) on plots of winter oilseed rape when exposed to honey bees (HB) with and without dispensers containing the entomopathogenic fungus *Metarhizium anisopliae* (F) at their hive entrances (modified after Carreck et al. 2006)

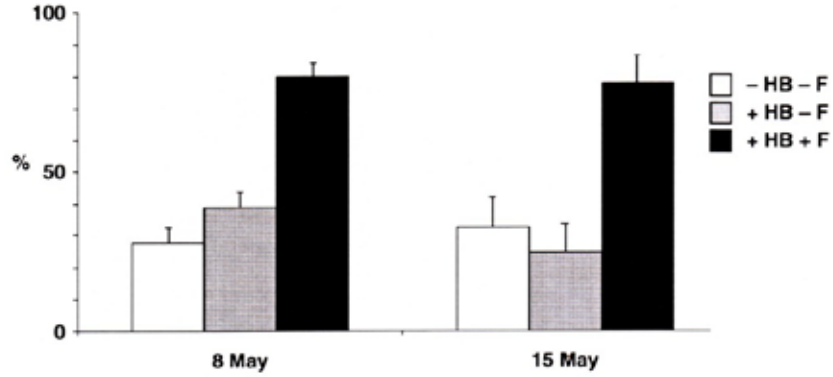


Fig. 14.4 Percentage mortality of adult seed weevils (*Ceutorhynchus obstrictus* syn. *C. assimilis*) on plots of winter oilseed rape when exposed to honey bees (HB) with and without dispensers containing the entomopathogenic fungus *Metarhizium anisopliae* (F) at their hive entrances (modified after Carreck et al. 2006)

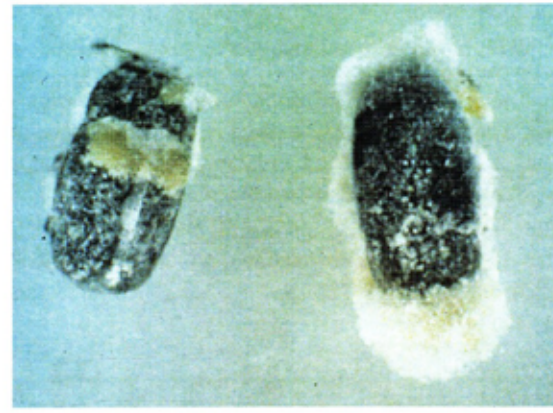


Fig. 14.5 Body of the pollen beetle, *Meligethes aeneus*, showing external conidiation of the fungus, confirming infection by *Metarhizium anisopliae* (Photo: Ingrid Williams)

their temperature tolerances and so may have different effects on honey bees within the brood nest, where the temperature is maintained at ca. 35°C, than on foragers at outside temperatures. Some isolates of *M. anisopliae* are being tested for the biological control of the parasitic mite *Varroa destructor* Anderson and Trueman in honey bee colonies (Shaw et al. 2002, Davidson et al. 2003, Kanga et al. 2003, Lodesani et al. 2003). Carreck et al. (2006) found that, although in field cages where honey bees were disseminating *M. anisopliae* inoculum some of the bees that died showed external conidiation when incubated, declines in colony population size appeared to be unrelated to fungal infection, as they were no greater in colonies disseminating fungal inoculum than in control plots with bees but no inoculum. Population decline

is usual in honey bee colonies, particularly large ones, when they are confined in field cages with limited forage (Pinzauti 1994). Further, if this strategy were to be used for pest control in oilseed rape crops some loss of honeybee foragers may be acceptable as their colonies are managed and to some extent therefore replaceable by beekeepers. The effect of *M. anisopliae* on bumblebees foraging on the crop remains to be investigated.

The effect of *M. anisopliae* on key parasitoids of the inflorescence pests of oilseed rape needs further investigation as Husberg and Hokkanen (2000) found that the hymenopterous larval endoparasitoids of the pollen beetle, *Phradis morionellus* (Holmgren) and *Diospilus capito* (Nees), both key agents in conservation biocontrol of the beetle (Ulber Chapter 2 this volume), were also susceptible, although to different extents, to spray treatment with the fungus.

14.7 Implications for Biocontrol-Based Integrated Management of Insect Pests of Oilseed Rape

Oilseed rape, as a widespread mass-flowering crop of agroecosystems of northern and central Europe, as well as in North America and other regions of the world, provides an abundant resource of pollen and nectar for bees. Many beekeepers move their honey bee colonies to oilseed rape crops during flowering; honey is a valuable by-product from the crop. Loss of food resources for bees in arable landscapes is probably a major cause of their decline over recent decades in many regions. The foraging activities of bees on the crop have been shown to improve both the quality and quantity of seed produced. Husbandry practices on the rape crop should therefore seek to sustain and not diminish bee populations.

Currently crop protection on oilseed rape, particularly against inflorescence pests such as the pollen beetle and the cabbage seed weevil, relies heavily on the application of pyrethroid insecticides. These kill beneficial insects, such as bees and parasitoids (Ulber et al. Chapter 13 this volume) foraging on the crop, particularly when applied during flowering. They also cause sub-lethal effects, although these have been little studied. The recent widespread development in many European countries of resistance to pyrethroids in the pollen beetle (Thieme et al. Chapter 12 this volume) has increased the urgency of developing integrated pest management strategies that minimise the use of insecticides on the crop, particularly during flowering. Further development of biocontrol strategies incorporating parasitoids and predators is essential to achieve this.

Honey bees also have potential for employment in biocontrol strategies. Their ability to vector the entomopathogenic fungus *M. anisopliae* for the control of inflorescence pests could perhaps be further enhanced by using an early-flowering cultivar of oilseed rape or turnip rape as a trap crop to concentrate both pest and honey bee populations. This would facilitate both concentration and horizontal transfer of the inoculum to its target pest populations before they move onto the oilseed rape main crop (Cook et al. 2006). However, to be of use in integrated pest management, any entomopathogenic fungus to be used should be benign both to

bees, needed to pollinate the crop, and to parasitoids of the pests which contribute to their biocontrol.

Acknowledgements This work was funded by Estonian targeted financing project No. SF 0170057s09 and by Estonian Science Foundation grants No 6722 and 7391.

References

Al-mazra'awi MS, Shipp L, Broadbent B, Kevan PG (2006) Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper. *Biol Control* 37: 89–97.

Alix A, Vergnet C (2007) Risk assessment to honey bees: A scheme developed in France for non-sprayed systemic compounds. *Pest Manag Sci* 63: 1069–1080.

Alves SB, Marchini LC, Pereira RM, Baumgratz LL (1996) Effects of some insect pathogens on the Africanized honey bee, *Apis mellifera* L. (Hym., Apidae). *J Appl Entomol* 120: 559–564.

Aupinel P, Fortini D, Michaud B, Marolleau F, Tasei JN, Odoux JF (2007) Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new in vitro standardized feeding method. *Pest Manag Sci* 63: 1090–1094.

Barnett EB, Charlton AJ, Fletcher MR (2007) Incidents of bee poisoning with pesticides in the United Kingdom, 1994–2003. *Pest Manag Sci* 63: 1051–1057.

Batra LR, Batra SWT, Bohart GE (1973) The mycoflora of domesticated and wild bees (Apoidea). *Mycopathol Mycol Appl* 49: 13–44.

Becker HC, Damgaard C, Karlsson B (1992) Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). *Theor Appl Genet* 84: 303–306.

Beckie HJ, Warwick SI, Nair H, Séguin-Swartz G (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecol Appl* 13: 1276–1294.

Beekman M, van Stratum P (1999) Respiration in bumblebee queens: Effect of life phase on the discontinuous ventilation cycle. *Ent Exp Appl* 92: 295–298.

Bell SA, Cresswell JE (1998) The phenology of gender in homogamous flowers: Temporal change in the residual sex function of flowers of oilseed rape *Brassica napus*. *Funct Ecol* 12: 298–306.

Belzunces LP, Vandame R, Xingfa Gu (2001) Joint effects of pyrethrinoid insecticides and azole fungicides on honeybee thermoregulation. In: Belzunces LP, Pelissier C, Lewis GB (eds.) *Hazards of pesticides to bees*. INRA, France.

Benton TG, Vickery JA, Wilson JD (2003) Farmland biodiversity: Is habitat heterogeneity the key? *Trends Ecol Evol* 18(4): 182–188.

Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351–354.

Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C (2003) Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bull Insectol* 56: 63–67.

Butt TM, Carreck NL, Ibrahim L, Williams IH (1998) Honey bee-mediated infection of pollen beetle (*Meligethes aeneus* Fab.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. *Biocontrol Sci Techn* 8: 533–538.

Butt TM, Ibrahim L, Ball BV, Clark SJ (1994) Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. *Biocontrol Sci Techn* 4: 207–214.

Carreck NL, Butt TM, Clark SJ, Ibrahim L, Isger EA, Pell JK, Williams IH (2006) Honey bees can disseminate a microbial control agent to more than one inflorescence pest of oilseed rape. *Biocontrol Sci Techn* 17: 179–191.

Carreck NL, Williams IH, Little DJ (1997) The movement of honey bee colonies for crop pollination and honey production by beekeepers in Great Britain. *Bee World* 78: 67–77.

Chown SL (2002) Respiratory water loss in insects. *Comp Biochem Phys A* 133: 791–804.

Chown SL, Gibbs AG, Hetz SK, Klok CJ, Lighton JRB, Marais E (2006) Discontinuous gas exchange in insects: A clarification of hypotheses and approaches. *Physiol Biochem Zool* 79: 333–343.

Cook SM, Awmack CS, Murray DA, Williams IH (2003) Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecol Entomol* 28: 622–627.

Cook SM, Smart LE, Martin JL, Murray DA, Watts NP, Williams IH (2006) Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomol Exp Appl* 119: 221–229.

Corbet SA, Williams IH, Osborne JL (1991) Bees and the pollination of crops and wild flowers in the European community. *Bee World* 72: 47–59.

Corbet SA, Willmer PG, Beament JW, Unwin DM, Prys-Jones OE (1979) Post secretory determinants of sugar concentration in nectar. *Plant Cell Environ* 2: 293–308.

Corbett JR (1974) *The biochemical mode of action of pesticides*. Academic Press, London, New York.

Cox RL, Wilson WT (1984) Effects of permethrin on the behaviour of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environ Entomol* 13: 375–378.

Cresswell JE, Davies TW, Patrick MA, Russell F, Pennel C, Vicot M, Lahoubi M (2004) Aerodynamics of wind pollination in a zoophilous flower, *Brassica napus*. *Funct Ecol* 18: 861–866.

Cresswell JE, Osborne L, Bell SA (2002) A model of pollinator-mediated gene flow between plant populations with numerical solutions for bumblebees pollinating oilseed rape. *Oikos* 98: 375–384.

Cutler GC, Scott-Dupree CD (2007) Exposure to clothianidin seed-treated canola has no long-term impact o honey bees. *Ecotoxicology* 100: 765–772.

Davidson G, Phelps K, Sunderland KD, Pell JK, Ball BV, Shaw K, Chandler D (2003) Temperature growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: Mesostigmata), using a nonlinear model of poikilotherm development. *J Appl Microbiol* 94: 816–825.

Davis AR, Sawhney VK, Fowke LC, Low NH (1994) Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectar size and nectar carbohydrate production and composition. *Apidologie* 25: 602–614.

Day S, Beyer R, Mercer A, Ogden S (1990) The nutrient composition of honey bee-collected pollen in Otago, New Zealand. *J Apic Res* 29: 138–146.

Decourtye A, Lacassie E, Pham-Delègue MH (2003) Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. *Pest Manag Sci* 59: 269–278.

Decourtye A, Le Metayer M, Pottiau H, Tisseur M, Odoux JF, Pham-Delegue MH (1999) Impairment of olfactory learning performances of *Apis mellifera* L. by long term ingestion of imidacloprid. In: Belzunces LP, Pélissier C, Lewis GB (eds.) *Hazards of pesticides to bees. 7th Int Symp ICPBR Bee Protection Group*. INRA, Avignon, France, pp 113–117.

Delaplane KS, Mayer DF (2000) *Crop pollination by bees*. CAB International, Wallingford, UK.

Devaux C, Klein EK, Lavigne C, Sausse C, Messéan A (2008) Environmental and landscape effects on cross-pollination rates observed at long distance among French oilseed rape *Brassica napus* commercial fields. *J Appl Ecol* 45: 803–812.

Dikshit AK, Lal OP, Sinha SR, Srivastava YN (2002) Safety evaluation, persistence and bioefficacy of Imidacloprid and beta cyfluthrin on okra. Part II. *Pestology* 26: 30–37.

Duncan FD, Krasnov B, McMaster M (2002) Novel case of a tenebrionid beetle using discontinuous gas exchange cycle when dehydrated. *Physiol Entomol* 27: 79–83.

Eisikowitch D (1981) Some aspects of pollination of oilseed rape (*Brassica napus* L.). *J Agric Sci* 96: 321–326.

Eurostat (2009) http://epp.eurostat.ec.europa.eu
Ferguson F (1987) Long term effects of systemic pesticides on honey bees. *Austr Beekeeper* 89: 49–54.
Frediani D, Pinzauti M, Caterini B (1987) Influence of the entomophilous pollination on the oilseed rape production (*Brassica napus* L. spp. *oleifera*). *Apicoltura* 3: 63–73.
Free JB (1993) *Insect pollination of crops*, 2nd edn. Academic Press, London, UK.
Free JB, Nuttall PM (1968) The pollination of oilseed rape (*Brassica napus*) and the behaviour of bees on the crop. *J Agric Sci* 71: 91–94.
Free JB, Williams IH (1972) The transport of pollen on the body hair of honeybees (*Apis mellifera* L.) and bumblebees (*Bombus* spp. L.). *J Appl Ecol* 9: 609–615.
Gabriel D, Tscharntke T (2007) Insect pollinated plants benefit from organic farming. *Agr Ecosyst Environ* 118: 43–48.
Gels JE, Held DW, Potter DA (2002) Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J Econ Entomol* 95: 722–728.
Gerolt P (1976) The mode of action of insecticides: Accelerated water loss and reduced respiration in insecticide-treated *Musca domestica* L. *Pestic Sci* 7: 604–620.
Gerolt P (1983) Insecticides: Their route to entry, mechanisms of transport and mode of action. *Biol Rev* 58: 233–274.
Gibbs AG, Johnson RA (2004) The role of discontinuous gas exchange in insects: The chtonic hypothesis does not hold water. *J Exp Biol* 207: 3477–3482.
Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. *Biol Conserv* 122: 1–8.
Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces LP (2001) Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiol Learn Mem* 76: 183–191.
Hall L, Topinka K, Huffman J, Davis L, Good A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Sci* 48: 688–694.
Harak M, Lamprecht I, Kuusik A, Hiiesaar K, Metspalu L, Tartes U (1999) Calorimetric investigations of insect metabolism and development under the influence of a toxic plant extract. *Thermochim Acta* 333: 39–48.
Hasler A, Maurizio A (1950) Ueber den Einfluss verschiedener Nährstoffe auf Blütenansatz, Nektarsekretion und Samenertrag von honigenden Pflanzen, speziell von Sommerraps (*Brassica napus* L.). *Schweiz Landw Mh* 6: 201–211.
Haynes KF (1988) Sublethal effects of neurotoxic insecticides on insect behaviour. *Annu Rev Entomol* 33: 149–168.
Herbert EW (1992) Honey bee nutrition. In: Graham JM (ed.) *The hive and the honey bee*. Dadant & Sons, Hamilton, Illinois, USA.
Hetz SK, Bradley TJ (2005) Insects breathe discontinuously to avoid oxygen toxicity. *Nature* 433: 516–519.
Hokkanen HMT, Menzler-Hokkanen I, Butt TM (2003) Pathogens of oilseed rape pests. In: Alford DV (ed.) *Biocontrol of oilseed rape pests*. Blackwell, Oxford, UK.
Hrassnigg N, Crailsheim K (1998) The influence of brood on the pollen consumption of worker bees (*Apis mellifera* L.). *J Insect Physiol* 44: 393–404.
Husberg G-B, Hokkanen HMT (2000) Effects of *Metarhizium anisopliae* on the pollen beetle *Meligethes aeneus* and its parasitoids *Phradis morionellus* and *Diospilus capito*. *Biocontrol* 46: 261–273.
Jagers op Akkerhuis GAJM, Kjær C, Damgaard C, Elmegaard N (1999b) Temperature-dependent, time-dose-effect model for pesticide effects on growing, herbivorous arthropods: Bioassays with dimethoate and cypermethrin. *Environ Toxicol Chem* 18: 2370–2378.
Jagers op Akkerhuis GAJM, Seidelin N, Kjaer C (1999a) Are we analysing knockdown in the right way? How independence of the knockdown-recovery process from mortality may affect measures for behavioural effects in pesticide bioassays. *Pestic Sci* 55: 62–68.

Jagers op Akkerhuis GAJM, Westerhof R, Straalen NMvan, Koeman JH (1995) Water balance, respiration and immobilisation in relation to deltamethrin poisoning and physical conditions in the epigeal spider *Oedothorax apicatus*. *Pestic Sci* 44: 123–130.

Jaycox E (1964) Effect on honey bees of nectar from systemic insecticides treated plants. *J Econ Entomol* 57: 31–35.

Johansen CA, Mayer DF (1990) *Pollinator protection: A bee and pesticide handbook*. Wicwas Press, Cheshire, Conn.

Jõgar K, Kuusik A, Metspalu L, Hiiesaar K, Grishakova M, Luik A (2008) Effects of Neem EC on gas exchange, tracheal ventilation, and water loss in diapausing pupae of *Pieris brassicae*. *Ent Exp Appl* 2: 165–173.

Kanga LHB, Jones WA, James RR (2003) Field trials using the fungal pathogen, *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) to control the ectoparasitic mite *Varroa destructor* (Acari: Varroidae) in honey bee, *Apis mellifera* (Hymenoptera: Apidae) colonies. *J Econ Entomol* 96: 1091–1099.

Karise R, Kuusik A, Mänd M, Metspalu L, Williams IH, Hiiesaar K, Luik A, Muljar R, Liiv K (2010) Gas exchange patterns of bumble bee foragers before and after exposing to lowered temperature. *J Insect Physiol* (in press).

Karise R, Mänd M, Koskor E, Bender A (2006) The effect of Neem EC on the pollen forage of the bumble bee *Bombus terrestris* L., *Proc Int Conf: Information Systems in Sustainable Agriculture, Agrienvironment and Food Technology, Volos, Greece, 2006*, pp 367–372.

Karise R, Mänd M, Viik E, Martin A-J, Lääniste P (2004) Flower visitors of spring oilseed rape in different cropping systems. *Latvian J Agronomy* 7: 6–11.

Karise R, Viik E, Mänd M (2007) Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape *Brassica napus* flowers in field conditions. *Pest Manag Sci* 63: 1085–1089.

Kestler P (1991) Cyclic CO_2 release as a physiological stress indicator in insects. *Comp Biochem Phys C* 100: 207–211.

Kevan PG, Eisikowitch D (1990) The effect of insect pollination on canola (*Brassica napus* L. cv. OAC Triton) seed germination. *Euphytica* 45: 33–41.

Klein AM, Steffan-Dewenter I, Tscharntke T (2003) Fruit set of highland coffee increases with the diversity of pollinating bees. *Proc R Soc B* 270: 955–961.

Klein A-M, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B* 274: 303–313.

Klowden MJ (2002) *Physiological systems in insects*. Academic Press, San Diego, USA.

Kovac H, Stabentheiner A, Hetz SK, Petz M, Crailsheim K (2007) Respiration of resting honeybees. *J Insect Physiol* 53: 1250–1261.

Kremen C, Williams NM, Bugg RL, Fay JP, Thorp RW (2004) The area requirements on an ecosystem service: Crop pollination by native bee communities in California. *Ecol Lett* 7: 1109–1119.

Kühne S (2008) Prospects and limits of botanical insecticides in organic farming. *Agronomski Glasnik* 4: 377–382.

Ladurner E, Bosch J, Maini S, Kemp WP (2003) A method to feed individual bees (Hymenoptera: Apiformes) known amounts of pesticides. *Apidologie* 34: 597–602.

Levy RI, Schneiderman HA (1966) Discontinuous respiration in insects. 2. The direct measurement and significance of changes in tracheal gas composition during the respiratory cycle of silkworm pupae. *J Insect Physiol* 12: 83–104.

Lighton JRB (1994) Discontinuous ventilation in terrestrial insects. *Physiol Zool* 67: 142–162.

Lighton JRB (1996) Discontinuous gas exchange in insects. *Annu Rev Entomol* 41: 309–324.

Lighton JRB (1998) Notes from the underground: Towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *Am Zool* 38: 483–491.

Lighton JRB (2007) Why insects evolved discontinuous gas exchange. *Curr Biol* 17: 645–647.

Lighton JRB, Lovegrove BGL (1990) A temperature-induced switch from diffusive to convective ventilation in the honeybee. *J Exp Biol* 154: 509–516.

Lighton JRB, Schilman PE, Holway DA (2004) The hyperoxic switch: Assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *J Exp Biol* 207: 4463–4471.

Lodesani M, Costa C, Nipoti P, Prodi A (2003) Use of three mitosporic fungi for the control of the honey bee parasitic mite *Varroa destructor* Anderson & Trueman. *Proc XXXVIIth Int Apic Cong, Ljubiana, Slovenia*, pp 422–423.

Marais E, Chown SL (2003) Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *J Exp Biol* 206: 4565–4574.

Mesquida J, Pham-Delegue MH, Marilleau R, le Metayer M, Renard M (1991) The floral secretion in male-sterile cybrid of winter rapeseed (*Brassica napus* L.). *Agronomie* 11: 217–227 (in French).

Mesquida J, Renard M (1979) Preliminary results on the pollination of male sterile winter rape and methods for production of hybrid seed. *Bull Inf Tech CETIOM* 65: 3–14.

Mesquida J, Renard M (1981) Pollinisation du colza d'hiver mâle-fertile et mâle-sterile (*Brassica napus* L. *var. oleifera* Metzger) par l'abeille domestique (*Apis m. mellifera* L.) Effects sur la phenologie et le rendement. *Apidologie* 12: 345–362.

Mesquida J, Renard M (1982) Etude de la dispersion du pollen par le vent et de l'importance de la pollinisation anemophilie chez le colza (*Brassica napus* L. *var. oleifera* Metzger). *Apidologie* 13: 353–367.

Mesquida J, Renard M, Pierre JS (1988) Rapeseed (*Brassica napus* L.) productivity: The effect of honey bees (*Apis mellifera* L.) and different pollination conditions in cage and field tests. *Apidologie* 19: 51–72.

Meyerhoff G (1958) The foraging behaviour of honeybee on rape. *Leipzig Bienenztg* 72: 164–165 (in German).

Miranda JE, Navickiene HMD, Nogueira-Couto RH, De Bortoli SA, Kato MJ, Bolzani VdaS, Furlan M (2003) Susceptibility of *Apis mellifera* (Hymenoptera: Apidae) to pellitorine, an amide isolated from *Piper tuberculatum* (Piperaceae). *Apidologie* 34: 409–415.

Mishra RC, Kaushic HD (1992) Effect of cross pollination on yield and oil content of *Brassica* spp. and *Eruca sativa* with pollination efficiency of honeybees. *Ann Entomol* 10: 33–37.

Muranjan PN, Gandhale DN, Chaudhari CS, Patil BD, Pokharkar DS, Naik RL (2006) Toxocity of ready-mix formulations of pyrethroids and ad-mixed insecticides on forager honey bee, *Apis cerana indica* Fabricius. *Ann Plant Prot Sci* 14(1) Print ISSN: 0971–3573.

Mänd M, Kuusik A, Martin AJ, Williams IH, Luik A, Karise R, Metspalu L, Hiiesaar K (2005) Discontinuous gas exchange cycles and active ventilation in pupae of the bumblebee *Bombus terrestris*. *Apidologie* 36: 561–570.

Mänd M, Kuusik A, Martin AJ, Williams IH, Luik A, Karise R, Metspalu L, Hiiesaar K (2006) Regular periods of abdominal contractions recorded from larvae of the bumblebee, *Bombus terrestris* L. (Hymenoptera: Apidae). *Eur J Entomol* 103: 319–322.

Mänd M, Mänd R, Williams IH (2002) Bumblebees in the agricultural landscape of Estonia. *Agr Ecosyst Environ* 89: 69–76.

Öckinger E, Smith HG (2007) Semi-natural grasslands as population sources for pollinating insects in agricultural landscapes. *J Appl Ecol* 44: 50–59.

Osborne JL, Williams IH, Corbet SA (1991) Bees, pollination and habitat change in the European community. *Bee World* 72: 99–116.

Pajot S (2001) Dossier Gaucho. *Abielles et Fleurs* 616: 160–165.

Peng G, Sutton JC, Kevan PG (1992) Effectiveness of honey bees for applying the biological control agent *Gliocladium roseum* to strawberry flowers to suppress *B. cinerea*. *Can J Plant Pathol* 14: 117–129.

Pernal SF, Currie RW (1998) Nectar quality in open-pollinated, pol CMS hybrid and dominant SI hybrid oilseed summer rape. *Can J Plant Sci* 78: 79–89.

Pertl M, Hauser TP, Damgaard C, Jørgensen RB (2002) Male fitness of oilseed rape (*B. napus*), weedy *B. rapa* and their F_1 hybrids when pollinating *B. rapa* seeds. *Heredity* 89: 212–218.

Pierre J, Mesquida J, Marilleau R, Pham-Delegue MH, Renard M (1999) Nectar secretion in winter oilseed rape, *Brassica napus* – quantitative and qualitative variability among 71 genotypes. *Plant Breed* 118: 471–476.

Pinochet X, Bertrand R (2000) Oilseed rape grain yield productivity increase with hybrid varietal types: A first balance sheet with post registration tests in France and Europe. *OCL-Oleagineux Corps Gras Lipides* 7: 11–16.

Pinzauti M (1994) Pollinator behaviour and activity in colonies of *Apis mellifera* in confined environments. *Ethol Ecol Evol* 3: 101–106.

Radchenko TG (1964) The influence of pollination on the crop and the quality of the seed of winter rape. *Bdzhil'nitstvo* 1: 68–74 (in Ukrainian).

Ramirez-Romero R, Chaufaux J, Pham-Delègue MH (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie* 36: 601–611.

Ramsay G, Thompson C, Squire G (2003) *Quantifying Landscape-Scale Gene Flow in Oilseed Rape*. Department for Environment, Food and Rural Affairs, London, UK.

Rathcke BJ (1992) Nectar distributions, pollinator behaviour, and plant reproductive success. In: Hunter MD, Ohgushi T, Price RPW (eds.) *The effect of Resource Distribution on Animal-Plant Interactions*. Academic Press, London, UK.

Riaz A, Li G, Quresh Z, Swati MS, Quiros CF (2001) Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. *Plant Breed* 120: 411–415.

Rieger M, Lamond M, Preston C, Powles SBN, Roush RT (2002) Pollen mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386–2388.

Rives M (1957) Etudes sur la selection du Colza d'hiver. *Ann Amelior Plantes* 1: 61–107.

Sabbahi R, De Olivera D, Marceau J (2005) Influence of honey bee (Hymenoptera: Apidae) density on the production of canola (Crucifera: Brassicacae). *J Econ Entomol* 98: 367–372.

Sandu DK, Waraich MK (1985) Yeasts associated with pollinating bees and flower nectar. *Microbial Ecol* 11: 51–58.

Scheffler JA, Parkinson R, Dale PJ (1995) Evaluating the effectiveness of isolation distances for field plots of oilseed rape (*Brassica napus*) using a herbicide-resistance transgene as a selectable marker. *Plant Breeding* 114: 317–321.

Schmuck R, Schöning R, Stork A, Schramel O (2001) Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* 57: 225–238.

Schricker B, Stephen WP (1970) The effect of sublethal doses of parathion on honeybee behavior. I. Oral administration and the communication dance. *J Apicult Res* 9: 141–153.

Sepp K, Mikk M, Mänd M, Truu J (2004) Bumblebee communities as an indicator for landscape monitoring in the agri-environmental programme. *Landscape Urban Plann* 67: 173–183.

Shaw KE, Davidson G, Clark SJ, Ball BV, Pell JK, Chandler D, Sunderland K (2002) Laboratory bioassays to assess the pathogenicity of mitosporic fungi to *Varroa destructor*, an ectoparasitic mite of the honeybee, *Apis mellifera*. *Biol Control* 24: 266–276.

Slama K (1999) Active regulation of insect respiration. *Ann Entomol Soc Am* 92: 916–929.

Slama K, Sobotnik J, Hanus R (2007) Respiratory concerts revealed by scanning microrespirography in a termite *Prorhinotermes simplex* (Isoptera: Rhinotermitidae). *J Insect Physiol* 53: 295–311.

Steffan-Dewenter I (2003) Seed set of male-sterile and male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density. *Apidologie* 34: 227–235.

Stoner A, Wilson WT, Harvey J (1985) Acephate (Orthene): Effects on honey bee queen, brood and worker survival. *Am Bee J* 125: 448–450.

Sur R, Stork A (2003) Uptake, translocation and metabolism of imidacloprid in plants. *Bull Insectol* 56: 35–40.

Tasei JN (2001) Effects of insect growth regulators on honey bees and non-*Apis* bees. *Apidologie* 32: 527–545.

Tasei JN, Carré C, Moscatelli B, Grondeau C (1988) Recherche de la DL50 de la deltaméthrine (Decis) chez: *Megachile rotundata* F., abeille pollinisatrice de la luzerne et des effets de doses infraléthales sur les adultes et les larves. *Apidologie* 19: 291–306.

Tasei JN, Lerin J, Ripault G (2000) Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during laboratory feeding test. *Pest Manag Sci* 56: 784–788.

Thompson HM (2001) Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* 32: 305–321.

Thompson HM (2003) Behavioural effects of pesticides in bees – their potential for use in risk assessment. *Ecotoxicology* 12: 317–330.

Thompson HM, Hunt LV (1999) Extrapolating from honeybees to bumble bees in pesticide risk assessment. *Ecotoxicology* 8: 147–166.

Thompson HM, Maus C (2007) The relevance of sublethal effects in honey bee testing for pesticide risk assessment. *Pest Manag Sci* 63: 1058–1061.

Thompson HM, Wilkins S, Battersby AH, Waite RJ, Wilkinson D (2005) The effects of four insect growth regulating (IGR) insecticides on honeybee (*Apis mellifera*) (L.) colony development, queen rearing and drone sperm production. *Ecotoxicology* 14: 757–769.

Thompson HM, Wilkins S, Battersby AH, Waite RJ, Wilkinson D (2007) Modelling long term effects of IGRs on honey bee colonies. *Pest Manag Sci* 63: 1081–1084.

Thomson SV, Shotwell KM, Vandenberg JD (1990) The distribution of antagonistic bacteria by honey bees for biological control of fire blight. *Phytopathol* 80: 1017.

Timmons AM, O'Brain ET, Chatters YM, Dubbels SJ, Wilkinson MJ (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. *Euphytica* 85: 417–423.

van der Steen JJM, de Ruijter A (1990) Test methods to determine the hazards of IGRs (insect growth regulators) on honey bee brood, using phenoxycarb as an example. *Proc 4th Int Symp Harmonization of Methods for Testing the Toxicity of Pesticides to Bees, May 15–18, Rez near Prague, Research Institute of Apiculture, Dol, Czechoslovakia 1990*, pp 88–91.

Vandame R, Meled M, Colin ME, Belzunces LP (1995) Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. *Environ Toxicol Chem* 14: 855–860.

Vänninen I, Tyni-Juslin J, Hokkanen H (2000) Persistence of augmented *Metarhizium aniosopliae* and *Beauveria bassiana* in Finnish agricultural soils. *Biocontrol* 45: 201–222.

Varis A-L (1995) Abundance, species composition and daily pattern of bees visiting field bean, goat's rue and turnip rape in southern Finland. *Agricult Sci in Finland* 4: 473–478.

Vogt JT, Appel AG (2000) Discontinuous gas exchange in the fire ant, *Solenopsis invicta* Buren: Caste differences and temperature effects. *J Insect Physiol* 46: 403–416.

Westcott L, Nelson D (2001) Canola pollination: An update. *Bee World* 82: 115–129.

Westphal C, Steffan-Dewenter I, Tscharntke T (2003) Mass flowering crops enhanced pollinator densities at a landscape scale. *Ecol Lett* 6: 961–965.

Westphal C, Steffan-Dewenter I, Tscharntke T (2009) Mass flowering oilseed rape improves early colony growth but not sexual reproduction of bumblebees. *J Appl Ecol* 46: 187–193.

Wilkinson MJ, Elliot LJ, Allainguillaume J, Shaw W, Norris C, Welters R, Alexander M, Sweet J, Mason DC (2003) Hybridization between *Brassica napus* and *B. rapa* on a national scale in the United Kingdom. *Science* 302: 457–459.

Williams IH (1978) The pollination requirements of swede rape (*Brassica napus*) and turnip rape (*Brassica campestris*). *J. Agric Sci* 91: 343–348.

Williams IH (1980) Oilseed rape and beekeeping, particular in Britain. *Bee World* 61(4): 141–153.

Williams IH (1984) The concentrations of air-borne rape pollen over a crop of oil-seed rape (*Brassica napus* L.). *J Agric Sci* 103: 353–357.

Williams IH (1985) The pollination of swede rape (*Brassica napus* L.). *Bee World* 66: 16–22.

Williams PH (1986) Environmental change and the distributions of British bumble bees (*Bombus* Latr.). *Bee World* 67: 50–61.

Williams IH (1994) The dependence of crop production within the European Union on pollination by honeybees. *Agricult Sci Rev* 6: 229–257.

Williams IH (1996) Aspects of bee diversity and crop pollination in the European Union. In: Matheson A, Buchmann SL, O'Toole C, Westrich C, Williams IH (eds.) *Conservation of Bees*. Academic Press for IBRA and the Linnean Society, London, UK.

Williams IH (2004) Advances in insect pest management of oilseed rape in Europe. In: Horowitz AR, Ishaaya I (eds.) *Insect pest management: Field and protected crops*. Springer-Verlag, Berlin, Heidelberg.

Williams IH, Büchs W, Hokkanen H, Menzler-Hokkanen I, Johnen A, Klukowski Z, Luik A, Nilsson C, Ulber B (2005) MASTER – Integrating biological control within IPM for winter oilseed rape across Europe. *Proc BCPC Int Cong, Crop Sci Techn, Glasgow, 31 Oct–2 Nov 2005*, pp 1, 301–308.

Williams IH, Carreck N, Little DJ (1993) Nectar sources for honey bees and the movement of honey bee colonies for crop pollination and honey production in England. *Bee World* 74: 160–175.

Williams IH, Cook VA (1982) The beekeeping potential of oilseed rape. *BBJ* 110: 68–70.

Williams IH, Corbet SA, Osborne JL (1991) Beekeeping, wild bees and pollination in the European community. *Bee World* 72(4): 170–180.

Williams IH, Free JB (1978) The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oilseed rape (*Brassica napus* L.). *J Agri Sci* 91: 453–459.

Williams IH, Free JB (1979) Compensation of oil-seed rape (*Brassica napus* L.) plants after damage to their buds and pods. *J Agr Sci* 92: 53–59.

Williams IH, Martin AP, Ferguson AW, Clark SJ (1987) The effect of insect pollination on plant development and seed production in winter oil-seed rape (*Brassica napus* L.). *J Agr Sci* 109: 135–139.

Williams IH, Martin AP, White RP (1986) The pollination requirements of oil-seed rape (*Brassica napus* L.). *J Agr Sci* 106: 27–30.

Williams IH, Simpkins J (1989) Honeybee pollination of the double low oil-seed rape line Ariana. *Asp Appl Biol* 23: 343–346.

Winston ML (1987) The biology of the honey bee. Harvard University Press, Cambridge, Massachusetts, USA.

Wobschall A, Hetz SK (2004) Oxygen uptake by convection and diffusion in diapausing moth pupae (*Attacus atlas*). *Int Cong Series* 1275: 157–164.

Yang EC, Chuang YC, Chen YL, Chang LH (2008) Abnormal foraging behaviour induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101: 1743–1748.

Yu H, Sutton JC (1997) Effectiveness of bumble bees and honey bees for delivering inoculum of *Gliocladium roseum* to raspberry flowers to control *B. cinerea*. *Biol Control* 10: 113–122.

Zehnder G, Gurr GM, Kühne S, Wade MR, Wratten SD, Wyss E (2007) Arthropod pest management in organic crops. *Annu Rev Entomol* 52: 57–80.

II

Viik, E., Mänd, M., Karise, R., Lääniste, P., Williams, I. H., Luik, A. 2012

THE IMPACT OF FOLIAR FERTILIZATION ON THE NUMBER OF BEES (APOIDEA) ON SPRING OILSEED RAPE

Žemdirbystė=Agriculture, 99 (1), 41-46

ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 99, No. 1 (2012), p. 41–46
UDK 633.853.494«321»631.816:581.33

The impact of foliar fertilization on the number of bees (*Apoidea*) on spring oilseed rape

Eneli VIIK, Marika MÄND, Reet KARISE, Peeter LÄÄNISTE, Ingrid H. WILLIAMS, Anne LUIK

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences
Kreutzwaldi 1a, 51014 Tartu, Estonia
E-mail: eneli.viik@pmk.agri.ee

Abstract

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop whose cultivation area has increased significantly in Estonia. It is predominantly autogamous but cross-pollination can have several positive effects, including higher seed yield. We studied the effect of fertilization with different foliar microfertilizers on the flower density and pollen and nectar production of spring oilseed rape as well as the impact of these factors on the abundance of flower visiting bees – honey bees, bumble bees and solitary bees.

Field experiments were carried out in 2004 and 2005. The field consisted of 32 plots (10 m^2 each): control plots (no mineral fertilizers used), plots fertilized with the complex fertilizer OptiCrop (NPK 21-08-12 + S + Mg + B + Ca) alone and plots treated with OptiCrop and one of the six foliar microfertilizers (Mn, S, Cu, B, Mg, Mo). There were four replicates of each treatment. Flower visiting bees were counted twice a week on sunny days. Flowers were counted at the same time on an area of 1 m^2 on each plot. Nectar production by the flowers was measured in the field by inserting a 1 µl capillary into the corolla tube of flowers isolated for 24 h. Pollen grains were counted from previously isolated flowers after dissolving the flower tissues.

The density of flower visiting bees (honey bees, bumble bees and solitary bees) on spring oilseed rape depended mainly on flower density. Fertilization increased not only the number of flowers but also the amount of nectar and pollen per flower. Additional foliar fertilization had no effect either on the number of flowers or the amount of pollen grains per flower. Nectar production per flower seemed to be inhibited by additional manganese. Therefore, to secure higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollination, spring oilseed rape should be given proper complex fertilization. Microfertilizers turned out to be useless in terms of increasing the number of pollinators.

Key words: *Brassica napus* L. var. *oleifera*, spring oilseed rape, *Apoidea*, flower density, pollen production, nectar production, foliar fertilization.

Introduction

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop, whose production area has increased significantly in northern countries of Europe (Treu, Emberlin, 2000), including Estonia. It is predominantly autogamous and visits of insect pollinators are not essential for the final seed yield (Williams et al., 1987). However, flower morphology favours first cross-pollination followed by self-pollination (Delaplane, Mayer, 2000). Adequate pollination can have positive effects such as a reduction of the flowering period, a reduction of raceme production, acceleration of ripening and an increase of seed weight (Williams et al., 1987). Cross-pollination also raises the seed yield (Steffan-Dewenter, 2003; Chifflet et al., 2011).

Large fields of oilseed rape in flower are important food resources for bees enhancing both nectar and pollen reserves abundantly (Westphal et al., 2009; Mänd et al., 2010). Oilseed rape is an especially attractive food plant for bees because of the high nectar production of its flowers and its high sugar content (Pierre et al., 1999). Adult bees use nectar to satisfy their energy and water needs. Pollen is collected by bees as their only source of protein and is used as food for the larvae. The pollen of oilseed rape contains more of the three most important amino acids for bee survival and development than other field crops flowering at the same time (Cook et al., 2003).

Oilseed rape is a fast growing crop which needs more nutrients than graminaceous crops. Considering other plant species, it has been found that soil fertilizer affects the concentration of amino acids in the floral nectar of corncockle, *Agrostemma githago* (Gardener, Gillman, 2001) and soil nitrogen has a positive effect on the pollen performance of *Cucurbita pepo* (Lau, Stephenson, 1993). Many studies have focused on the effect of fertilization (Sidlauskas, Bernotas, 2003; Szulc et al., 2003; Rathke et al., 2006) and pollinators (Steffan-Dewenter, 2003; Sabbahi et al., 2005) on seed yield of oilseed rape as well as on the effect of ambient temperature conditions on honey bee foraging activity (Blažytė-Čereškienė et al., 2010). However, none of these studies have dealt with the impact of fertilization on the resource of the bee food (nectar and pollen production) provided by oilseed rape and on the number of the most important pollinators – bees. Taking into account several benefits of cross-

pollination (Williams et al., 1987) and the pollinators' contribution to yield increase (Sabbahi et al., 2005), this gap in knowledge needs to be filled. In this context, the present study examines the effect of foliar fertilization on the flower density and nectar productivity of spring oilseed rape and on the number of pollen grains per flower in relation to the abundance of flower visiting bees – honey bees, bumble bees and solitary bees.

Materials and methods

Study plots. The study was carried out in an experimental field of the Estonian University of Life Sciences near Tartu, Estonia, during the flowering period of oilseed rape in 2004 and 2005. The spring oilseed rape variety 'Mascot', bred and produced by the Swedish company "Weibull", was used. Technical data of the variety: crude fat content 40–43%, 1000 seed weight 3.5–4.5 g, glucosinolate content 20 µmol g^{-1}, lodging resistance 6–8 points, plant height 98–108 cm, growth period 90–108 days (Velička, 2003). The soil in the study area was slightly acidic (pH_{KCl} 6.2) *Stagnic Luvisol* (FAO classification LV st, 2006) with loamy texture: humus content 2.4%, P – 77.66 mg kg^{-1}, K – 169.8 mg kg^{-1}, Ca – 5648 mg kg^{-1}, S – 13.54 mg kg^{-1}.

In 2004, spring oilseed rape was sown on 5 May and in 2005 on 9 May at a rate of 200 viable seeds m^{-2}, sowing depth 2–3 (4) cm, pre-crop being potato. In 2004 and 2005, the field consisted of 32 plots (10 m^2 each). Control plots received no fertilizer; the other plots received a complex fertilizer alone or the complex fertilizer plus one of the six microfertilizers. There were four replicates of each treatment. The treatments were: 1) 0 (no mineral fertilizers), 2) OptiCrop (Opti) (only the mineral complex fertilizer OptiCrop NPK 21-08-12 + S + Mg + B + Ca, the amount of nitrogen applied 120 kg ha^{-1}), 3) Opti + HydroPlus™ Boron (Opti + B) (consumption rate 2 l ha^{-1}), 4) Opti + HydroPlus™ Micro Copper (Opti + Cu) (consumption rate 0.5 l ha^{-1}), 5) Opti + Hydromag 300 (Opti + Mg) (consumption rate 7 1 ha^{-1}), 6) Opti + HydroPlus™ Micro Manganese (Opti + Mn) (consumption rate 1 l ha^{-1}), 7) Opti + HydroPlus™ Micro Molybdenum (Opti + Mo) (consumption rate 0.25 l ha^{-1}), 8) Opti + Sulphur F3000 (Opti + S) (consumption rate 7 1 ha^{-1}).

Prior to sowing, the whole field was sprayed with the soil-applied herbicide EK Trifluralin (0.15 l ha^{-1}). The mineral complex fertilizer OptiCrop NPK 21-08-12 + S + Mg + B + Ca, the amount of nitrogen applied 120 kg ha^{-1}, was used (except for treatment 0). Liquid microfertilizers (spray volume 400 l ha^{-1}) were foliar-applied when the plants had reached the growth stage 27–31 according to the BBCH scale (Lancashire et al., 1991).

Evaluation of flower visiting bees: honey bees, bumble bees and solitary bees. Flower visiting bees were counted during the flowering period of the crop (5–22 July 2004 and 28 June to 18 July 2005) on each 10 m^2 plot twice a week (altogether 6 observation days in 2004 and 7 observation days in 2005) by walking slowly along the study plots and recording all bees visiting the flowers of oilseed rape. The observations were made on sunny days between 11:00 and 15:00 when temperature was above 16ºC and wind speed did not exceed 6 m s^{-1}.

Evaluation of flower density. Flowers were counted simultaneously with flower visiting bees on an area of 1 m^2 on each plot which was divided into 4 subplots (50 × 50 cm) and the data were summarized.

Evaluation of nectar production. Nectar was collected from five flowers in each plot three times during the flowering period of the crop in 2004. The collection was carried out in late morning at full flowering of the plants. Each flower was previously covered with a voile bag for 24 h to exclude floral visitors and to prevent nectar consumption the day before nectar measurement. Nectar production was measured in the field by inserting a 1 µl capillary into the flower corolla tube. It should be noted that nectar productivity can only be measured when there is no rainfall during 24 h. As in 2005 there was little rain on almost all days of flowering period of spring oilseed rape, nectar production was analysed only for 2004.

Evaluation of pollen production. In 2004 and 2005, after anthesis, pollen production was quantified for 5 flowers in each plot at the same time as flower visiting bees and flowers were counted. The flowers were collected randomly from the plant main raceme and stored separately. These racemes were previously isolated to avoid consumption of the pollen by pollen beetles (*Meligethes* sp.). The flowers with pollen were later acetolysed (Faegri, Iverson, 1989) to digest both the floral tissue and pollen content, leaving pollen exines intact. Separated pollen was dispersed in distilled water (1 ml). The pollen grains were counted with a light microscope using a Fuchs-Rosenthal chamber (3.2 mm^3). These data were used to calculate the number of pollen grains per flower.

Climate conditions. The flowering period of spring oilseed rape was warmer in 2004 (July 19.6ºC) and colder in 2005 (July 16.5ºC) than the mean of the past ten years (July 17.3ºC). Ambient temperature was measured every time before the evaluation of the number of flower visiting bees and flowers and nectar and pollen production at the level of the flowers. In 2004, air temperature fluctuated from 21.5ºC on the first observation day (5 July) to 26ºC on the forth observation day (15 July). In 2005, the lowest air temperature was recorded on first and last observation days (28 June and 18 July; 17.8ºC and 18.8ºC, respectively). In 2005, the flowering period was rainy with only two days without any precipitation. In 2004 15 days were without any rain. The amount of precipitation in July 2004 was 87.8 mm and in July 2005, 113.2 mm. The mean of the past ten years was 81 mm in July.

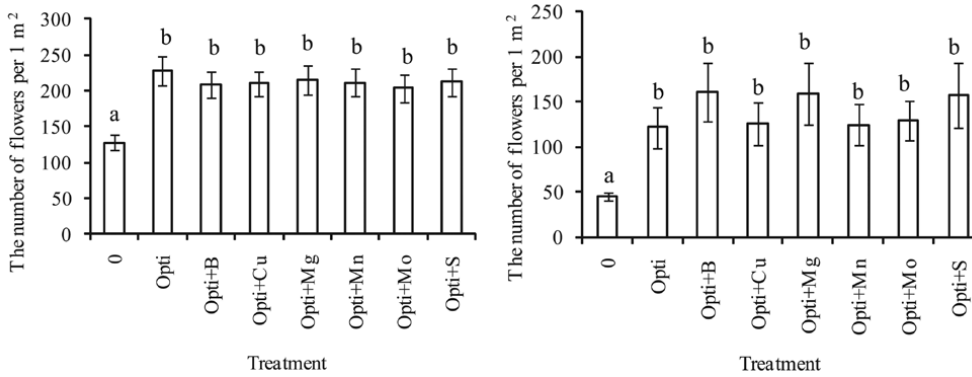
Statistical data analysis. Statistical analyses were performed using *Statistica 7*. The impact of different treatments on the number of flowers, nectar and pollen production and on the number of flower visiting bees was analysed with *ANOVA* – where necessary data were normalised. The differences between means were inspected using Fisher's protected significant difference post hoc analysis. The significance of interactions between year and treatment on pollen production and the number of bees were analysed with *factorial ANOVA*. The relationship between bees and the food resource was analysed with Pearson correlation analysis – where necessary data were normalised.

Results and discussion

Flower density on plots with different treatments. In both years (2004 and 2005), the abundance of flowers was significantly higher on fertilized than on unfertilized plots ($F(7, 184) = 2.83$, $p = 0.01$ in 2004; $F(7, 216) = 2.85$, $p = 0.01$ in 2005). Oilseed rape is a fast growing crop which needs a high amount of nutrients from the soil; otherwise its growth will slow down and, as a result, the number of flowers produced is also lower. In the case of resource deficiency, oilseed rape plants probably preserve the size of flowers rather than the number of flowers (Cresswell et al., 2001).

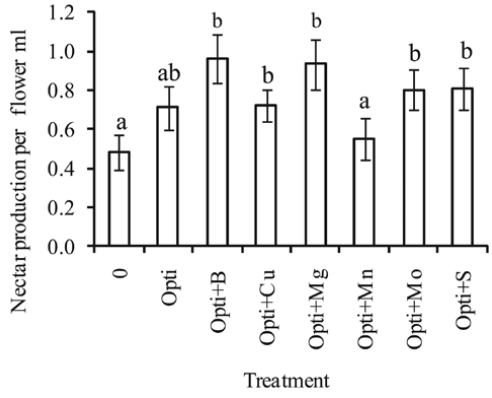
There were no significant differences between differently fertilized plots, including plots fertilized with the complex fertilizer OptiCrop alone (Fig. 1). Thus, the number of flowers depended directly on complex fertilization and addition of different foliar microfertilizers to the complex fertilizer OptiCrop did not have any significant impact on increasing the number of flowers.

Nectar production on plots with different treatments. Fertilization influences the nectar production of oilseed rape flowers. Except for fertilization with manganese or with the complex fertilizer OptiCrop alone, the production of nectar in 2004 was significantly higher on fertilized than on unfertilized plots (F (7, 312) = 2.48, $p = 0.02$). Unlike flower productivity, nectar production gains from foliar fertilization, except that supplementary manganese appeared to inhibit nectar production (Fig. 2). Plots fertilized with OptiCrop plus manganese had significantly lower nectar production than those fertilized with OptiCrop plus one of the other five microfertilizers. Flowers from plants fertilized with manganese had even less nectar than those fertilized with pure OptiCrop. Manganese increases plant height, leaf area per plant and dry weight of the aerial parts (Ali et al., 2011), and apparently, plants contribute less to nectar production.



Note. The letters above the boxes indicate statistically significant differences between treatments (*ANOVA*, Fisher LSD test). The boxes indicate the mean value and the whiskers indicate the standard error of the mean.

Figure 1. The number of flowers on plots with different treatments in 2004 (left) and 2005 (right)



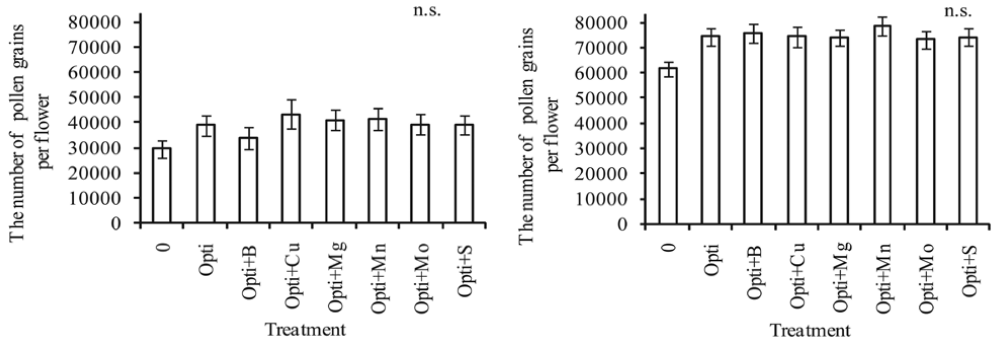
Note. The letters above the boxes indicate statistically significant differences between treatments (*ANOVA*, Fisher LSD test). The boxes indicate the mean value and the whiskers indicate the standard error of the mean.

Figure 2. Nectar production of spring oilseed rape flowers on plots with different treatments in 2004

Several authors have studied the nectar production of oilseed rape flowers depending on the varietal (Mohr, Jay, 1990; Kotowski, 2001) and genetic differences (Pierre et al., 1999) but not the effect of fertilization on nectar production. As several factors affecting nectar production and nectar standing crop are documented, e.g., evaporation and absorption (Corbet, 2003), final conclusions cannot be drawn on the basis of one study year, although a preliminary trend is evident. The topic of the effect of fertilization on nectar production needs further research.

Pollen production on the plots with different treatments. In both years (2004 and 2005), there were no significant differences in pollen production between differently treated plots (F (7, 248) = 1.15, $p = 0.33$ in 2004; F (7, 344) = 2.02, $p = 0.05$ in 2005). However, in both years, especially in 2005, the pollen production was higher on fertilized than on unfertilized plots (Fig. 3). Still, the difference was not statistically significant, probably because of the high variability of pollen production. When summarizing over the two years, the effect of treatment became significant (Table 1). In addition, there was no statistically significant interaction between year and treatment on the number of pollen grains produced per flower, which means that the impact of different treatments followed the same trend in both years being higher on fertilized than on unfertilized plots.

Pollen dissemination by pollinators (Hayter, Cresswell, 2006) and the influence of other factors on pollen transfer and gene flow (Beckie et al., 2003; Devaux et al., 2008) have received considerable attention recently in connection with potential problems associated with the adoption of genetically modified oilseed rape. However, as pollinators visit flowers to have some reward, the effect of fertilization on pollen production, which in turn can affect the number of pollinators, deserves attention as well.



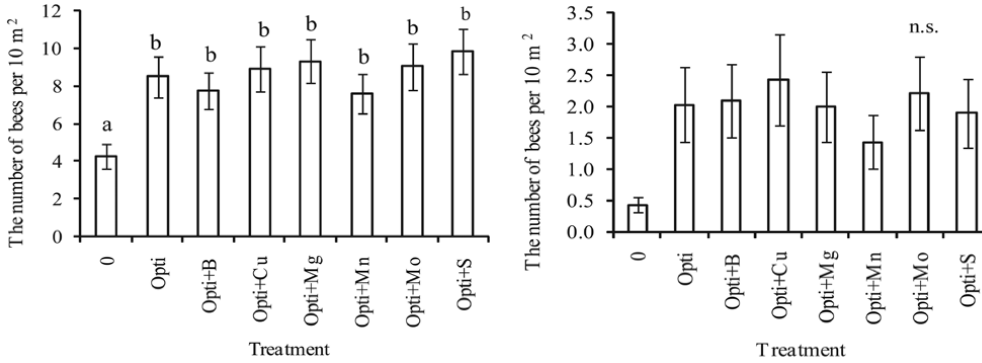
Note. The boxes indicate the mean value and the whiskers indicate the standard error of the mean; n.s. – statistically not significant.

Figure 3. The number of pollen grains per flower on the plots with different treatments in 2004 (left) and 2005 (right)

Table 1. Factorial *ANOVA* table of F-values showing the effect of year and treatment on the number of pollen grains per flower in 2004 and 2005

Effect	df	SS	F	*p*
Year	1	19543	374.5	<0.01
Treatment	7	3643	374.5	<0.01
Interaction between year and treatment	7	6709	0.4	0.92

The number of flower visiting bees on the plots with different treatments. In 2004, the number of flower visiting bees was significantly higher on fertilized than on unfertilized plots ($F(7, 184) = 2.62$, $p = 0.01$). Similar results were obtained in 2005 but the differences were not statistically significant ($F(7, 216) = 1.24$, $p = 0.28$) (Fig. 4). When summarizing over the two years, the effect of treatment was significant (Table 2). Again, there was no statistically significant interaction between year and treatment on the number of flower visiting bees, which means that the impact of different treatments followed the same trend in both years being higher on fertilized than on unfertilized plots.



Notes. The letters above the boxes indicate statistically significant differences between different treatments (*ANOVA* Fisher LSD); n.s. – statistically not significant. The boxes indicate the mean value and the whiskers indicate the standard error of the mean. Note that there are differences in the scale values of the y-axes.

Figure 4. The mean number of flower visiting bees on the plots with different treatments in 2004 (left) and 2005 (right)

Table 2. Factorial *ANOVA* table of F-values showing the effect of year and treatment on the number of bees in 2004 and 2005

Effect	df	SS	F	*p*
Year	1	4155	239.8	<0.01
Treatment	7	487	4.0	<0.01
Interaction between year and treatment	7	138	1.1	0.34

Relations between flower visiting bees and the food resource of spring oilseed rape. A significant positive correlation between the number of flower visiting bees and the number of flowers was found in both years ($r = 0.59$, $p < 0.01$ in 2004; $r = 0.69$, $p < 0.01$ in 2005). There was also a moderate correlation between nectar production and the number of flower visiting bees ($r = 0.41$, $p < 0.01$). For pollen production, a weak correlation was found in 2005 ($r = 0.21$, $p < 0.01$), but not in 2004 ($r = -0.01$, $p = 0.93$).

The economically most important and abundant pollinators of spring oilseed rape are bees (Klein et al., 2007). Considering the fact that bees visit flowers in search of food, the number of bees in the field is affected by existing food resources: the density of flowers and nectar and pollen content in them. Most bees collect only two food items from flowers: nectar, which provides bees with energy, and pollen, which provides them with protein necessary for growth of larvae (Rasheed, Harder, 1997). According to an optimal foraging theory, bees try to maximize the benefit and minimize the costs (Pettersson, Sjödin, 2000). Hence the food collected from the flower – the reward – has to exceed the energy spent on flying.

The positive correlation between the number of flower visiting bees and the number of flowers found in this experiment shows that bees consider the abundance of the food resource while looking for food, preferring areas with higher flower density. Karise et al. (2007) also found that the density of oilseed rape flowers most likely played a major role in choice of foraging area. It is energetically more profitable to choose denser flower areas in order to expend less energy in flying between flowers (Cartar, Real, 1997). As the nectar of oilseed rape flowers can be replenished within half an hour of depletion (Pierre et al., 1999), encountering empty flowers is unlikely. Oilseed rape is a favourable food plant for bees because its flowers provide copiously pollen and nectar. High-density flower patches may serve as a sign of presence of vigorous plants which are able to provide abundant food for bees (Karise et al., 2007).

Conclusions

Spring oilseed rape (*Brassica napus* L. var. *oleifera*) is an important oilseed crop, whose production area has increased significantly in northern Europe, including Estonia. Spring oilseed rape is predominantly autogamous but cross-pollination can have several positive effects, including higher seed yield. Hence it is profitable to encourage high number of pollinators in oilseed rape fields. The results of the current study allowed us to make the following conclusions:

1. The density of flower visiting bees – honey bees, bumble bees and solitary bees on spring oilseed rape depended mainly on flower density.

2. Fertilization increased not only the number of flowers but also the amount of nectar and pollen per flower.

3. Additional foliar fertilization had no effect either on the number of flowers or on the amount of pollen grains per flower. Nectar production per flower appeared to be inhibited by additional manganese.

4. To secure a higher number of pollinators for achieving higher seed yield and other benefits deriving from cross-pollination spring oilseed rape should receive proper complex fertilization. Microfertilizers turned out to be useless in terms of increasing the number of pollinators.

Acknowledgements

This research was funded by the Estonian Science Foundation grants 7391 and 8895, Target Financing SF170057s09 and the Estonian Ministry of Agriculture.

Received 09 09 2011
Accepted 10 01 2012

References

Ali S. O. M., Ei-Hiti S. M. J., Rezazadeh S., Yekta M. Effect of potassium with or without micronutrients on plant growth and alkaloids content in deadly nightshade (*Atropa bella-donna* L.). Part 1. Plant growth and biomass // Zeitschrift Fur Arznei- und Gewurzpflanzen. – 2011, vol. 16, iss. 2, p. 70–74

Beckie H. J., Warwick S. I., Nair H., Séguin-Swartz G. Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*) // Ecological Application. – 2003, vol. 13, iss. 5, p. 1276–1294

Blažytė-Čereškienė L., Vaitkevičienė G., Venskutonytė S., Būda V. Honey bee foraging in spring oilseed rape crops under high ambient temperature conditions // Žemdirbystė=Agriculture. – 2010, vol. 97, No. 1, p. 61–70

Cartar R. V., Real L. A. Habitat structure and animal movement: the behaviour of bumblebees in uniform and random spatial resource distributions // Oecologia. – 1997, vol. 112, iss. 3, p. 430–434

Chifflet R., Klein E. K., Lavigne C., Le Fèon V., Ricroch A. E., Lecomte J., Vassière B. E. Spatial scale of insect-mediated pollen dispersal in oilseed rape in an open agricultural landscape // Journal of Applied Ecology. – 2011, vol. 48, iss. 3, p. 689–696

Cook S. M., Awmack C. S., Murray D. A., Williams I. H. Are honey bees' foraging preferences affected by pollen amino acid composition? // Ecological Entomology. – 2003, vol. 28, iss. 5, p. 622–627

Corbet S. A. Nectar sugar content: estimating standing crop and secretion rate in the field // Apidologie. – 2003, vol. 34, iss. 1, p. 1–10

Cresswell J. E., Hagen C., Woolnough J. M. Attributes of individual flowers of *Brassica napus* L. are affected by defoliation but not by intraspecific competition // Annals of Botany. – 2001, vol. 88, iss. 1, p. 111–117

Delaplane K. S., Mayer D. F. Crop pollination by bees. – Washington, USA, 2000, 352 p.

Devaux C., Klein E. K., Lavigne C., Sausse C., Messean A. Environmental and landscape effects on cross-pollination rates observed at long distance among French oilseed rape *Brassica napus* commercial fields // Journal of Applied Ecology. – 2008, vol. 45, iss. 3, p. 803–812

Faegri K., Iverson J. Textbook of pollen analysis, 4th ed. – Chichester, UK, 1989, 328 p.

FAO. World reference base for soil resources 2006. – Rome, Italy, 2006, 132 p.

Gardener M. C., Gillman M. P. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, *Agrostemma githago* (*Caryophyllaceae*) // Oikos. – 2001, vol. 92, iss. 1, p. 101–106

Hayter K. E., Cresswell J. E. The influence of pollinator abundance on the dynamics and efficiency of pollination in agricultural *Brassica napus*: implications for landscape-scale gene dispersial // Journal of Applied Ecology. – 2006, vol. 43, iss. 6, p. 1196–1202

Karise R., Viik E., Mänd M. Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape (*Brassica napus*) flowers in field conditions // Pest Management Science. – 2007, vol. 63, iss. 11, p. 1085–1089

Klein A. M., Vassière B. E., Cane J. H., Steffan-Dewenter I., Cunningham S. A., Kremen C., Tscharntke T. Importance of pollinators in changing landscapes for world crops // Proceedings of the Royal Society B: Biological Sciences. – 2007, vol. 274, iss. 1608, p. 303–313

Kotowski Z. Results of the investigations into nectar secretion and pollen production of new cultivars of rape (*Brassica napus* L.) // Acta Horticulturae. – 2001, vol. 561, p. 127–129

Lancashire P. D., Bleiholder H., Boom T. van den, Langelüddeke P., Strauss R., Weber E., Witzenberger A. A uniform decimal code for growth stages of crops and weeds // Annals of Applied Biology. – 1991, vol. 119, iss. 3, p. 561–601

Lau T. C., Stephenson A. G. Effects of soil nitrogen on pollen production, pollen grain size, and pollen performance in *Cucurbita pepo* (*Cucurbitaceae*) // American Journal of Botany. – 1993, vol. 80, iss. 7, p. 763–768

Mohr N. A., Jay S. C. Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. // Journal of Apicultural Research. – 1990, vol. 29, iss. 2, p. 95–100

Mänd M., Williams I. H., Viik E., Karise R. Oilseed rape, bees and integrated pest management // Biocontrol-based integrated management of oilseed rape pests / I. H. Williams (ed.). – London, New York, 2010, p. 357–379

Pettersson M. W., Sjödin E. Effects of experimental plant density reductions on plant choice and foraging behaviour of bees (*Hymenoptera: Apoidea*) // Acta Agriculturae Scandinavica, Section B: Plant Soil Science. – 2000, vol. 50, iss. 1, p. 40–46

Pierre J., Mesquida J., Marilleau R., Pham-Delegue M. H., Renard M. Nectar secretion in winter oilseed rape, *Brassica napus* – quantitative and qualitative variability among 71 genotypes // Plant Breeding. – 1999, vol. 118, iss. 6, p. 471–476

Rasheed S. A., Harder L. D. Economic motivation for plant species preferences of pollen-collecting bumble bees // Ecological Entomology. – 1997, vol. 22, iss. 2, p. 209–219

Rathke B. W., Behrens T., Diepenbrock W. Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): a review // Agriculture, Ecosystems and Environment. – 2006, vol. 117, iss. 2–3, p. 80–108

Sabbahi R., de Oliveira D., Marceau J. Influence of honey bee (*Hymenoptera*: *Apidae*) density on the production of canola (*Crucifera*: *Brassicaceae*) // Journal of Economic Entomology. – 2005, vol. 98, iss. 2, p. 367–372

Sildauskas G., Bernotas S. Some factors affecting seed yield of spring oilseed rape (*Brassica napus* L.) // Agronomy Research. – 2003, vol. 1, iss. 2, p. 229–243

Steffan-Dewenter I. Seed set of male-sterile and male-fertile oilseed rape (*Brassica napus*) in relation to pollinator density // Apidologie. – 2003, vol. 34, iss. 3, p. 227–235

Szulc P. M., Drozdowska L., Kachlicki P. Effect of sulphur on the yield and content of glucosionolates in spring oilseed rape seeds // Electronic Journal of Polish Agricultural Universities. – 2003, vol. 6, iss. 2. <http://www.ejpau.media.pl /volume6/ issue2/agronomy/abs-01.html> [accessed 11 04 2011]

Treu R., Emberlin J. Pollen dispersal in the crops Maize (*Zea mays*), oil seed rape (*Brassica napus* ssp. *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*) // A report for the Soil Association from the National Pollen Research Unit. – Worcester, UK, 2000, 57 p.

Velička R. Rape: summary of monograph, presented for hibernation conference, Lithuanian University of Agriculture. – Kaunas, 2003, 78 p. (in Lithuanian)

Westphal C., Steffan-Dewenter I., Tscharntke T. Mass flowering oilseed rape improves early colony growth but not sexual reproduction of bumblebees // Journal of Applied Ecology. – 2009, vol. 46, iss. 1, p. 187–193

Williams I. H., Martin A. P., White R. P. The effect of insect pollination on plant development and seed production in winter oilseed rape (*Brassica napus* L.) // Journal of Agricultural Science. – 1987, vol. 109, iss. 1, p. 135–139

ISSN 1392-3196
Žemdirbystė=Agriculture, vol. 99, No. 1 (2012), p. 41–46
UDK 633.853.494«321»631.816:581.33

Tręšimo per lapus įtaka bičių (*Apoidea*) kiekiui vasariniuose rapsuose

E. Viik, M. Mänd, R. Karise, P. Lääniste, I. H. Williams, A. Luik

Estijos gyvybės mokslų universiteto Žemės ūkio ir aplinkos mokslų institutas

Santrauka

Vasarinis rapsas (*Brassica napus* L. var. *oleifera*) yra svarbus aliejinis augalas, kurio auginimo plotai Estijoje smarkiai padidėjo. Šis augalas yra savidulkis, ir tai yra teigiamas veiksnys, ypač jo sėklų derliui. Tirta tręšimo įvairiomis lapų mikrotrąšomis įtaka vasarinių aliejinių rapsų žiedų tankumui ir žiedadulkių formavimuisi bei nektaro išsiskyrimui, taip pat šių veiksnių įtaka žiedus lankančių bičių gausai.
Lauko bandymai vykdyti 2004 ir 2005 m. Lauką sudarė 32 laukeliai (10 m^2): kontroliniai (netręšti mineralinėmis trąšomis), tręšti tik kompleksinėmis trąšomis OptiCrop (NPK 21-08-12 + S + Mg + B + Ca), ir tręšti OptiCrop bei vienomis iš šešių mikrotrąšų (Mn, S, Cu, B, Mg, Mo). Kiekvienas variantas turėjo keturis pakartojimus. Žiedus lankančios bitės skaičiuotos du kartus per savaitę saulėtomis dienomis. Žiedai skaičiuoti tuo pačiu metu kiekvieno laukelio 1 m^2 plote. Nektaro išsiskyrimas matuotas lauke, 1 µl kapiliarą įstačius į 24 valandas izoliuotų žiedų vainikėlio vamzdelį. Žiedadulkių grūdeliai skaičiuoti ištirpinus prieš tai izoliuotų žiedų audinius.
Žiedus lankančių medunešių, kamanių ir pavienių bičių tankumas ant vasarinių rapsų daugiausia priklausė nuo žiedų tankumo. Tręšimas padidino ne tik žiedų skaičių, bet ir nektaro bei žiedadulkių kiekį viename žiede. Papildomas tręšimas per lapus neturėjo įtakos nei žiedų, nei žiedadulkių grūdelių kiekiui. Vieno žiedo nektaro skyrimąsi slopino papildomas tręšimas manganu. Todėl, siekiant užtikrinti didesnį kiekį apdulkintojų ir gauti didesnį sėklų derlių bei kitą kryžmadulkos teikiamą naudą, rapsus reikėtų tręšti tinkamomis kompleksinėmis trąšomis. Apdulkintojų kiekiui mikrotrąšos nebuvo efektyvios.

Reikšminiai žodžiai: *Brassica napus* L. var. *oleifera*, vasariniai rapsai, *Apoidea*, žiedų tankumas, žiedadulkių formavimasis, nektaro išsiskyrimas, tręšimas per lapus.

III

Karise, R., **Viik, E.**, Mänd, M. 2007

IMPACT OF ALPHA-CYPERMETHRIN ON HONEY BEES FORAGING ON SPRING OILSEED RAPE *BRASSICA NAPUS* FLOWERS IN FIELD CONDITIONS

Pest Management Science, 63, 1085-1089

Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape (*Brassica napus)* flowers in field conditions†

Reet Karise,* Eneli Viik and Marika Mänd
Institute of Agricultural and Environmental Sciences, Estonian Agricultural University, Kreutzwaldi 1, 51014, Tartu, Estonia

Abstract

BACKGROUND: Cruciferous oil-bearing crops have gained in importance worldwide. The expansion of the growing area of these crops has caused a proliferation of pests. Exposure to organophosphate, carbamate and pyrethroid insecticides has been associated with bee poisoning in food crops. This study examines the repellent effect of alpha-cypermethrin on the number of foraging honey bees, *Apis mellifera* L., on fields of spring oilseed rape, *Brassica napus* L. var. *oleifera*.

RESULTS: The first experiment was conducted on differently sprayed 10 m^2 experimental plots where alpha-cypermethrin was applied at different times. Another experiment was conducted on a 4 ha seed production field divided into two parts: one part was treated with alpha-cypermethrin and the other was not treated with this insecticide. The results show that there was no difference in the number of honey bees between alpha-cypermethrin-treated and untreated patches. The result persisted through three observation years, regardless of varying flower and honey bee densities.

CONCLUSION: No repellent effect of the insecticide on honey bees was found even 24 h after spraying. The density of oilseed rape flowers most likely played a major role in choosing the foraging area.

Keywords: alpha-cypermethrin; *Brassica napus* L. var. *oleifera*; *Apis mellifera* L.; foraging; repellence

1 INTRODUCTION

The continuous growth of the human population has increased the need for agricultural products. During the last 50 years the growing area of cruciferous oil-bearing crops has greatly increased.[1] Vegetable oils are needed not only in food production but also as a raw material for fuel. Northern agricultural areas are unsuitable for the effective cultivation of most oil crops, but oilseed rape, *Brassica napus* L. var. *oleifera*, is easy to establish and grow in northern temperate climates.

A major problem with cultivating spring oilseed rape in northern Europe is that damage caused by the key pest, the pollen beetle *Meligethes aeneus* F., is increasing. Hokkanen[2] has explained the increase in the numbers of pollen beetle by ecological changes: initially, when the host plants were sparse, the high reproductive rate of the insect was of no benefit for it; when the number of host plants became unlimiting, however, their high fecundity became advantageous. Owing to the increased occurrence of pests in oilseed rape, the use of pesticides has become an almost inevitable part of cultivating these crops.

Oilseed rape plants are very attractive to pollinating insects.[3,4] In the case of conventional farming, where pesticides are widely used, the high attractiveness of a plant species may enhance the hazards of pesticide poisoning to bees. Bee poisoning incidents have been frequently associated with exposure to pesticides.[5,6] Bees may come into contact with poisonous compounds through contaminated flower resources, direct contact with poison or exposure to residues.[6]

Application of insecticides is often not permitted during the flowering period of a given crop. Even when insecticides are not sprayed on flowers but on flower buds, the residues of the compounds still contaminate nectar and pollen in sublethal doses via both active and passive transport.[5] Many insecticides have been described as safe to bees because they do not kill them, although sublethal doses may affect pollinators by decreasing their foraging and navigation abilities.[6] Some pesticides do not affect adult bees but affect brood, so that young adults emerging from cocoons may have malformed wings or other deformations.[7] However, some insecticides may be regarded as safe because they repel bees, although in some instances,

* Correspondence to: Reet Karise, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014, Tartu, Estonia
E-mail: reet.karise@emu.ee
† This paper was presented at the 9th International Symposium Hazards of Pesticides to Bees
(*Received 28 February 2006; revised version received 23 November 2006; accepted 1 December 2006*)
Published online 19 September 2007; DOI: 10.1002/ps.1445

such as in the case of oilseed rape, the attractiveness of a food resource may override the repellent effect.[8] The effect of cultivation methods on the abundance of bees has been studied at landscape scale. Morandin and Winston[9] have shown that the abundance of bees within organic crops is higher than in conventional and genetically modified crops. One of the explanations they offered for this observation is that organic crops are smaller in area, and therefore their environment could be more suitable for natural bee populations. These results concur with the studies by Mänd *et al*.[10] and Sepp *et al*.[11] However, there is a lack of data concerning bee abundance on insecticide-treated and untreated crops when the crops are situated next to each other. Alpha-cypermethrin is a non-systemic insecticide with contact and stomach action that may reduce the foraging ability of bees[12] and is reported to be repellent to them for 48 h.[5] Hence, it can be assumed that, owing to repellency, the number of honey bees should be lower on insecticide-treated food resource patches for at least 24 h after treatment. This study examines the repellent effect of alpha-cypermethrin on the number of foraging honey bees on spring oilseed rape fields.

2 MATERIALS AND METHODS

Two field experiments were carried out on spring oilseed rape crops to study the repellent effect of the insecticide alpha-cypermethrin on the density of honey bees. The experiments were conducted near Tartu, Estonia, in 2003–2005. In both experiments, the rape cultivar was 'Maskot', and a commercial alpha-cypermethrin 50 g L^{-1} EC (Fastac; BASF, Limburgerhof, Germany) was used at a rate of 0.15 L ha^{-1} (7.5 g AI ha^{-1}).

2.1 Experiment 1: effect of alpha-cypermethrin treatment intensity on the number of honey bees

This experiment was performed to evaluate the impact of alpha-cypermethrin on the number of foraging honey bees on small patches of spring oilseed rape treated once or twice (at different times) with the insecticide. The observation area consisted of a 5 ha field of summer wheat where a regular array of patches of spring oilseed rape was sown. The design of the experiment was a randomized block with twelve $1 \times 10\,m^2$ plots with a distance of 1 m between each. Three treatments were used: unsprayed, once sprayed and sprayed twice, each replicated 4 times. In the sprayed-once treatment the insecticide was applied when rape plants were in the growth stage of 2–4 true leaves (GS 10, according to Lancashire *et al*.[13]). For the twice-sprayed treatment, the first spray was applied at the same time as the once-sprayed plots with an additional application at the stage of first flowers (GS 61–62). The insecticide was applied using a manually operated sprayer, and, during spraying, plastic screens prevented the contamination of neighbouring plots. The insecticide treatments were conducted only on days when wind speed did not exceed 1–2 m s^{-1}. The cultivation methods between the treatments were identical.

In all years, the observation period lasted throughout July, i.e. the flowering period of oilseed rape. The lengths of flowering periods differed according to weather conditions and lasted from 2 weeks (2004) to 3.5 weeks (2005). During bee counts, the observer walked slowly along the plot and recorded all honey bees foraging on the oilseed rape. The number of open flowers was determined on 1 m^2 quadrats within each plot. Counts were made twice weekly during the flowering period, starting at 24 h after the last spray application. All bee counts were made on days when there was no rain, fog or strong wind and air temperature was over 16 °C at around midday (11.00–16.00 h).

2.2 Experiment 2: honey bee abundance before and after alpha-cypermethrin treatment

The second experiment was carried out on a seed production crop of spring oilseed rape to test the abundance of honey bees before and after insecticide application. The experiment was conducted in July 2003. A spring oilseed rape field (4 ha) was divided into two parts (approximately 2 ha): one part was treated with alpha-cypermethrin and the other was left untreated. Within both fields, seven $1 \times 10\,m^2$ observation plots were marked. Six honey bee colonies were brought close to the crops (200 m away) 2 days before flowering started (late bud stage, GS 60). The insecticide was applied using a motorized field sprayer when the plants were at the mid-flowering stage (GS 65–66). During spraying, wind speed did not exceed 1–2 m s^{-1}. To prevent direct poisoning of honey bees, the hives were closed before the insecticide application and kept closed for 24 h. No visible mortality was detected in close proximity to the hives during the experiment. The counting of flowers (on 1 m^2 per plot) and bees (on the whole plot, 10 m^2) was made 8 days before and 1 day and 8 days after the insecticide treatment using the methods described above.

2.3 Data analysis

To test for the effects of the treatments and years on the number of flowers and the number of bees, one-way and two-way analysis of variance (ANOVA) was used. The number of flowers on different observation plots varied both from day to day and throughout the flowering period. Therefore, when estimating the mean density of honey bees, their number was not taken per unit area but per 1000 flowers. Because the data of the first experiment were not distributed normally, Spearman's correlation was used to test for correlation between number of flowers and number of bees. To compare the abundance of bees and flowers on seed production crops, the *t*-test was used. The accepted level of significance was 5% in all cases.

Pest Manag Sci **63**:1085–1089 (2007)
DOI: 10.1002/ps

3 RESULTS

3.1 Experiment 1: effect of alpha-cypermethrin treatment intensity on the number of honey bees

In all years, there was no significant difference in the number of bees per 1000 flowers between the treatments either during the whole observation period (Fig. 1) (2003: $F_{2,38} = 0.3$, $P = 0.7$; 2004: $F_{2,29} = 0.9$, $P = 0.4$; 2005: $F_{2,27} = 0.7$, $P = 0.5$) or on the first observation day, i.e. 24 h after the second spraying (2003: $F_{2,9} = 0.5$, $P = 0.6$; 2004: $F_{2,9} = 1.6$, $P = 0.3$; 2005: $F_{2,9} = 0.2$, $P = 0.8$). Yet there was a significant difference in total number of bees between the years ($F_{2,177} = 3.7$, $P = 0.03$). Flower densities differed significantly between the treatments in all years (2003: $F_{2,57} = 5.2$, $P = 0.008$; 2004: $F_{2,33} = 8.4$, $P = 0.001$; 2005: $F_{2,81} = 8.2$, $P = 0.001$). An interesting trend was found: in the case of lower flower densities, the number of bees did not depend on the number of flowers, but statistically significant positive correlations became apparent at a certain level of flower density (Fig. 2).

3.2 Experiment 2: honey bee abundance before and after alpha-cypermethrin treatment

The number of bees per 1000 flowers did not differ between the untreated and treated crops either 1 week before ($t = 1.7$, df = 12, $P = 0.12$) or 1 week after ($t = 0.2$, df = 12, $P = 0.9$) the application of the insecticide (Fig. 3). However, 24 h after spraying, the number of honey bees per 1000 flowers for the treated crop was significantly higher than for the untreated crop ($t = 4.4$, df = 12, $P = 0.001$). An investigation was carried out to determine whether these differences in the abundance of honey bees between the crops were induced by the differences in flower densities. Indeed, in the middle of the flowering period (counted 24 h after spraying) the density of flowers in the treated crop was significantly higher than in the untreated crop ($t = 2.2$, df = 12, $P = 0.048$). At the same time, the number of oilseed rape flowers did not differ significantly between the untreated and treated crops at the beginning and at the end of the flowering period (accordingly: $t = 1.5$, df = 12, $P = 0.2$; $t = 0.04$, df = 12, $P = 0.9$). When comparing the abundance of honey bees for the observation days, the number of bees was significantly lower for both crops 24 h after spraying (untreated: $F_{2,18} = 16.4$, $P = 0.001$; treated: $F_{2,18} = 3.3$, $P = 0.05$) (Fig. 3).

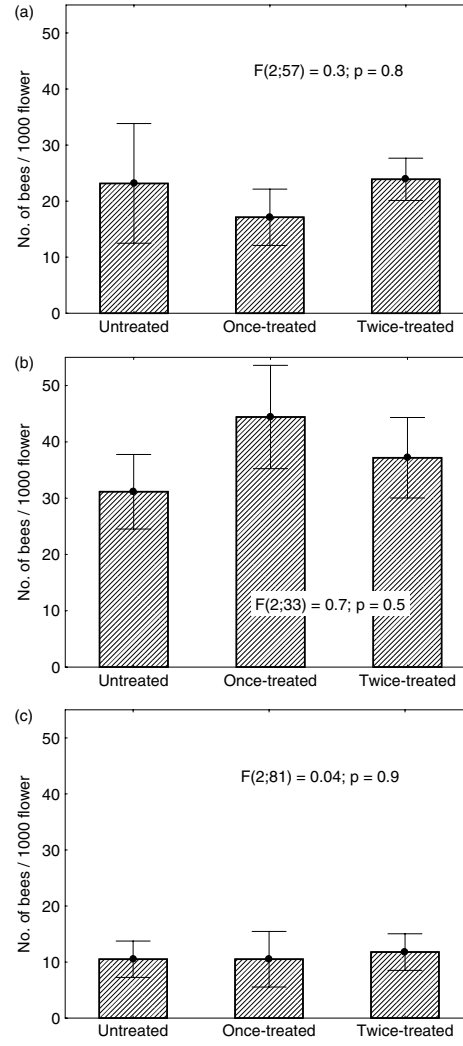


Figure 1. Number of honey bees per 1000 flowers on oilseed rape crops treated with alpha-cypermethrin or not treated with alpha-cypermethrin: (a) 2003; (b) 2004; (c) 2005. Means with standard error are given.

4 DISCUSSION

This study showed that there was no difference in the number of foraging honey bees between the patches treated with alpha-cypermethrin and those not treated with the insecticide. The result persisted through three observation years regardless of varying flower and honey bee densities. No repellent effect of the insecticide on honey bees was found even 24 h after spraying. The density of oilseed rape flowers most likely played a major role in choosing the foraging area.

Pyrethroids are known as the insecticides most repellent to bees.[8] Pyrethroid repellency can also reduce the foraging activity of bees.[14] Alpha-cypermethrin has been reported to maintain repellency to bees for 48 h after treatment.[5] However, most studies on repellency have been performed in laboratory or semi-field conditions. In field conditions, the repellency of pyrethroids may be lower than suggested by semi-field experiments.[8] In field studies, Mayer and

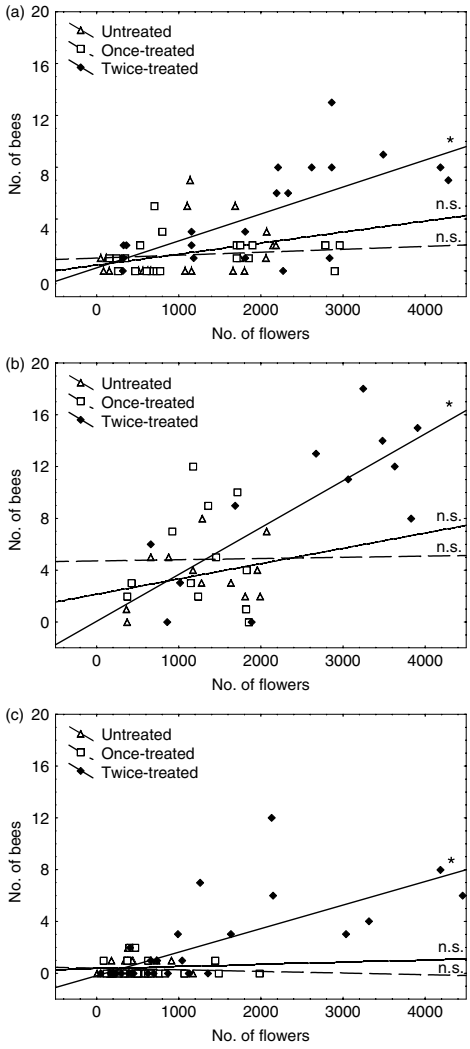


Figure 2. Spearman's correlations between the number of honey bees and the number of flowers on the experimental plots (10 m^2): (a) 2003; (b) 2004; (c) 2005; * – $P < 0.05$; n.s. – not significant.

Lunden[15] did not find any repellency to bees for alpha-cypermethrin applied at the field rate to flowering oilseed rape. Shires *et al*.[16] found that, when sprayed on oilseed rape during periods of peak honey bee foraging activity, alpha-cypermethrin caused a slight decline in the level of foraging and in the levels of collected pollen.[8] Evidence for repellency may also be questioned by the detection of relatively high residues of cypermethrin in honey and wax.[8] The present results tend to confirm that alpha-cypermethrin does not show repellency for honey bees in field conditions. If

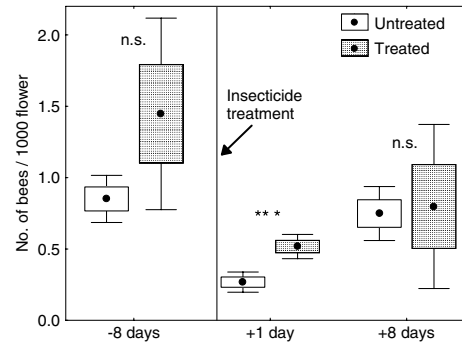


Figure 3. Number of honey bees per 1000 flowers on three observation days on seed production crops adjacent to each other. Means with standard error and standard *1.96 are given. *** – $P < 0.001$; n.s. – not significant.

any repellency does occur with respect to this insecticide, the attractiveness of the flower resource may override it.

The results of the first experiment showed that application of the insecticide at the beginning of flowering had no effect on the number of foraging honey bees per unit of flowers. Irrespective of the variable number of bees available in different years, the trends remained the same. The relative number of honey bees was connected with floral density: on dense observation plots, the numbers of bees and flowers were positively correlated, whereas on sparse patches no such correlation was found. According to the theory of optimal foraging, animals distribute among differently rewarding food resources so that the average amount of food per specimen remains equal.[17] In spite of this theory, in the first experiment, dense patches of oilseed rape were even more attractive for the bees. The data of the second experiment also uphold the result that the bees visited rich food patches more often than expected on the basis of flower resources.

Rape plants are known to be a favoured food source for bees owing to their high nectar production rate[3] and valuable pollen amino acid content.[4] It is also known that honey bees recruit nestmates to profitable foraging sites. Newly recruited bees fly directly from the hive to the vicinity of a food source, and then proceed to search for its exact location using odour and other cues.[18] The patches with higher flower densities may trigger more recruitment of nestmates on fields, as might have occurred in our second experiment. However, in the first experiment this could hardly affect the results because the area itself and the experimental patches were too small, and the patches were situated between each other, which would not permit exact identification of profitable small patches through waggle dance.

In the second experiment, 24 h after spraying there was a decline in the number of foragers not only

Pest Manag Sci **63**:1085–1089 (2007)
DOI: 10.1002/ps

on the treated but also on the untreated crop when compared with the rest of the observation days. As the abundance of honey bees decreased on both fields, it can be assumed that this was not related to treatment but more likely to climatic conditions and/or the start of flowering of some other attractive food plant species (e.g. leguminous) nearby. The end of July is the period when the aftermath of clover starts flowering on pastures or meadows and may attract bees away from rape crops.

Coming into direct contact with alpha-cypermethrin, or its residues, may cause death or sublethal effects in bees. The contact may be either direct (residues on leaf surfaces) or indirect (spray contamination of the nectar or pollen).[6] It has been shown that the residues on leaf surfaces are toxic for more than 3 days following insecticide application and may kill up to 25% of bees that come into contact with them.[19] There is at least one study that shows the presence of residues of alpha-cypermethrin in small quantities ($0.01\ mg\ kg^{-1}$) in the pollen of oilseed rape after insecticide application ($10\ g\ AI\ ha^{-1}$).[12] The compound has an LD_{50} of $0.319\ \mu g\ AI\ bee^{-1}$.[20] There is also evidence for the existence of alpha-cypermethrin residues in dead honey bees.[21] The present experiments indicate that honey bee food crop preference does not depend on the presence of insecticide residues on flowers but rather on the flower abundance of the crop plant. The alpha-cypermethrin formulation Fastac is commonly used to control pollen beetles in oilseed rape. Controlling this pest contributes to higher flower densities as the damage caused by the larvae to the flowering structures is prevented. Therefore, treated crops may often have high flower densities and therefore are more attractive to bees than crop areas damaged by the beetle. In field conditions, honey bees can become contaminated with residues of alpha-cypermethrin even if the hives have been kept closed for some time after spraying. The foraging ability of honey bees depends on their physiological state. Therefore, it is evident that reliable data are needed with respect to the effects of sublethal doses of the insecticide on the transpiration and respiration of the bees.

ACKNOWLEDGEMENTS

The authors are grateful to M Ajaots and P Lääniste for crop husbandry, to IH Williams for help in refining the English of this article and to two anonymous referees for useful comments and suggestions. This work was supported by the Estonian Scientific Foundation (Grants no. 5737, 6722).

REFERENCES

1 *Production Yearbook, 57. FAO Statistics Series* No. 177. Food and Agriculture Organization of the United Nations, Rome, 340 pp (2003).
2 Hokkanen HMT, The making of a pest: recruitment of *Meligethes aeneus* onto oilseed Brassicas. *Entomol Exp Appl* **95**:141–149 (2000).
3 Free JB, *Insect Pollination of Crops*. Academic Press, London–New York, pp. 172–180 (1993).
4 Cook SM, Awmack CS, Murray DA and Williams IH, Are honey bees' foraging preferences affected by pollen amino acid composition? *Ecol Entomol* **28**:622–627 (2003).
5 Thompson HM, Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* **32**:305–321 (2001).
6 Gels JE, Held DW and Potter DA, Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J Econ Entomol* **95**:722–728 (2002).
7 Tasei JN, Effects of insect growth regulators on honey bees and non-*Apis* bees. *Apidologie* **32**:527–545 (2001).
8 Thompson HM, Behavioural effects of pesticides in bees – their potential for use in risk assessment. *Ecotoxicology* **12**:317–330 (2003).
9 Morandin LA and Winston ML, Wild bee abundance and seed production in conventional, organic and genetically modified canola. *Ecol Appl* **15**:871–881 (2005).
10 Mänd M, Geherman V, Luik A, Martin AJ, Mikk M, Paimetova V, *et al*, Bumble bee diversity on ecological and conventional dairy farms. *Acta Biol Univ Daugavp* **1**:21–25 (2001).
11 Sepp K, Mikk M, Mänd M and Truu J, Bumble bee communities as an indicator for landscape monitoring in the agri-environmental programme. *Landscape Urban Plan* **67**:173–183 (2004).
12 van Heemstra-Lequin EAH and van Esch GT, *International Programme on Chemical Safety, Environmental Health Criteria 142 1992–1992.* [Online]. Available: http://www.inchem.org/documents/ehc/ehc/ehc142.htm [19 December 2005].
13 Lancashire PD, Bleiholder H, Van Den Boom T, Langeluddeke P, Stauss S, Weber E, *et al*, A uniform decimal code for growth stages of crops and weeds. *Ann Appl Biol* **119**:561–601 (1991).
14 Mueller-Beilschmidt D, Toxicology and environmental fate of synthetic pyrethroids. *J Pestic Reform* **10**:32–37 (1990).
15 Mayer DF and Lunden JD, Field and laboratory tests of the effects of fipronil on adult female bees of *Apis mellifera, Megachile rotundata* and *Nomia melanderi*. *J Apicult Res* **38**:191–197 (1999).
16 Shires SW, Le Blanc J, Murray A, Forbes S and Debray P, A field trial to assess the effects of a new pyrethroid insecticide, WL85871, on foraging honeybees in oilseed rape. *J Apicult Res* **23**:217–226 (1984).
17 Alonso JC, Alonso JA, Bautista LM and Muñoz-Pulido R, Patch use in cranes: a field test of optimal foraging predictions. *Anim Behav* **49**:1367–1379 (1995).
18 Dyer FC, The biology of the dance language. *Annu Rev Entomol* **47**:917–949 (2002).
19 Cox C, Cypermethrin. *J Pestic Reform* **16**:15–20 (1996).
20 *Material Safety Data Sheet: Alphaguard 100EC – 2003.* [Online]. Nufarm UK Limited. Available: http://www.interfarm.co.uk/safety_data_sheets/SDS%20Alphaguard%20100EC.pdf [19 December 2005].
21 Barnett EA, Fletcher MR, Hunter K and Sharp EA, Pesticide poisoning of animals 2000: investigation of suspected incidents in the United Kingdom. CSL, Department for Environment, Food and Rural Affairs, Sand Hutton, York, UK (2002).

IV

Muljar, R., Karise, R., **Viik, E.**, Kuusik, A., Mänd, M., Williams, I. H., Metspalu, L., Hiiesaar, K., Luik, A., Must, A.

EFFECTS OF FASTAC 50 EC ON BUMBLE BEE *BOMBUS TERRESTRIS* L. RESPIRATION: DGE DISAPPEARANCE DOES NOT LEAD TO INCREASING WATER LOSS

Journal of Insect Physiology (submitted)

Effects of Fastac 50 EC on bumble bee *Bombus terrestris* L. respiration: DGE disappearance does not lead to increasing water loss

Riin Muljar, Reet Karise*, Eneli Viik, Aare Kuusik, Marika Mänd, Ingrid Williams, Luule Metspalu, Külli Hiiesaar, Anne Must and Anne Luik

Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Department of Plant Protection, Kreutzwaldi 1, Tartu 51014, Estonia
*Author for correspondence (reet.karise@emu.ee)

Short title: Fastac effect on bumble bee respiration

SUMMARY

Sublethal effects of pesticides in insects can be observed through physiological changes, which are commonly estimated by metabolic rate and respiratory patterns, more precisely by the patterns of discontinuous gas-exchange (DGE) cycles. The aim of the present research was to study the effect of some low concentrations of Fastac 50 EC on the cycles of CO_2 release and respiratory water loss rates (WLR) in bumble bee *Bombus terrestris* foragers. Bumble bees were dipped into 0.004% and 0.002% Fastac 50 EC solution. Flow-through respirometry was used to record the respiration and WLR three hours before and after the treatment. The respirometry was combined with infrared actography to enable simultaneous recording of abdominal movements. Our results show that Fastac 50 EC has a dose dependent after-effect on bumble bee respiratory rhythms and muscle activity but doesn't affect WLR. Treatment with 0.004% Fastac 50 EC solution resulted in disappearance of the respiration cycles; also the lifespan of treated bumble bees was significantly shorter. Treatment with 0.002% Fastac 50 EC solution had no significant effect on respiration patterns or longevity. We found no evidence for the DGE cycles functioning as a water saving mechanism, our results rather support the oxidative damage hypothesis.

Key words: Fastac 50 EC, DGE, WLR, *Bombus terrestris* L.

* Corresponding author. Tel.: + 372 7313 396; Fax: + 372 7313 351
E-mail address: reet.karise@emu.ee (R. Karise)

INTRODUCTION

The abundance of native pollinators has declined rapidly over recent years in intensively managed agricultural landscapes (Mänd et al., 2002; Carvell et al., 2006; Potts et al., 2010). Among other reasons the intensive use of pesticides has been claimed to play an important role in this decline (Wickramasinghe et al., 2004; Potts et al., 2010; Stokstad, 2012). Insecticide application on flowering crops is mainly prohibited, although many products are allowed to be used at times when honey bees are not foraging. Unfortunately, these restrictions do not prevent bee contact with pesticide residues and do not consider behavioural aspects of bumble bees and other wild bees (Corbet et al., 1993; Thompson, 2001; Karise et al., 2007). Also, compared to lethal doses, sub-lethal doses of toxicants might be even more detrimental to bee populations causing chronic effects which, over a longer time-scale and in interaction with other stressors, may lead to decline of the species (Thompson, 2001, 2003).

The effects of sub-lethal pesticide doses can sometimes be observed and proved only through physiological changes in insects. The physiological state of an insect is commonly estimated and characterized by the metabolic rate and respiratory patterns, more precisely by the patterns of discontinuous gas-exchange (DGE) cycles (Kestler 1985, 1991). DGE is a nerve controlled system that reacts easily to slight changes in stress level.

Cyclic release of CO_2 during gas exchange (Kestler, 1971, 1985) is common in many insect species. This is a range of respiratory gas exchange patterns from continuous to periodic. The pattern is called discontinuous (DGE) (Lighton, 1996; Hetz and Bradley 2005; Chown et al., 2006) when it involves the closing of the spiracles of the insect tracheae (constriction phase, C) during which no CO_2 release occurs; the C-phase is followed by a period of intermittent CO_2 release (flutter phase, F) and, thereafter, a rapid opening of spiracles (open phase, O). The O-phase often coincides with contraction of abdominal muscles or active ventilation. According to Kestler (2003), this active ventilation is a strategy to conserve water. The cyclic gas exchange pattern (CGE) (Lighton, 1996; Marais and Chown, 2003; Gibbs and Johnson, 2004; Marais et al., 2005) has no C-phase, the opening of spiracles is alternated by a F-period with a low level of CO_2 release. In CGE, the cycle length is shorter and CO_2 release rarely decreases to zero. The precise pattern of cyclic respiration in insects depends on many factors: metabolic rate (Moerbitz and Hetz, 2010), insect species (Chown 2011) with its intrinsic needs for environmental conditions (Quinlan and Hadley, 1993; Basson and Terblanche, 2011), individual traits (Gray and Chown, 2008; Woods, 2011), stressors (Kestler, 1991; Zafeiridou and Theophilidis, 2006) and to the conditions

(Karise et al., 2010) the specimen is exposed to during the experiment. Most likely several factors work together to influence the expression of DGE cycles (Chown, 2002).

There are different hypotheses and contradictory explanations about the function of DGE, as reviewed by Chown (2002) and Chown et al. (2006). The newest hypotheses consider oxidative damage (Hetz and Bradley, 2005), signalling role for reactive oxygen species (Boardman et al., 2012) and neural regulation of the ventilation patterns (Matthews and White, 2011). Still, the most widely discussed has been the hygric hypothesis, which, starting with Buck (1958) has later been supported by many other researches (Kestler, 1980, 1982; Slama, 1988, 1999; Lighton et al., 1993; Terblanche et al., 2008). Despite some counter-arguments (Lighton et al., 2004; Lighton and Turner, 2008), the hygric hypothesis is still the one with most support (Schimpf et al., 2009, 2012; Williams et al., 2010).

Despite many different hypotheses on the function of DGE none exclude others, rather it suggests a basis for combined existence (Förster and Hetz, 2010). There is proof that DGE cycles do confer a fitness benefit. Schimpf et al. (2012) showed that desert insects that exchange gases discontinuously are more likely to survive desiccating conditions than those that do not. Although respiratory water loss usually represents only a small fraction of total water loss (Lighton, 1994; Chown, 2002; Dingha et al., 2005; Lamprecht et al., 2009) the lower water loss due to DGE may be significant in extending survival in some conditions (Schimpf et al., 2012). The pesticide derived excessive diuresis in insects may lead to increasing stress becoming determinative in surviving toxicosis.

Pyrethroids are often sprayed on the flowering oilseed crops to control the pollen beetle *Meligethes aeneus* (Fabricius, 1775) which attacks buds and flowers. The beginning of flowering in turn attracts large numbers of pollinators which may come into contact with pesticides or their residues on flowers. The objective of the present investigation was to clarify whether the effect of an insecticide results from water loss, as it could be assumed according to the hygric hypothesis, or whether there are other factors that play an important role. Our precise aim was to study the effect of some low concentrations of Fastac 50 EC (a.i. alpha-cypermethrin) on the cycles of CO_2 release and respiratory water loss rate (WLR) of bumble bee *Bombus terrestris* (Linnaeus, 1758) foragers.

MATERIALS AND METHODS

Insects

Colonies (Natupol hives) of the bumble bee *B. terrestris* were purchased from Koppert Biological Systems B.V. (Berkel en Rodenrijs, The Netherlands). The hives were kept at room temperature and the bees fed with dried honey bee pollen and a sugar solution (30%). The bees used in the experiment were caught as they emerged naturally from the hive entrance tunnel; it means that all of them were foragers.

Respirometry

An infrared gas analyser (IRGA, Infralyt-4, VEB, Junkalor, Dessau), adapted for entomological research, was used in the first experiment, to record the CO_2 signals and metabolic rates (VCO_2 ml h^{-1}) at 8°C. The IRGA was calibrated at different flow rates using calibration gases (Trägergase, VEB, Junkalor, Dessau) with gas injection (Kuusik et al., 2002; Martin et al., 2004; Mänd et al., 2005, 2006). The rate of carbon dioxide release was measured (VCO_2 ml h^{-1}) at an air flow rate of 120 ml min^{-1}, a pressure compensated URAS 26 (ABB Analytical, Frankfurt, Germany), covering a measuring range of 0 to 500 ppm. The data from the analyzer were sampled at a rate of 10 Hz to PC via the analog output. The CO_2 and H_2O were eliminated from the flow-through system air by DRIERITE and a molecular sieve.

The LI-7000 differential CO_2/H_2O Analyzer (LiCor, Lincoln, Nebraska, USA), designed for laboratory and field research applications, was used in the second experiment, to record water loss (VH_2O µl h^{-1}) parallel to the bursts of CO_2 releases in bumble bee foragers at 18°C. Air flow in LI-7000 was regulated at 166 ml min^{-1} (10 l h^{-1}). The CO_2 and H_2O were eliminated from the air used in the flow-through system by NaOH and $Mg(ClO_{42})$. The IRGA was calibrated using NIST-traceable standard gases (for CO_2).

Infrared-actography

The LI-7000 was combined with an infrared (IR) actograph to record abdominal movements. The actograph has also been used as an insect IR cardiograph or optocardiograph (Hetz, 1994; Hetz et al., 1999; Mänd et al., 2006; Karise et al., 2010). Two IR-emitting diodes (TSA6203) were placed on one side (ventral side of the insect abdomen) and two sensor diodes (BP104) were placed on the opposite side of the insect chamber. Abdominal movements caused changes in the light transmitters, which were converted into voltages and recorded as spikes.

Treatments

A commercial formulation of alpha-cypermethrin (Fastac 50 EC, a.i. 50 g/l, BASF SE, D-67056 Ludwigshafen, Germany) was used to measure its effect on bumble bee respiratory patterns and water loss. We diluted the Fastac 50 EC to 0.04% (20 ppm of alpha-cypermethrin), which corresponds to the registered field rate in Estonia of 20 g a.i. ha^{-1}. For our experiments, the field dosage of Fastac 50 EC was diluted with distilled water to 0.004% (2 ppm of alpha-cypermethrin) and 0.002% (1 ppm of alpha-cypermethrin) which are accordingly 10 and 20 times lower concentrations than recommended for treating flowering rape fields against pests. The bumble bees were dipped into the alpha-cypermethrin solution or distilled water as control for 10 seconds (Saba, 1971). Following dipping, each bee was air-dried on filter paper. This dipping method is widely used in various insect toxicology experiments with differing solvents or submergence times (5 sec to 1 min) by both insect larvae (Isayama et al., 2005; Cetin et al., 2006; Erler et al., 2010) and adults (Sibul et al., 2004; Azimi et al., 2009). In the case of bumble bees, the dipping method has been used as an alternative method in contact tests (van der Steen, 2001).

The measurements

The measurements lasted for six hours per individual bumble bee. All individuals were measured in the flow-through respirometer for three hours after which the insect chamber was opened and the bumble bee taken out for treatment. The treatment, according to the prescribed scheme (different concentrations of Fastac 50 EC or distilled water), was carried out immediately and the bee was placed back into the insect chamber for the next three hours.

In the first experiment, the metabolic rate and the frequency of bursts of CO_2 releases of *B. terrestris* foragers were measured at 8°C. Bumble bees are very active insects and tend to maintain high body temperature by shivering and contractions of flight muscles. The temperature was chosen to prevent flight muscle activity in the bumble bees (Goller and Esch, 1990; Kuusik et al., 2002) and eventually the regular DGE appeared in most of the individuals.

In the second experiment, muscle activity, respiration rate and WLR were measured at 18°C. Bumble bees often experience this temperature when foraging. For bumble bees it is important to keep their thoracic temperature high for several reasons: to minimise pre-flight warm-up time when exploiting different inflorescences and to minimise escape time when avoiding predators (Nieh et al., 2006). That is why many bumble bee individuals shorten the

length of the DGE cycles or do not show DGE at all at 18°C. Therefore, we did not count the clear cycles of discontinuous gas exchange at this temperature; instead, we examined the change in the respiratory and abdominal activity patterns. The higher metabolic rate increases also the WLR of the insect; therefore the differences in WLR should be more easily detectable.

The dose of alpha-cypermethrin bumble bees received (measured from ground-up bumble bee bodies) was 0.995 ± 0.227 μg/g (0.004%) and 0.87 ± 0.18 μg/g (0.002%) (analyzed by Agricultural Research Centre, Laboratory for Residues and Contaminants, Teaduse 4/6, Saku, 75501 Harjumaa, Estonia). The method used in the chemical analysis was EN 12393-1,2,3: 1998 GC-ECD/NPD, GC-MS, LC-MS/MS; Norwegian Crop Research Institute Pesticide Lab, M04.

The longevity of bumble bees

Bumble bees treated with Fastac 50 EC solutions of both concentrations or distilled water, as described above, were kept at room temperature in the dark. Each bee was placed in a separate chamber and provided with 30% sugar solution as food. The bumble bees were checked daily until death. The bumble bees were considered dead when they did not move antennae or legs and did not respond to tactile stimulation. Then death was confirmed using LI-7000 (Jõgar et al., 2008).

Data acquisition and statistics

Computerised data acquisition and analysis were performed using the DAS 1401 A/D (analog-digital) hardware and the software TestPoint (Keitley, Metrabyte, USA) with a sampling rate of 10 Hz. The LI-7000 analyser was connected to a computer to record CO_2 production in parts per million (ppm) using LiCor software. Mean metabolic rates were automatically calculated by a statistical program by averaging data over 3 h periods after excess CO_2 and H_2O which entered the system during handling had left the system. Paired t-tests and Kruskal-Wallis test were used in statistical analysis (StatSoft ver.10, Inc./USA). Mean values are presented with ± s.e.m.

RESULTS

The experiment at 8°C

The untreated resting bumble bee foragers exhibited rhythmic gas exchange patterns at low temperature. The results of all the treatments are presented in Table 1. The solution with 0.004% Fastac 50 EC changed the respiratory patterns. The numbers of bursts of CO_2 releases and the mean metabolic rates decreased significantly. Treating the bees with 0.002% solution also caused a decrease in the numbers of bursts of CO_2 releases, although the difference was not statistically significant. The mean metabolic rate decreased significantly. Dipping the bumble bees into distilled water as a control affected neither the frequency of bursts of CO_2 releases nor the mean metabolic rate (Table 1).

The experiment at 18°C

At 18°C, bumble bees were more active: the time to calm down and show CGE or DGE cycles were longer. During the first three hours of the experiments (before the treatment) the bumble bees showed different patterns of muscle activity (not locomotor activity). This was directly dependent on which activity type the specimen belonged to (R. Karise, unpublished). Some bumble bees need more time to calm down before showing regular CGE or DGE; others show the discontinuous respiration pattern already 10-30 minutes after inserting the bee into the insect chamber. Usually longer or shorter periods of CGE or DGE interchange the periods of active ventilation.

Treating the bees with 0.004 % Fastac 50 EC solution caused rapid disappearance of both rhythmic release of CO_2 and muscle activity (Fig. 1A,B). In DGE, the bumble bee uses muscle work only during the short O period to aid gas exchange; after treatment regular cycles disappeared and a long-lasting muscle tremor appeared. The change in metabolic rates after the treatment was significant: in one individual the metabolic rate increased, in others it decreased. Fastac 50 EC had no significant effect on WLR (Table 2).

Treatment with 0.002% Fastac 50 EC solution did not disrupt either the regular bursts of CO_2 releases or muscle activity (Fig. 2A,B). However, the DGE was replaced by CGE (Fig. 3A,B), by which the level of CO_2 release did not reach near zero as happens during DGE. Similarly to the effect of the stronger solution, the metabolic rates of the bumble bees decreased significantly. The WLR did not change significantly (Table 2).

Dipping the bumble bees into distilled water disrupted neither the DGE, if it had been present before the treatment, nor the muscle activity of the bumble bee foragers. The metabolic rate and WLR did not change significantly (Table 2).

Respiratory transpiration constituted only a small part, less than 10% of total transpiration in the bumble bee foragers. Also during activity, the H_2O release was not recognisably higher compared to the WLR in the C-phase. The simultaneous measurement ensured the exact coincidence of the bursts of CO_2 and H_2O release (Fig. 4).

The effect of Fastac 50 EC on bumble bee longevity

The mortality rate of bumble bees treated with different Fastac 50 EC solutions was affected by the solution concentration (H (2, N=30)=11.736 p=0.003). Most individuals treated with the higher concentration solution (0.004%) died within 1-3 days, although individual variation was observed (one specimen lived for 8 days, another 16 days). The bee which lived for 16 days after the treatment was also repeatedly controlled in the respirometer, which showed that the normal DGE or CGE recurred 48 hours after treatment and this pattern persisted at least until day 4. Also muscle activity recurred on day 3. On day 6, there was neither DGE nor regular CGE. Treatment with 0.002% solution did not shorten the life span of the bees significantly compared to those treated with distilled water ($p>0.05$) (Fig. 5).

DISCUSSION

Our results show that Fastac 50 EC has a dose dependent after-effect on bumble bee respiratory rhythms, metabolic rate and muscle activity but has no effect on WLR. The regular periods of discontinuous or cyclic gas exchange disappeared during the first 30 minutes after treatment with 0.004% Fastac 50 EC solution. This treatment also shortened the lifespan of bumble bees. Contact with 0.002% Fastac 50 EC solution did not provoke that kind of drastic disappearance of rhythmic gas exchange and the longevity of bumble bees did not change compared to control bees treated with distilled water.

The existence and nature of carbon dioxide emission patterns depends on many factors. These include environmental conditions (Kestler, 1971; Dingha et al., 2005; Terblanche et al., 2008; Karise et al., 2010), metabolic rate (Kestler, 1991; Sibul et al., 2004; Jõgar et al., 2006), the life stage of the insect (Beekman and Stratum, 1999; Mänd et al., 2005, 2006) and several stress factors (Kestler, 1991; Lighton and Lovegrove, 1990; Kovac et al., 2007). Normally bumble bees show DGE cycles as a sign of calming down or resting. The events of calming down are clearly observed on the respirograms of bumble bees (Karise et al., 2010).

Kestler (1991) claims that negative stressors raise standard metabolic rate of resting insects. Jõgar et al. (2006) also described the rise in metabolic rates after treatment with Neem EC in Colorado potato beetles. By contrast, our results show a decline in metabolic rates of

bumble bees after contact with Fastac, a pyrethroid insecticide. Some other researches also interpret the reduction in metabolic rate as a generalized response to stressors (e.g., toxins, insecticides, heat and cold) that could lead to a reduction in respiratory water loss (Hoffmann and Parsons, 1989; Chown and Gaston, 1999). Sibul et al. (2004), however, did not see any change in metabolic rates of pine weevils after contact with Neem EC. These results suggest that the effect of pesticides on metabolic rates of insects depends largely on both insect species and pesticide formulation.

According to Kestler (1991), the pathological CO_2 release patterns can be divided into phases: latency phase with closed-flutter-ventilation (CFV), followed by continuous respiration with small irregular bursts of CO_2 releases. Kestler considers this as a reversible excitation phase being a typical stress index for sublethal doses of neurotoxic pesticides. The reversible excitation phase devolves to an irreversible excitation phase with no bursts of cyclic CO_2 release. At that time, the spiracles stay open and are paralysed.

We found clear alteration in respiratory rhythms of bumble bees after treatment with alpha-cypermethrin, the neurotoxic active ingredient of Fastac 50 EC. Contact with the 0.004% solution caused rapid disappearance of the respiration cycles in most of the foragers. Contact with the 0.002% solution of Fastac 50 EC changed the classical CFO cycles to FV cycles within about the first 30 minutes, later the bouts of CO_2 releases disappeared. If the large bouts of CO_2 releases occurred after treatment, these were rather FV cycles instead of CFO cycles. Two specimens out of six showed large bursts of CO_2 releases after the treatment, others showed varying rates of released CO_2 of a relatively low but smooth level. We saw the shift from cyclic towards continuous respiratory behaviour along with decreasing metabolic rate due to non-ability of bumble bees to keep the spiracles closed. The diminishing muscle work after the treatment with the neurotoxic chemical (Zafeiridou and Theophilidis, 2006; Woodman et al., 2008) is most likely the result of paralysis, not the result of calming down. In unstressed insects the decreasing metabolic rate is a sign of calming down and therefore the shift towards classical DGE should appear (Bradley, 2007; Gray and Chown, 2008; Moerbitz and Hetz, 2010).

It seems reasonable to conclude that a dose of 0.004% Fastac 50 EC is not sub-lethal, but lethal. For most individuals, the symptoms of intoxication were irreversible. The fact that at least two specimens lived for longer (8 and 16 days), shows that this concentration must be near the lethal dose for bumble bees but indicates also the heterogeneity of *B. terrestris* population in the context of alpha-cypermethrin immunity. We interpret that, according to Kestler´s (1991) classification, the bumble bees must have been in reversible excitation phase

only. The three hour period must have been too short to see total recovery from the intoxication. We saw the reappearance of the regular DGE in the bumble bees which survived the higher dose and lived for 8 or 16 days after treatment.

In spite of significantly decreasing metabolic rate, total water loss did not differ significantly after dipping the bees into distilled water or into the Fastac 50 EC solution of either concentration. However, the WLR showed a trend to increase after treatment of the bees with the 0.004% solution, while decreasing after treatment with 0.002% solution or distilled water. The decreasing WLR is normal when metabolic rate decreases. At lower metabolic rate the gas exchange including WLR is lower. The slightly higher WLR after the treatment with 0.004% Fastac 50 EC solution was not caused by muscular excitation, since this would have been seen on the actograph recordings. We suppose that, due to paralysis, the spiracles of the bumble bees may have been open (continuous CO_2 release) after treatment and along with the outflow of CO_2, the water vapour was also washed out from the tissues of moribund insects. Total water loss has been showed to be higher during continuous, compared to discontinuous, CO_2 release (Matthews and White, 2012).

Several studies reveal that respiratory water loss comprises mostly a small fraction of total water loss, even when the spiracles are open (Quinlan and Hadley, 1993; Quinlan and Lighton, 1999; Chown, 2002; Gibbs and Johnsson, 2004; Lighton et al., 2004). We suppose that, for bumble bees, respiratory water loss probably does not play a very important role and the non-ability to DGE and desiccation thereafter was not the direct cause of death. The importance of respiratory water loss differs between insect species (Lamprecht et al., 2009) depending more or less on water permeability of the cuticle. Bumble bees feed mostly on liquid food and therefore they need to discharge excess water, and the water permeability of their cuticle is high (Nicolson, 2009). A characteristic of bee water balance is the rapid mobilisation of ingested dietary water from the crop to the haemolymph, allowing rapid correction of haemolymph osmotic pressure (Willmer, 1986). Besides, in larger bees like *Xylocopa* and *Bombus sp*, the metabolic water may be in excess during flight and occasionally these bees eliminate water by spitting or by defaecation (Bertsch, 1984; Willmer and Stone, 1997). Because of these characteristics of bumble bee physiology, which allow them to be less judicious about respiratory patterns, and based on our results, we do not believe that death resulted from desiccation, even if the pyrethroid had increased the diuretic event. Still, the DGE cycles may confer a fitness benefit for the bumble bee *B. terrestris*. We did not find proof for the theory of DGE cycles functioning as a water saving mechanism; rather our results support the oxidative damage hypothesis (Hetz and Bradley, 2005). Probably, the

intoxicated bumble bees were paralysed and their spiracles were open: the freely entering oxygen could have been the key factor diminishing their fitness. This kind of research may benefit from precise observation under the microscope on the behaviour of the spiracles during intoxication.

ACKNOWLEDGEMENTS

We thank Enno Merivee and Sirje Mitt for valuable assistance and advice on the statistical analysis.

FUNDING

This research was supported by target financing of the Estonian Ministry of Education and Research [SF0170057s09]; and Estonian Science Foundation [grant numbers 7391, 9449 and 9450].

REFERENCES

Azimi, M., Pourmirza, A. A., Safaralizadeh, M. H. and Mohitazar, G. (2009). Studies on the Lethal Effects of Spinosad on Adults of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) with Two Bioassay Methods. *Asian J. Biol. Sci.* **2**, 1-6.

Basson, C. H. and Terblanche, J. S. (2011). Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *J. Insect Physiol.* **57**, 433-443.

Beekman, M. and van Stratum, P. (1999). Respiration in bumblebee queens: effect of life phase on the discontinuous ventilation cycle. *Entomol. Exp. Appl.* **92**, 295–298.

Bertsch, A. (1984). Foraging in male bumblebees (*Bombus lucorum* L.): maximising energy or minimising water load? *Oecologia* **62**, 325-336.

Boardman, L., Terblanche, J. S., Hetz, S. K., Marais, E. and Chown, S. L. (2012). Reactive oxygen species production and discontinuous gas exchange in insects. *Proc. Biol. Sci.* **279**, 893-901.

Bradley, T. J. (2007). Control of the respiratory pattern in insects. *Adv. Exp. Med. Biol.* **618**, 211–228.

Buck, J. (1958). Cyclic CO_2 release in insects. IV. A theory of mechanism. *Biol. Bull.* **114**, 118-140.

Carvell, C., Roy, D. B., Smart, S., Pywell, R. F., Preston, C. D. and Goulson, D. (2006). Declines in forage availability for bumblebees at a national scale. *Biol. Conserv*. **132**, 481–489.

Cetin, H., Erler, F. and Yanikoglu, A. (2006). Larvicidal activity of novaluron, a chitin synthesis inhibitor, agains the housefly, *Musca domestica*. *J. Insect Sci.* **6**, 1–4.

Chown, S. L. (2002). Respiratory water loss in insects. *Comp. Biochem. Phys.* **133**, 791-804.

Chown, S. L. (2011). Discontinuous gas exchange: new perspectives on evolutionary origins and ecological explanations. *Funct. Ecol.* **25**, 1163-1168.

Chown, S. L. and Gaston, K. J. (1999). Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biol. Rev.* **74**, 87-120.

Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B. and Marais, E. (2006). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool.* **79**, 333–343.

Corbet, S. A., Fussell, M., Ake, R., Fraser, A., Gunson, C., Savage, A. and Smith, K. (1993). Temperature and pollinating activity of social bees. *Ecol. Entomol.* **18**, 17–30.

Dingha, B. N., Appel, A. G. and Eubanks, M. D. (2005). Discontinuous carbon dioxide release in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), and its effect on respiratory transpiration. *J. Insect Physiol.* **51**, 825–836.

Erler, F., Cetin, H., Saribasak, H. and Serttas, A. (2010). Laboratory and field evaluations of some botanical pesticides against the cedar leaf moth, *Acleris undulana*. *J. Pest Sci*. **83**, 265–272.

Förster, T. D. and Hetz, S. K. (2010). Spiracle activity in moth pupae – the role of oxygen and carbon dioxide revisited. *J. Insect Physiol.* **56**, 492–501.

Gibbs, A. G. and Johnson, R. A. (2004). The role of discontinuous gas exchange in insects: the chtonic hypothesis does not hold water. *J. Exp. Biol.* **207**, 3477–3482.

Goller, F. and Esch, H. (1990). Comparative study of chill-coma temperatures and muscle potentials in insect flight muscles. *J. Exp. Biol*. **150**, 221–231.

Gray, E. M. and Chown, S. L. (2008). Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: A case study from a highly variable cockroach, *Perisphaeria sp*. *J. Insect Physiol.* **54**, 169-179.

Hetz, S. K. and Bradley, T. J. (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature*. **433**, 516–519.

Hetz, S. K., Psota, E. and Wasserthal, L. T. (1999). Roles of aorta, ostia and tracheae in heartbeat and respiratory gas exchange in pupae of *Troides rhadamantus* Staudinger 1888 and *Ornithoptera priamus* L. 1758 (Lepidoptera, Papilionidae). *Int. J. Insect Morphol.* 28, 131-144.

Hetz, S. K., Wasserthal, L. T., Hermann, S., Kaden, H. and Oelssner, W. (1994). Direct oxygen measurements in the tracheal system of lepidopterous pupae using miniaturized amperometric sensors. *Bioelectroch. Bioener.* **33**, 165-170.

Hoffmann, A. A. and Parsons, P. A. (1989). Selection for increased desiccation resistance in *Drosophila melanogaster*: Additive genetic control and correlated responses for other stresses. *Genetics.* **122**, 837-845.

Isayama, S., Saito, S., Kuroda, K., Umeda, K. and Kasamatsu, K. (2005). Pyridalyl, a novel insecticide: potency and insecticidal selectivity. *Arch. Insect Biochem. Physiol.* **58**, 226–33.

Jõgar, K., Kuusik, A., Metspalu, L., Hiiesaar, K., Grishakova, M. and Luik, A. (2008). Effects of Neem EC on gas exchange, tracheal ventilation, and water loss in diapausing pupae of *Pieris brassicae*. *Entomol. Exp. Appl.* **126**, 165–173.

Jõgar, K., Kuusik, A., Metspalu, L., Hiiesaar, K., Luik, A. and Grishakova, M. (2006). Results of treatments with natural insecticidal substances on the development and physiological state of insects. *Agron. Res.* **4**, 203-210.

Karise, R., Kuusik, A., Mänd, M., Metspalu, L., Williams, I. H., Hiiesaar, K., Luik, A., Muljar, R. and Liiv, K. (2010). Gas exchange patterns of bumble bee foragers before and after exposing to lowered temperature. *J. Insect Physiol.* **56**, 529-535.

Karise, R., Viik, E. and Mänd, M. (2007). Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape *Brassica napus* flowers in field conditions. *Pest Manag. Sci.* **63**, 1085–1089.

Kestler P. (1991). Cyclic CO_2 release as a physiological stress indicator in insects. *Comp. Biochem. Physiol.* **100C**, 207–211.

Kestler, P. (1971). Die Diskontinuierliche Ventilation bei *Periplaneta americana* L. und Anderen Insekten. Dissertation, Julius-Maximilians-Universität Würzburg, 232 pp.

Kestler, P. (1980). Saugventilation verhindert bei Insekten die Wasserabgabe aus dem Tracheensystem. *Verh. dt. zool. Ges.* **1980,** 306.

Kestler, P. (1982). Wie verhindern Stigmen den Wasserverlust? *Verh. Dtsch. Zool. Ges.* **1982**, 262.

Kestler, P. (1985). Respiration and respiratory water loss. In *Environmental Physiology and Biochemistry of Insects* (ed. K. H. Hoffmann), pp. 137–189. Berlin: Springer-Verlag.

Kestler, P. (2003). Physiological gas exchange strategies for spiracular control. *Comp. Biochem. Physiol.* **134A Suppl.**, 73-74.

Kovac, H., Stabentheiner, A., Hetz, S. K., Petz, M. and Crailsheim, K. (2007). Respiration of resting honeybees. *J. Insect Physiol.* **53**, 1250–1261.

Kuusik, A., Martin, A.-J., Mänd, M., Hiiesaar, K., Metspalu, L. and Tartes, U. (2002). Interrelations of gas exchange cycles, body movements and heartbeats in the foragers of bumblebee *Bombus terrestris* (Hymenoptera: Apidae) at low temperatures. *Eur. J. Entomol.* **99**, 209–214.

Lamprecht, I., Seymour, R. S., White, C. R., Matthews, P. G. D. and Wadsö, L. (2009). Discontinuous ventilation in the rhinoceros beetle *Oryctes nasicornis*: Direct and indirect calorimetry. *J. Therm. Anal. Calorim.* **95(3),** 743-747.

Lighton, J. R. B. (1994). Discontinuous ventilation in terrestrial insects. *Physiol. Zool.* **67**, 142–162.

Lighton, J. R. B. (1996). Discontinuous gas exchange in insects. *Annu. Rev. Entomol.* **41**, 309–324.

Lighton, J. R. B. and Lovegrove, B. G. L. (1990). A temperature-induced switch from diffusive to convective ventilation in the honeybee. *J. Exp. Biol.* **154**, 509–516.

Lighton, J. R. B. and Turner, R. J. (2008). The hygric hypothesis does not hold water: abolition of discontinuous gas exchange cycles does not affect water loss in the ant *Camponotus vicinus*. *J. Exp. Biol.* **211**, 563–567.

Lighton, J. R. B., Fielden, L. J. and Rechav, Y. (1993). Discontinuous ventilation in a non-insect, the tick *Amblyomma marmoreum* (Acari, Ixodidae): characterization and metabolic modulation. *J. Exp. Biol.* **180**, 229–245.

Lighton, J. R. B., Schilman, P. E. and Holway, D. A. (2004). The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *J. Exp. Biol.* **207**, 4463–4471.

Mänd, M., Kuusik, A., Martin A.-J., Williams, I. H., Luik, A., Karise, R., Metspalu L. and Hiiesaar, K. (2006). Regular periods of abdominal contractions recorded from larvae of the bumblebee, *Bombus terrestris* L. (Hymenoptera: Apidae). *Eur. J. Entomol.* **103**, 319–322.

Mänd, M., Kuusik, A., Martin, A.-J., Williams, I. H., Luik, A., Karise, R., Metspalu, L. and Hiiesaar, K. (2005). Discontinuous gas exchange cycles and active ventilation in pupae of the bumblebee *Bombus terrestris*. *Apidologie* **36**, 561–570.

Mänd, M., Mänd, R. and Williams, I. H. (2002). Bumblebees in the agricultural landscape of Estonia. *Agr. Ecosyst. Environ.* **89**, 69–76.

Marais, E. and Chown, S. L. (2003). Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. *J. Exp. Biol.* **206**, 4565–4574.

Marais, E., Klok, C. J., Terblanche, J. S. and Chown, S. L. (2005). Insect gas exchange patterns: a phylogenetic perspective. *J. Exp. Biol.* **208**, 4495–4507.

Martin, A.-J., Kuusik, A., Mänd, M., Metspalu, L. and Tartes, U. (2004). Respiratory patterns in nurses of the red wood ant *Formica polyctena* (Hymenoptera, Formicidae). *Insect. Soc.* **51**, 62–66.

Matthews, P. G. D. and White, C. R. (2011). Discontinuous gas exchange in insects: Is it all in their heads? *Am. Nat.* **177**, 130-134.

Matthews, P. G. D. and White, C. R. (2012). Discontinuous gas exchange, water loss, and metabolism in *Protaetia cretica* (Cetoniinae, Scarabaeidae). *Physiol. Biochem. Zool*. **85(2),** 174-182.

Moerbitz, C. and Hetz, S. K. (2010). Tradeoffs between metabolic rate and spiracular conductance in discontinuous gas exchange of *Samia cynthia* (Lepidoptera, Saturniidae). *J. Insect Physiol.* **56**, 536-542.

Nicolson, S. W. (2009). Water homeostasis in bees, with the emphasis on sociality. *J. Exp. Biol.* **212**, 429-434.

Nieh, J. C., Leon, A., Cameron, S. and Vandame, R. (2006). Hot bumble bees at good food: thoracic temperature of feeding *Bombus wilmattae* foragers is tuned to sugar concentration. *J. Exp. Biol.* **209**, 4185-4192.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* **25 (6)**, 345 – 353.

Quinlan, M. C. and Hadley, N. F. (1993). Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. *Physiol. Zool.* **66**, 628 -642.

Quinlan, M. C. and Lighton, J. R. B. (1999). Respiratory physiology and water relations of three species of Pogonomyrmex harvester ants (Hymenoptera: Formicidae). *Physiol. Entomol.* **24**, 293-302.

Saba, F. (1971). A simple test method for evaluating response to toxicants in mite populations. *J. Econ. Entomol.* **64**, 321.

Schimpf, N. G., Matthews, P. G. D. and White, C. R. (2012). Cockroaches that Exchange respiratory gases discontinuously survive food and water restriction. *Evolution*. **66 (2),** 597-604.

Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R. (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212**, 2773–2780.

Sibul, I., Kuusik, A. and Voolma, K. (2004). Monitoring of gas exchange cycles and ventilatory movements in the pine weevil *Hylobius abietis*: respiratory failures evoked by a botanical insecticide. *Entomol. Exp. Appl*. **110(2),** 173-179.

Slama, K. (1988). A new look at insect respiration. *Biol. Bul.* **175**, 289–300.

Slama, K. (1999). Active regulation of insect respiration. *Ann. Entomol. Soc. Am.* **92**, 916–929.

Stokstad, E. (2012). Field research on bees raises concern about low-dose pesticides. *Science* **30: 335 (6076),** 1555.

Terblanche, J. S., Clusella-Trullas, S., Deere, J. A. and Chown, S. L. (2008). Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. *J. Insect Physiol.* **54**, 114-127.

Thompson, H. M. (2001). Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* **32**, 305–321.

Thompson, H. M. (2003). Behavioural effects of pesticides in bees – their potential for use in risk assessment. *Ecotoxicology*. **12**, 317–330.

van der Steen, J. J. M. (2001). Review of the methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* **32**, 399–406.

Wickramasinghe, L. P., Harris, S., Jones, G. and Jennings, N. V. (2004). Abundance and species richness of nocturnal insects on organic and conventional farms: effects of agricultural intensification on bat foraging. *Conserv. Biol.* **18**, 1283–1292.

Williams, C. M., Pelini, S. L., Hellmann, J. J. and Sinclair, B. J. (2010). Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. *Biol. Lett.* **6**, 274–277.

Willmer, P. and Stone, G. (1997). Temperature and water relations in desert bees. *J. Therm. Biol.* **22(6),** 453-465.

Willmer, P. G. (1986). Foraging patterns and water balance: problems of optimisation for a xerophilic bee, *Chalicodoma sicula*. *J. Animal Ecol.* **55**, 941-962.

Woodman, J. D., Haritos, V. S. and Cooper, P. D. (2008). Effects of phosphine on the neural regulation of gas exchange in *Periplaneta americana*. *Comp. Biochem. Physiol. C* **147**, 271-277.

Woods, H. A. (2011). Breathing, bugs, and brains: conceptual unification? *Funct. Ecol*. **25**, 1161–1162.

Zafeiridou, G. and Theophilidis, G. (2006). A simple method for monitoring he respiratory rhythm in intact insects and assessing the neurotoxicity of insecticides. *Pestic. Biochem. Physiol.* **86**, 211-217.

Fig. 1. (A) The periods of DGE observable in the left part of the figure (before the treatment) are terminated by the treatment (marked by arrow) with 0.004% Fastac 50 EC solution. (B) Simultaneous recording of the IR-actograph shows the disappearance of activity periods after the treatment. (C) Detail of the shaded area in A demonstrating continuous respiration.

Fig. 2. (A) The alternating periods of DGE and activity, observable in the left part of the figure (before the treatment), are replaced after the treatment with 0.002% Fastac 50 EC solution (marked by arrow), by periods of CGE, observable in the right part of the figure. (B) Simultaneous recording of the IR-actograph shows that this solution does not cause muscle paralysis, although the irregular rhythmic activity is supressed. The rectangles at A indicate the sections zoomed out on the Figs 3A and 3B.

Fig. 3. (A) The section of Fig. 2A (shaded area, left part) demonstrates the DGE pattern, where a modification from short C-phase to long C-phase can be seen. (B) The section of Fig. 2A (shaded area, right part) demonstrates the CGE rhythms, during which the CO_2 release does not reach near zero.

Fig. 4. Simultaneous recording of WLR (upper trace) and CO_2 release (lower trace) in bumble bee foragers. Clear DGC with smaller and larger bursts of CO_2 are observable in the left part of the figure and in the right part of the figure a brief activity period can be seen.

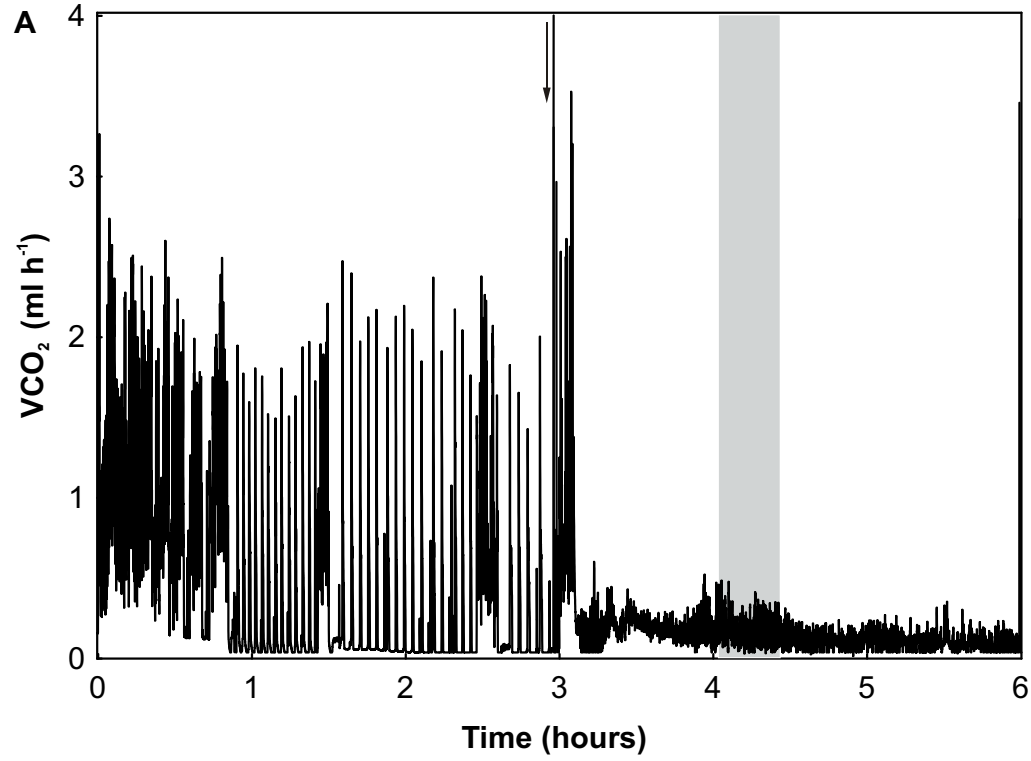
Fig. 5. The longevity of bumble bee foragers after treatment with with 0.002% and 0.004% Fastac 50 EC solution and distilled water. Different letters upon the boxes indicate statistically significant differences between groups.

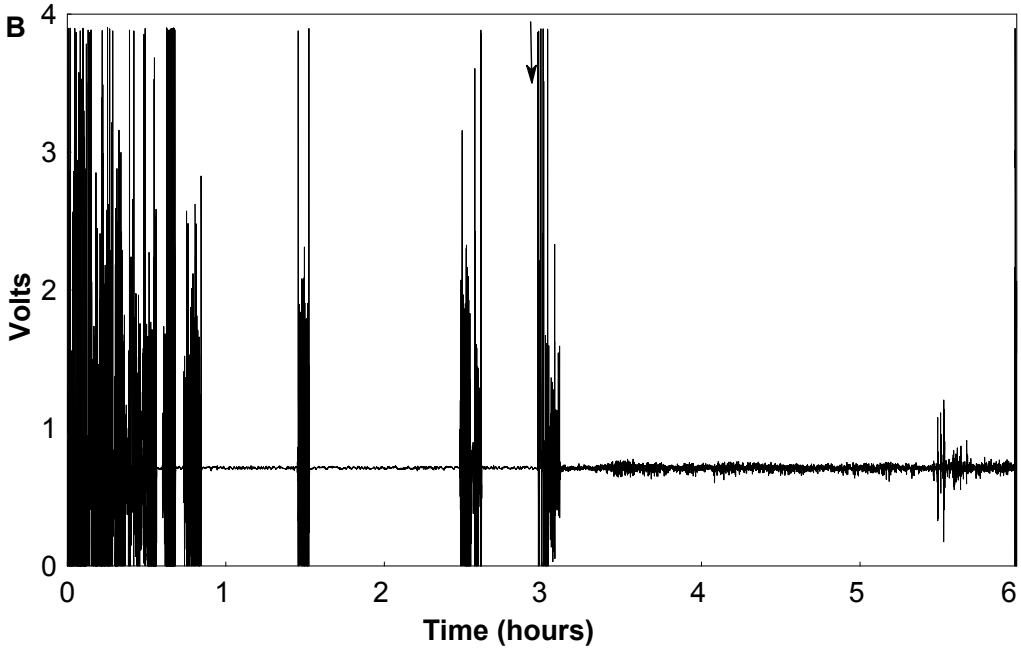
Table 1. *The mean metabolic rates (VCO_2 ml h^{-1}) and the numbers of bursts of CO_2 releases of forager bumble bees treated with 0.002% and 0.004% Fastac 50 EC solution and distilled water as control at 8°C*

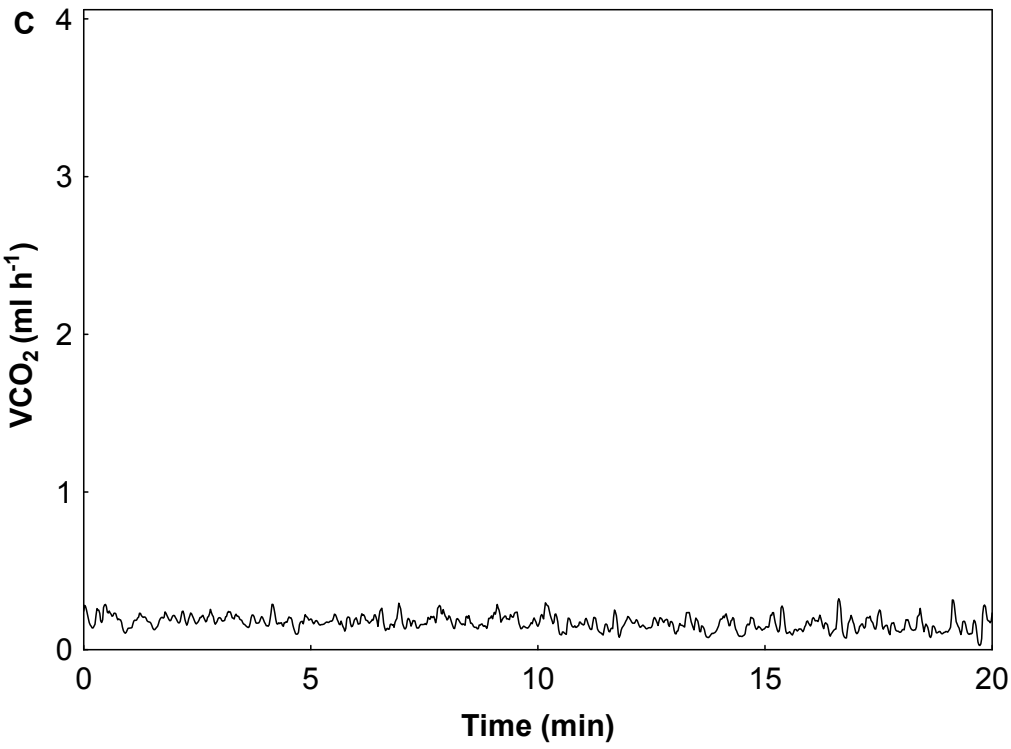
Treatment	Indiv.	Metabolic rate (VCO_2 ml h^{-1})		No of bursts of CO_2 releases	
		Before	After	Before	After
0.004%	1	0.177	0.162	8	1
Fastac 50	2	0.178	0.133	7	2
EC	3	0.164	0.151	18	0
	4	0.203	0.134	22	3
	5	0.182	0.141	19	0
	6	0.153	0.116	9	2
		t=4.318 df=5 **p=0.008**		t=4.49 df=5 **p=0.006**	
0.002%	1	0.213	0.204	9	4
Fastac 50	2	0.201	0.202	5	1
EC	3	0.189	0.183	34	14
	4	0.193	0.184	3	2
	5	0.185	0.176	12	11
	6	0.201	0.196	8	6
		t=3.853 df=5 **p=0.012**		t=1.85 df=5 p=0.124	
Dist. water	1	0.197	0.197	20	15
	2	0.193	0.116	18	11
	3	0.205	0.204	7	4
	4	0.154	0.103	9	9
	5	0.168	0.168	16	11
	6	0.186	0.183	6	10
		t=1.605 df=5 p=0.169		t=1.62 df=5 p=0.166	

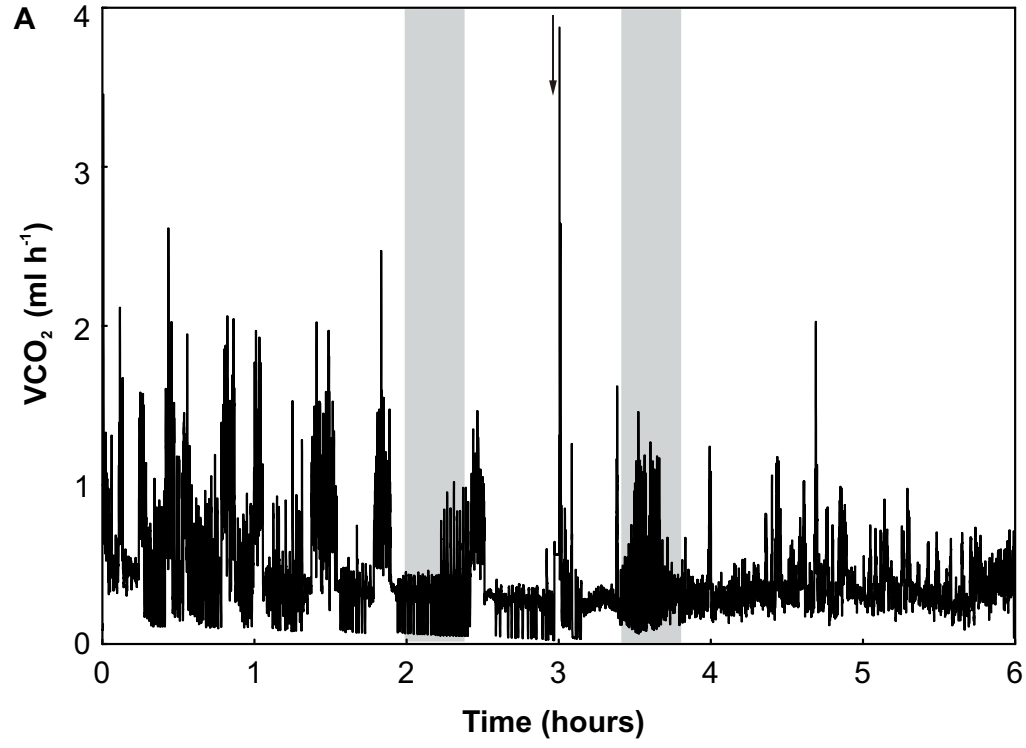
Table 2. *The mean metabolic rates (VCO_2 ml h^{-1}) and WLR (VH_2O µl h^{-1}) of forager bumble bees treated with 0.002% and 0.004% Fastac 50 EC solution and distilled water as control at 18° C*

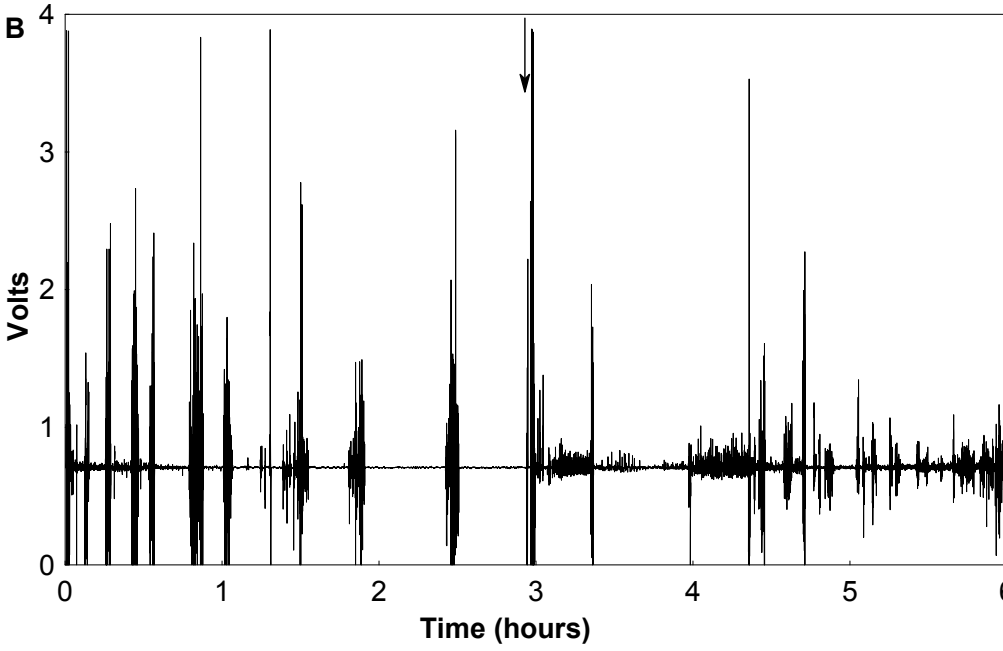
Treatment	Indiv.	Metabolic rate (VCO_2 ml h^{-1})		WLR (VH_2O µl h^{-1})	
		Before	After	Before	After
0.004%	1	0.267	0.318	1.94	2.10
Fastac 50	2	0.270	0.169	1.66	3.52
EC	3	0.466	0.224	2.34	1.46
	4	0.411	0.236	2.30	6.10
	5	0.469	0.329	1.80	1.15
	6	0.376	0.255	2.01	2.85
		t= 3.036 df=5 **p=0.029**		t= -1.171 df=5 p=0.294	
0.002%	1	0.625	0.376	2.68	4.66
Fastac 50	2	0.581	0.494	3.49	2.93
EC	3	0.660	0.219	0.14	0.22
	4	0.695	0.146	4.06	1.37
	5	0.335	0.188	1.39	0.57
	6	0.617	0.259	8.23	1.81
		t= 4.217 df=5 **p=0.008**		t= 1.194 df=5 p=0.286	
Dist. water	1	1.290	0.078	3.19	3.01
	2	0.061	0.024	8.13	3.24
	3	0.114	0.064	2.84	2.45
	4	0.089	0.050	2.79	2.83
	5	0.152	0.119	6.35	4.17
	6	0.415	0.198	5.23	3.75
		t=1.378 df=5 p=0.227		t= 1.993 df=5 p=0.103	

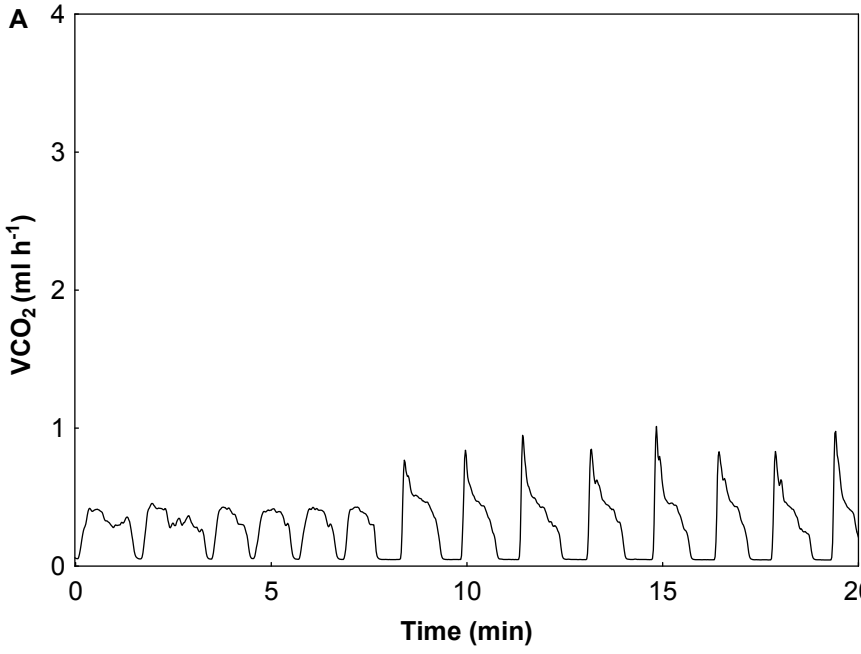

A
4
3
2
1
0
VCO_2 (ml h^{-1})
0
1
2
3
4
5
6
Time (hours)

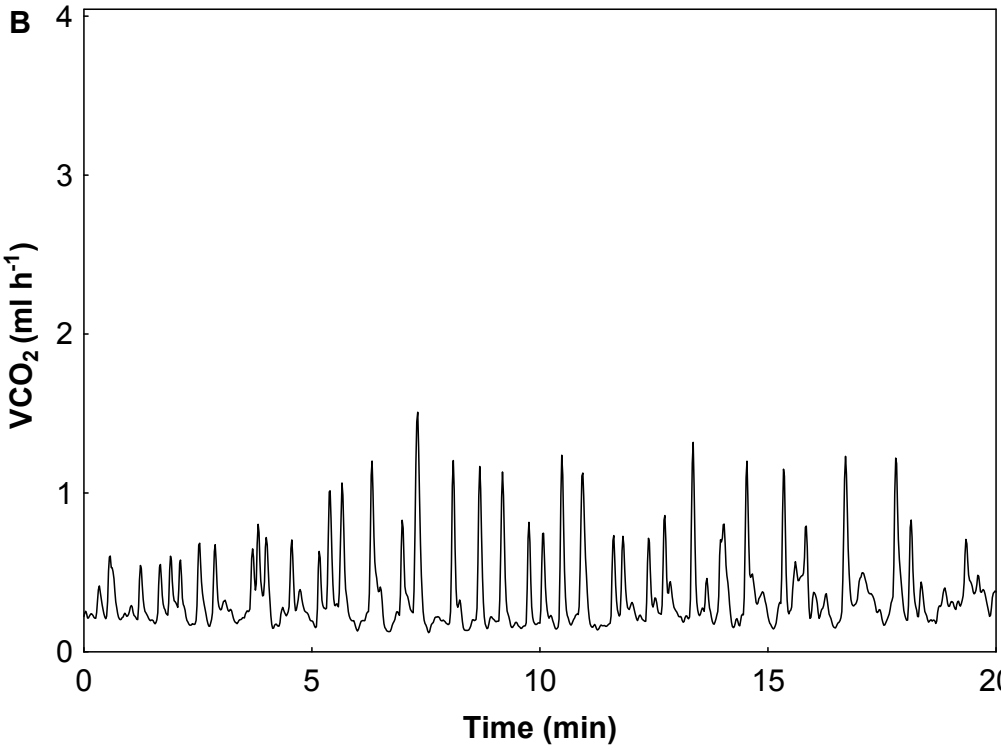

B
4
3
2
1
0
Volts
0
1
2
3
4
5
6
Time (hours)

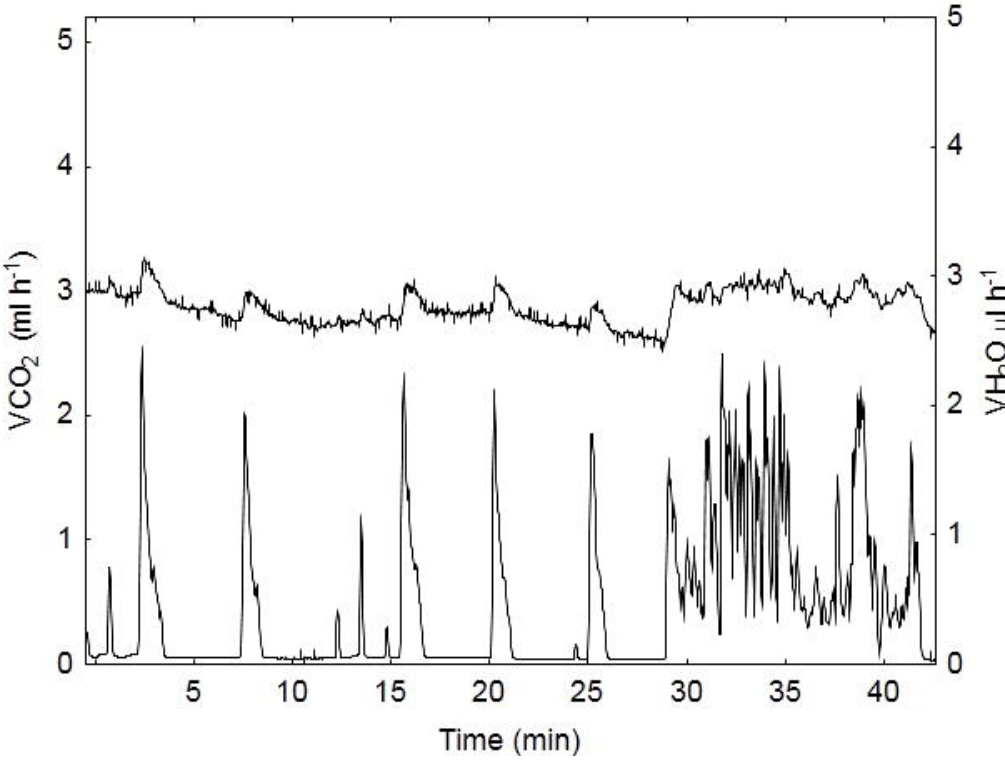

C
4
3
2
1
0
VCO2 (ml h-1)
0
5
10
15
20
Time (min)

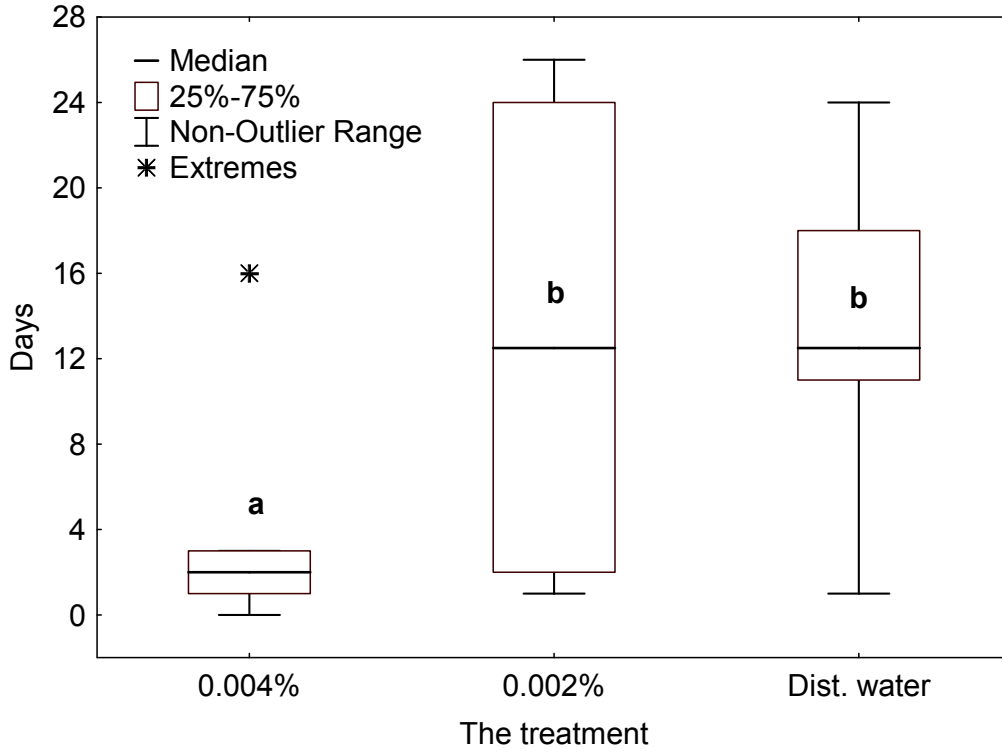

A
4
3
2
1
0
VCO2 (ml h-1)
0
1
2
3
4
5
6
Time (hours)


B
Volts
Time (hours)
0
1
2
3
4
5
6


A
VCO2 (ml h-1)
Time (min)
0
5
10
15
20


B
4
3
2
1
0
VCO_2 (ml h^{-1})
0
5
10
15
20
Time (min)


5
4
3
2
1
0
VCO_2 (ml h^{-1})
VH_2O µl h^{-1}
5
10
15
20
25
30
35
40
Time (min)


Median
25%-75%
Non-Outlier Range
Extremes
Days
28
24
20
16
12
8
4
0
a
b
b
0.004%
0.002%
Dist. water
The treatment

CURRICULUM VITAE

First name: Eneli
Surname: Viik
Citizenship: Estonian
Date of birth: 20.07.1982

Employment:
Agricultural Research Centre, Department of Agricultural Research and Monitoring,
Riia 24D, 51010, Tartu, Estonia
Phone: +372 7 480 941,
e-mail: eneli.viik@pmk.agri.ee
Position: Chief specialist

Academic degree:
Master's degree (MSc) "The impact of foliar fertilizing on the number of pollinators of spring oilseed rape", Estonian University of Life Sciences, 2006

Education:

2006–2012	Estonian University of Life Sciences, doctoral studies, entomology
2004-2006	Estonian University of Life Sciences, master studies, plant protection
2000-2004	Estonian Agricultural University, bachelor studies, landscape protection and management
1997-2000	Jõgeva Co-educational Gymnasium
1989-1997	Sadala Middle School

Foreign languages: English

Professional Employment:

2007–present	Agricultural Research Centre, chief specialist

Current research program:
Biosciences and Environment, Agricultural Sciences (The effect of growing technologies on insect visitors of oilseed rape flowers)

Participation in research projects:

2012–2015	ESF grant No 9450: „Impact of pesticide residues on the foraging behaviour and physiology of pollinators". PhD student
2011–2012	ESF grant No 8895: „Impact of host plants on the major pests of cruciferous plants and their parasitoids in different cropping systems". PhD student
2009–…	TF project SF0170057s09: „Plant protection for sustainable crop production". Investigator
2008–2011	ESF grant No 7391: „Foraging behaviour of pollinators in farmland: use of bumble bee colonies for increasing the seed yield of entomophilous crops". PhD student
2006–2008	TF project SF0172655s04: „Development of environmentally friendly plant protection II". Investigator
2004–2007	ESF grant No 5737: „Foraging behaviour of pollinators in farmland: use of bumble bee colonies for increasing the seed yield of entomophilous crops". MSc student, PhD student

Organisation membership Estonian Plant Protection Organization – member since 2004

Awards: Diploma in the field of agricultural sciences for scientific study "The effect of different growing technologies to the number of pollinators of spring oilseed rape"

Training and special courses

20.04–23.04.2009	NordForsk workshop "Mutualistic interactions", University of Copenhagen, Denmark, venue: Estonia
04.11–09.11.2007	BeeNOVA PhD course "Insect Pathology", University of Copenhagen, Denmark
01.09–08.09.2007	NOVA-BOVA intensive course "Beekeeping Techniques in Cold Climates", Latvia University of Agriculture, Latvia
20.05–27.05.2007	NOVA-BOVA PhD course "Weed Biology and Management", Lithuanian University of Agriculture, Lithuania
09.09–16.09.2006	NOVA PhD course "Insect Pollinators and Pollination Ecology", University of Helsinki, Finland

14.03–22.03.2006	NOVA-BOVA MSc course "Agroecology in the Baltic States Today", Lithuanian University of Agriculture, Latvia University of Agriculture, Estonian University of Life Sciences, Lithuania, Latvia, Estonia
10.10–14.10.2005	NOVA-BOVA postgraduate course "Non-Chemical Weed Control", Lithuanin University of Agriculture, Lithuania
06.09–12.09.2004	PhD course "Social Insects", Swedish University of Agricultural Sciences, Sweden

ELULOOKIRJELDUS

Nimi: Eneli Viik
Kodakondsus: Eesti
Sünniaeg: 20.07.1982

Töökoht: Põllumajandusuuringute Keskus,
Põllumajandusseire ja uuringute osakond,
Riia 24D, 51010, Tartu
Telefon: +372 7 480 941,
e-mail: eneli.viik@pmk.agri.ee

Ametikoht: Peaspetsialist

Teaduskraad: Põllumajandusteaduste magister (MSc) taimekaitse erialal, väitekiri "Juurevälise väetamise mõju suvirapsi tolmeldajate arvukusele", EMÜ, 2006

Hariduskäik:

2006–2012	EMÜ, doktorantuur, entomoloogia
2004–2006	EMÜ, magistriõpe, taimekaitse
2000–2004	EPMÜ, bakalaureuseõpe, maastikukaitse ja -hooldus
1997–2000	Jõgeva Ühisgümnaasium
1989–1997	Sadala Põhikool

Keelteoskus: Inglise keel

Teenistuskäik:

2007–	Põllumajandusuuringute Keskus, peaspetsialist

Teadustöö põhisuunad:
Bio- ja keskkonnateadused, põllumajandusteadus. Kasvatustehnoloogiate mõju suvirapsi õisi külastavatele putukatele

Osalemine uurimisprojektides:

2012–2015	ETF grant nr 9450: „Pestitsiidijääkide mõju tolmeldajate korjekäitumisele ja füsioloogiale". Doktorant
2011–2012	ETF grant nr 8895: „Peremeestaimede mõju ristõieliste õlikultuuride võtmekahjuritele ja nende parasitoididele

	erinevates viljelustingimustes“. Doktorant
2009–…	Sihtfinantseeritav teema SF0170057s09: „Taimekaitse jätkusuutlikule taimekasvatusele“, Täitja
2008–2011	ETF grant nr 7391: „Tolmeldajate korjekäitumine põllumajandusmaastikes: põllumajandusliku tegevuse mõju“. Doktorant
2006–2008	Sihtfinantseeritav teema SF0172655s04: „Keskkonnasäästliku taimekaitsetehnoloogia arendamine II“. Täitja
2004–2007	ETF grant nr 5737: „Tolmeldajate korjekäitumine põllumajandusmaastikes: kimalasperede kasutamine entomofiilsete kultuurtaimede seemnesaagi tõstmiseks”. Magistrant, doktorant

Organisatsiooniline tegevus:

Eesti Taimekaitse Selts – liige alates 2004

Teaduspreemiad:

Diplom põllumajandusteaduste valdkonnas teadustöö “Erinevate kasvatustehnoloogiate mõju suvirapsi tolmeldajate arvukusele” eest

Erialane enesetäiendus:

20.04–23.04.2009	NordForsk seminar “*Mutualistic interactions*”, Kopenhaageni Ülikool, Taani, toimumiskoht: Eesti
04.11–09.11.2007	BeeNOVA doktorikursus “*Insect Pathology*”, Kopenhaageni Ülikool, Taani
01.09–08.09.2007	NOVA-BOVA intensiivkursus “*Beekeeping Techniques in Cold Climates*”, Läti Põllumajandusülikool, Läti
20.05–27.05.2007	NOVA-BOVA doktorikursus “*Weed Biology and Management*”, Leedu Põllumajandusülikool, Leedu
09.09–16.09.2006	NOVA doktorikursus “*Insect Pollinators and Pollination Ecology*”, Helsingi Ülikool, Soome
14.03–22.03.2006	NOVA-BOVA magistrantide kursus “*Agroecology in the Baltic States Today*”, Leedu Põllumajandusülikool, Läti Põllumajandusülikool, Eesti Maaülikool, Leedu, Läti, Eesti

10.10–14.10.2005	NOVA-BOVA magistrantide kursus "*Non-Chemical Weed Control*", Leedu Põllumajandusülikool, Leedu
06.09–12.09.2004	Doktorikursus "*Social Insects*", Rootsi Põllumajandusteaduste Ülikool, Rootsi

LIST OF PUBLICATIONS

1.1. Publications indexed in the ISI Web of Science database:

Muljar, R., Karise, R., **Viik, E.**, Kuusik A., Mänd, M.,Williams, I. H., Metspalu, L., Hiiesaar K., Luik, A., Must, A. Effects of Fastac 50 EC on bumble bee *Bombus terrestris* L. respiration: DGE disappearance does not lead to increasing water loss. Journal of Insect Physiology (submitted)

Viik, E., Mänd, M., Karise, R., Lääniste, P., Williams, I. H., Luik, A. 2012. The impact of foliar fertilization on the number of bees (Apoidea) on spring oilseed rape. Žemdirbystė=Agriculture, 99 (1), 41-46.

Karise, R., **Viik, E.**, Mänd, M. 2007. Impact of alpha-cypermethrin on honey bees foraging on spring oilseed rape (*Brassica napus*) flowers in field conditions. Pest Management Science, 63, 1085–1089.

1.2. Papers publised in other peer-reviewed international journals with a registered code:

Muljar, R., **Viik, E.**, Marja, R., Svilponis, E., Jõgar, K., Karise, R., Mänd, M. 2010. The effect of field size on the number of bumble bees. Agronomy Research, 8, 351–357.

Koskor, E., Muljar, R., Drenkhan, K., Karise, R., Bender, A., **Viik, E.**, Luik, A., Mänd, M. 2009. The chronic effect of the botanical insecticide Neem EC on the pollen forage of the bumble bee *Bombus terrestris* L. Agronomy Research, 7, 341–346.

1.3. Papers in Estonian and in other peer-reviewed research journals with a local editorial board:

Karise, R., Mänd, M., **Viik, E.**, Martin, A.-J., Lääniste, P. 2004. Flower visitors on spring oilseed rape in different cropping system. Latvian Journal of Agronomy, 7, 6–11.

Mänd, M., Karise, R., **Viik, E.**, Metspalu, L., Lääniste, P., Luik, A. 2004. The effect of microfertilisers on the number of pollen beetles on spring oilseed rape. Latvian Journal of Agronomy, 7, 30–33.

3.1. Papers published in books listed in the ISI Web of Proceedings:

Mänd, M., Williams, I.H., **Viik, E.**, Karise, R. 2010. Oilseed rape, bees and integrated pest management. In: (Ed. Williams, I.H.) Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer Dordrecht Heidelberg, London, New York, p. 357–379.

3.2. Papers published in books by Estonian or foreign publishers not listed in the ISI Web of Proceedings:

Mõtte, M., Raa, I., **Viik, E.** 2011. MAKi meetmete rakendamise analüüs. Trükises: (koostanud Aamisepp, M., Matveev, E.) Põllumajandus ja maaelu 2011. Maamajanduse Infokeskus, Jäneda, lk. 48–56.

3.4. Articles/presentations published in conference proceedings not listed in the ISI Web of Proceedings:

Köster, T., Vask, K., Koorberg, P., Selge, I., **Viik, E.** 2009. Do We Need Broad and Shallow Agri-Environment Schemes? – Outcomes of Expost Evaluation of Estonian Rural Development Plan 2004-2006. In: Rural Development 2009. The Fourth International Scientific Conference. Proceedings I. Lithuania: Lithuanian University of Agriculture, p. 219-224.

3.5. Articles/presentations published in local conference proceedings:

Viik, E., Mänd, M., Karise, R., Koskor, E., Jõgar, K., Kevväi, R., Martin, A., Grishakova, M. 2007. Põllumajandusliku keskkonnameetme rakendamise mõju kimalaste liigirikkusele. Agronoomia 2007, 145–148.

Karise, R., **Viik, E.**, Mänd, M. 2006. Insektitsiidide mõju mesilaste korjekäitumisele. Agronoomia 2006, 241–244.

Mänd, M., Metspalu, L., **Viik, E.**, Lääniste, P., Luik, A. 2006. Lehekaudse väetamise mõju hiilamardika arvukusele suvirapsil. Agronoomia 2006, 232–235.

Koskor, E., Mänd, M., Karise, R., **Viik, E.,** Bender, A., Viiralt, R. 2005. Karukimalase (*Bombus terrestris* L.) hommikuse ja õhtuse õietolmukorje erinevused. Agronoomia 2005, 234–236.

Karise, R., Mänd, M., Ivask, M., **Viik, E.**, Koskor, E. 2005. Kimalase õietolmukämpude kalorsus ja seda mõjutavad tegurid. Transactions of the EAU 231–233.

Viik, E., Mänd, M., Rebane, A., Karise, R., Koskor, E. 2005. Lehekaudse väetamise mõju suvirapsi õite nektariproduktsioonile. Transactions of the EAU, 93–95.

Mänd, M., **Viik, E.**, Karise, R., Krikuhhin, T., Koskor, E., Lääniste, P. 2005. Väetamise mõju suvirapsi õietolmu produktsioonile. Agronoomia 2005, 96–98.

Karise, R., Mänd, M., **Viik, E.**, Lääniste, P., Martin, A-J. 2004. Mesilaselaadsete arvukus suvirapsil erinevate viljelusviiside korral. Agronoomia 2004, 88–90.

6.3. Popular science articles:

Viik, E., Mänd, M. 2008. Eesti põllumajandusmaastiku seire: kimalasi soodustavad väiksemad põllud. Mahepõllumajanduse Leht, 46 (5), 4.

Viik, E., Vask, K. 2008. ELi põllumajanduspoliitika vajab hindamist. Mahepõllumajanduse Leht, 43 (2), 12–14.

Viik, E., Vask, K. 2008. EL-i põllumajanduspoliitika vajab hindamist. Maamajandus, august 2008, 42–44.