



Bee-together

Free conference on pollinators with emphasis to stimulate interactions in the field

Date: 21 December 2010

Venue: Faculty Bioscience Engineering
Aula Oehoe (orals), Agora (posters-lunch/reception)
Coupure Links 653, Ghent



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Oral programme

9.15 Introduction

9.15 – 9.40 **Ivan Meeus:** Molecular detection of bumblebee parasites and pathogens

9.40 – 10.05 **Lina De Smet:** MLPA® as diagnostic tool for the honey bee viruses

10.05 – 10.30 **Gal Yarden:** Remebee – RNAi based product-line targeting major culprits in beekeeping

10.30 – 11.00 Coffee/tea break + posters

11.00 – 11.25 **Felix Wäckers:** Optimizing biocontrol and pollination services through informed landscape management

11.25 – 11.50 **Denis Michez:** Physiological constraint in host-plant specialization among specialist and generalist wild bees

11.50 – 12.15 **Sjef van der Steen:** Vitellogenin, *Varroa destructor*, pollen flow and honey bee (*Apis mellifera* L.) colony development

12.15 – 14.00 Lunch (free) + posters

14.00 – 14.25 **Uli Ernst:** Evidence for specialisation in worker policing in the honeybee *Apis mellifera*

14.25 – 14.50 **Dries Cardoen:** Microarray analysis provides unprecedented insight into the detailed physiology of reproductive and non-reproductive honeybee workers

14.50 – 15.15 **Matthias C. Van Vaerenbergh:** Mass spectrometry as a powerful tool for Hymenoptera venom analysis

15.15 – 15.45 Coffee/tea break + posters

15.45 – 16.10 **Julie Tobback:** The effect of age, task and insecticides on the *foraging* gene expression in the bumblebee *Bombus terrestris*

16.10 – 16.35 **Veerle Mommaerts:** *Bombus terrestris* as entomovector in greenhouse strawberries

16.35 – 17.00 **Patrick Lhomme:** Born in an alien nest: how do cuckoo bumblebee offspring avoid host recognition?

17.00 – 18.30 Closing & Reception (free)

Poster programme

1. **Bumblebee decline and parasite invasion: parasite prevalence in Argentinean bumblebees**, Marina Arbetman¹, Ivan Meeus², Hande Bayraktar², Carolina Morales¹, Marcelo Aizen¹, Guy Smagghe²
2. **A survey of parasite prevalence in wild bees**, Ivan Meeus¹, Hande Bayraktar¹, Dries Laget², Guy Smagghe¹
3. **Identification of sublethal effects of crop protection products on honey bees (*Apis mellifera*) and their impact on bee behavior and colony health**, T. Beliën¹, J. Kellers¹, K. Heylen², J. Billen², L. Arckens², R. Huybrechts², B. Gobin^{1,*}
4. **Host-plant specialisation in Colletidae (Hymenoptera, Apoidea): Does physiology interface with ecology?**, Maryse Vanderplanck¹, Georges Lognay² and Denis Michez¹
5. **The altruistic infertility of honeybees: a physiological approach**, Dries Cardoen¹, Uli Ernst^{1,3}, Dirk de Graaf², Tom Wenseleers³, Peter Verleyen¹, Liliane Schoofs¹
6. **Exploring the functionality of *Nasonia vitripennis*' unknown venom proteins**, Ellen L. Danneels, Ellen Formesyn, Frans Jacobs, Dirk C. de Graaf
7. **Development of multiplex PCR technology with microsatellite primers to study genetic structure of bumblebee populations**, Kevin Maebe, Ivan Meeus, Guy Smagghe
8. **A laboratory assay to evaluate sublethal effects on the foraging behaviour of the pollinator *Bombus terrestris***, Veerle Mommaerts¹, Guido Sterk², Guy Smagghe^{1,3}
9. **Side-effects of bio-fungicides and bio-insecticides on the pollinator *Bombus terrestris***, Veerle Mommaerts¹, Kris Jans², Guido Sterk², Lucien Hoffmann³, Sarah Labiese⁴ and Guy Smagghe^{1,4}
10. **Advancing Bee Taxonomy in Sub-Saharan Africa through *Abc Taxa* and Global Taxonomic Initiative on wild bees in Ethiopia and Burundi**, Connal Eardley^{1,2}, Michael Kuhlmann³, Alain Pauly⁴, Yves Samyn⁵
11. **Do *Aconitum* plants protect their pollen against herbivory?**, Dorothee Roelants¹, G. Glauser², Pierre Rasmont¹
12. **Parameters of bumblebee foraging/feeding behaviour for sugar water in a two-way choice assay and relation with its nutrient value**, Ellen Van Campenhout¹, Veerle Mommaerts¹, Kris Jans², Guy Smagghe^{1,3}
13. **Diversity in protein glycosylation among insect species**, Gianni Vandenborre^{1,2}, Els J.M. Van Damme² Bart Ghesquière^{3,4}, Gerben Menschaert⁵, Rameshwaram Nagender Rao^{1,2}, Kris Gevaert^{3,4}, Guy Smagghe¹
14. **The abnormal bumblebees scarcity syndrom: Are heat waves leading to local extinctions of bumblebees?**, Pierre Rasmont and Stéphanie Iserbyt

Oral
Abstracts
(alphabetically)

Microarray analysis provides unprecedented insight into the detailed physiology of reproductive and non-reproductive honeybee workers

Dries Cardoen¹, Uli Ernst^{1,3}, Liliane Schoofs¹, Dirk de Graaf², Tom Wenseleers³, Peter Verleyen¹

¹ Research Group of Differential Genomics and Proteomics, K.U.Leuven, Naamsestraat 59, 3000 Leuven, Belgium; dries.cardoen@bio.kuleuven.be

² Laboratory of Zoophysiology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

³ Laboratory of Entomology, K.U.Leuven, Naamsestraat 59, 3000 Leuven, Belgium

The success of a honeybee colony depends largely on the reproductive altruism of the worker bees, which usually remain sterile, and instead help their mother queen to reproduce. As yet, little is known about the physiological basis of this spectacular form of altruism. In this study, we compared gene expression patterns among whole bodies of 16 laying and 16 non-laying 18-day old honeybee workers using a whole-genome microarray. Our results demonstrate that there were massive differences in gene expression between these two sets of workers, with a total of 1292 genes being differentially expressed, of which 740 being up-regulated in fertile workers and 552 being up-regulated in sterile workers. GO enrichment analysis demonstrated that ca. one quarter of all the GO-terms enriched in egg-laying workers were linked to oogenesis, mitosis or meiosis, whereas the GO-terms enriched in non-laying workers were associated with muscle contraction, increased metabolism and flight behavior. Interestingly, these results therefore suggest that non-laying workers were foraging, whereas laying workers of the same age were not, in accord with theoretical predictions that reproductive workers should tend to carry out less work and less risky tasks inside the colony. In addition, we also discovered several genes of interest involved in odorant reception, steroid synthesis, neuropeptide signaling and many others. Several of these genes of interest are situated within the region of previously identified QTLs linked to differences in worker reproductive capacity in honeybees. Overall, our results provide unprecedented insight into the detailed physiology of non-reproductive honeybee workers.

Remebee – RNAi based product-line targeting major culprits in beekeeping

Gal Yarden¹

¹ Beeologics, EVP Business Development; www.beeologics.com; gal@beeologics.com

Three new RNAi-based applications for honeybee are presented by Beeologics for specific, safe, naturally occurring based compounds and efficient solutions for the major culprit and pathogens including: viruses, Nosema and Varroa mite. The use of naturally occurring and target specific biotechnology called RNAi, has brought a new hope for the beekeeping industry after years of colony loss.

Beeologics has established a simple and relatively inexpensive procedure to produce large quantities of dsRNA homologous to target pest or pathogen sequences. Remebee™ is the company's leading RNAi product produced in-vitro which is homologous to honeybee viral sequences. The exogenously supplied Remebee mimics the natural mechanism involved in viral replication, within the honeybee cells. In large scale field trials, the gene silencing mechanism induced by Remebee that was fed to the bees had shown to be highly effective in preventing honeybee mortality from the Israel Acute Paralysis Virus (IAPV).

The Colony Collapse Disorder (CCD) phenomenon is still not fully understood or agreed upon; however, there is a strong consensus that pathogens and pests are major contributing factors to Colony losses. Viruses, microsporeidia such as the Nosema ceranea and the Varroa mite are considered the top three pathogenic contributors to the phenomena. Beeologics has developed a generic technology platform which is utilized to introduce a full RNAi product-line targeting all three culprits.

The uniqueness of RNAi enables scientists to develop silencing strategies with new organisms. Basic elements of the new products' design including: mechanism of action proof of action, regulation, ongoing development and dissemination will be discussed.

Oral presentation at Bee-together, Ghent, 21 December 2010

MLPA[®] as diagnostic tool for the honey bee viruses

Lina De Smet and Dirk C. de Graaf

Lab. Zoophysiology, Ghent University, Ledeganstraat 35, B-9000 Gent, Belgium;
lina.desmet@ugent.be; dirk.degraaf@ugent.be

Insects, with solitary and social bees as most important group, play a crucial role in the reproduction of many crops and wild plants. Unfortunately, beekeeping is a declining industry. One of the principal reasons for the decline in managed honeybee colonies is extensive and unpredictable colony death. These losses are defined by a rapid loss of adult worker bees in colonies and the lack of apparent symptoms, leading to the nebulous label of ‘Colony Collapse Disorder’ (CCD). When an apiculturist identifies symptoms at the colony level, and then starts diagnostic procedures to identify the disease and initiate a treatment, it often comes too late to save or cure the colony. Consequently, there is a clear need for fast, reliable, sensitive and cheap diagnostic tools that alert the beekeeper to potential problems before colony level symptoms appear.

The aim of the BEEDOC DIAGNOSTIC DEPARTMENT is to develop diagnostic tools which detect for pathogens and pesticides exposure. These will cover three levels of application: research grade; extension grade and field grade. For the research grade diagnostic tool we will develop a DNA chip for rapid screening of gene expression related to honeybee detoxification, nutritional and immune status and to a broad range of pathogens. We will develop a colorimetric DNA chip to avoid the need for expensive instruments such as a laser scanner. The extension grade diagnostic tool will be based on PCR. Here we would like to develop a multiplex ligation-dependent probe amplification (MLPA[®]) based method. This method makes a nucleic acid sample suitable for a multiplex PCR reaction, in which up to 45 specific nucleic acid sequences are amplified simultaneously, using a single PCR primer pair. The third assay which will be developed is a field grade diagnostic tool such as dip-and-read-sticks which will be based on qualitative immunochromatography. The last assay will be developed in collaboration with VITA[®].

We were able to develop an MLPA diagnostic tool for the detection of 10 viruses. The genome sequences from some viruses are related to be detected in a specific manner and therefore in these cases we decide on the development of family probes which detect a group of viruses. We were able to design a specific probe to detect Acute Bee Paralysis Virus, Chronic Bee Paralysis Virus, Black Queen Cell virus, Slow Bee Paralysis Virus and Sacbrood Virus. A probe for the detection of the family including Acute Bee Paralysis Virus, Israeli Acute Paralysis Virus and Kashmir Virus and one for the detection of the family including Deformed Wing Virus, Kakugo Virus and Varoa Destructor Virus was designed. In a first attempt using the different probes we were able to detect viruses in positive controls and it seems that the probes are specific and do not show cross reaction.

Evidence for specialisation in worker policing in the honeybee *Apis mellifera*

Uli Ernst^{1,4}, Dries Cardoen¹, Francis Ratnieks², Dirk de Graaf³, Liliane Schoofs¹, Peter Verleyen¹, Tom Wenseleers⁴

¹ Research group of Functional Genomics and Proteomics, Department of Biology, K.U.Leuven, Naamsestraat 59, B-3000 Leuven, Belgium; uli.ernst@bio.kuleuven.be

² Department of Biology and Environmental Science, University of Sussex, Sussex House, Brighton, BN1 9RHUK, United Kingdom

³ Department of Sciences, Laboratory of Zoophysiology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

⁴ Laboratory of Entomology, Department of Biology, K.U.Leuven, Naamsestraat 59, 3000 Leuven, Belgium

Theory predicts that there is potential for conflict over male parentage in honeybees (*Apis*). Indeed, even in queenright colonies, some workers are laying male eggs. However, most of these eggs are removed by other workers, which is called worker policing. Due to highly efficient policing in which over 99% of the worker-laid eggs are killed, the incentive for workers to lay eggs in the presence of the queen is small, and only few workers have activated ovaries under normal circumstances. The evolutionary reasons behind worker policing are well understood: ultimately, workers benefit more from raising brothers (sons of the mother-queen) than sons of half-sisters. Yet, relatively little detailed behavioural data is available; for instance, it is not known which bees are removing worker laid eggs. Here, we provide more insight into the organisation of worker policing, by following individually marked bees inside an observation hive. We show that policing workers vary in age between 10 and 36 days with an average of 21 days. The large variation in the amount of eggs removed indicates a specialisation of some individuals in policing. Genetic analyses reveal that bees from different patrines differ also in the amount of policing, again suggesting that there is specialisation in worker policing in honeybees.

Oral presentation at Bee-together, Ghent, 21 December 2010

Born in an alien nest: how do cuckoo bumblebee offspring avoid host recognition?

Patrick Lhomme, Pierre Rasmont

Laboratory of Zoology, University of Mons, Place du Parc 20, 7000 Mons, Belgium;
patrick.lhomme@umons.ac.be; pierre.rasmont@umons.ac.be

In social insects, the high energetic costs of brood care have promoted the evolution of cheaters that exploit worker force of conspecifics or heterospecifics. In bumblebees, all the species of the subgenus *Psithyrus* have lost their worker caste and are completely dependent on hosts to produce their sexuals. One of the most striking challenges for these social parasites is to avoid the detection and rejection by their hosts. Many studies have shown how the *Psithyrus* queens overcome host recognition systems to enter successfully into host colonies. Once a social parasite has successfully usurped a host nest, its emerging offspring still face the same challenge of avoiding host recognition. We assume that cuckoo bumblebee females might camouflage themselves by decreasing pheromonal glands production and acquiring host nest odor. However young males already produce high amounts of species specific sex pheromones even at emergence. Host workers might be able to recognize them. Therefore, *Psithyrus* males require another strategy to prevent host agonistic behaviors. The aim of this study is to find how males of the cuckoo bumblebee *Bombus vestalis* fool *Bombus terrestris* workers during their intranidal life, using behavioral recognition assays and chemical analyses of their sex pheromones. Our results show that *B. vestalis* males seem to produce a repellent allomone to protect them from attacking host workers.

Oral presentation at Bee-together, Ghent, 21 December 2010

Molecular detection of bumblebee parasites and pathogens

Ivan Meeus¹, Dirk C. de Graaf², Guy Smagghe¹

¹ Lab of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure 653, 9000 Ghent Belgium; ivan.meeus@ugent.be; guy.smagghe@ugent.be

² Lab of Zoophysiology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; dirk.degraaf@ugent.be

Bumblebees are commercially reared and transported worldwide mainly for pollination of greenhouse tomatoes. Pathogen transmission from reared bumblebees to native bumblebees has been reported. The introduction of these non-native pathogenic species is regarded as a significant threat for wild life biodiversity and could thus be contributing to current loss of bumblebees species worldwide. We developed different cost-effective diagnostic techniques for the detection of bumblebee pathogens, essential for rearing facilities and governmental organizations to assure pathogen-free bumblebees by use of thorough sanitary controls.

Physiological constraint in host-plant specialization among specialist and generalist wild bees

Denis Michez¹, Maryse Vanderplanck¹, Pierre Rasmont¹, George Lognay²

¹ Université de Mons, Laboratoire de Zoologie, Place du parc 20, 7000 Mons, Belgium; e-mail: denis.michez@umons.ac.be

² Université de Liège, Gembloux agro-bio tech, Département de chimie analytique, Place des déportés, 5030 Gembloux, Belgium

Females of bees do not forage randomly on flowering plants. Specialized behaviors have been selected in social and solitary species to optimize foraging on suitable pollen. In social species like honeybees and bumblebees, host-plant specialization is often observed at individual, temporal, geographical and/or generational scale. Specialist behaviors (called *floral constancy*) result here from individual learning abilities. But in many solitary species like melittid bees, pollen specialization is innate and inherited in all individuals. The taxonomic fidelity of females (called *oligolecty*) is constant throughout their life, their geographic range and across generations. Many physiological and morphological adaptations to oligolecty have been described but constraints and ultimate causes of this kind of host-plant specialization are still poorly understood. More than selection for optimal foraging, oligolecty could result from the need of peculiar chemicals (sterols, proteins, ...) in pollen which reduce the range of suitable hosts. We present here our first analyzes of pollen composition of some common bee host-plants and their likely impact on bee development.

***Bombus terrestris* as entomovector in greenhouse strawberries**

Veerle Mommaerts¹, Kurt Put², Guy Smagghe^{1,3}

¹ Department of Biology, Faculty of Sciences, Free University of Brussels, Pleinlaan 2, Belgium; veerle.mommaerts@vub.ac.be

² Biobest NV, Ilse Velden 18, Westerlo, Belgium; kurt.put@biobest.be

³ Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium; guy.smagghe@ugent.be

Bumblebees are widely used as commercial pollinators but might also fulfil a role as entomovector against economical important crop diseases. The present study investigated the capacity of this technology with *Bombus terrestris* as vector to reduce grey mould *Botrytis cinerea* incidence in greenhouse strawberries. Hereto flowers were manually inoculated with the plant pathogen *B. cinerea* and a model microbiological control agent (MCA) Prestop-Mix and Prestop-Mix combined with Maizena-Plus (corn starch) were loaded in a newly developed two-way bumblebee dispenser. Major results were as follows. First, the loading of the MCA caused no adverse effects towards bumblebee (survival and foraging activity). Second, vectoring of Prestop-Mix by bumblebees resulted in a higher crop production as 71% of the flowers developed into healthy red strawberries at picking (pre-harvest yield) compared to 54% in the controls. Also, strawberries were better protected as 79% of the picked berries remained free of *B. cinerea* after a 2-day-incubation (post-harvest yield), while this was only 43% in the control. Furthermore, the total yield (pre-harvest x post-harvest) was 2-2.5 times higher in plants exposed to bumblebees vectoring Prestop-Mix than in the controls (24%). Third, the addition of the diluent Maizena-Plus to Prestop-Mix at 1:1 (w/w) resulted in a similar yield to that of Prestop-Mix used alone and in no negative effects on the bumblebees, flowers and berries.

In conclusion, this greenhouse study provides strong evidence that *B. terrestris* bumblebees can vector a MCA to reduce *B. cinerea* incidence in greenhouse strawberries, resulting in higher yields.

The effect of age, task and insecticides on the *foraging* gene expression in the bumblebee *Bombus terrestris*

Julie Tobback¹, Veerle Mommaerts², Hans Peter Vandersmissen¹, Guy Smagghe^{2,3}, Roger Huybrechts¹

¹ Research group Insect Physiology and Molecular Ethology, Department of Biology, Faculty of Sciences, KULeuven, Belgium; julie.tobback@bio.kuleuven.be; hanspeter.vandersmissen@bio.kuleuven.be; roger.huybrechts@bio.kuleuven.be

² Department of Biology, Faculty of Sciences, Free University of Brussels, Belgium; vmommaer@vub.ac.be;

³ Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium; guy.smagghe@ugent.be;

The *foraging* gene, encoding a cGMP dependent protein kinase, has been found to be involved in age-dependent division of labour in social insects. In contrast, task allocation in the bumblebee *Bombus terrestris* is mainly determined by size. Our results confirm the existence of a *foraging* homologue and a high conservation amongst insect PKGs. Quantitative real time PCR studies revealed a higher expression in foraging worker bees as compared to nursing bees. More importantly, in both nurses and foragers, *Btfor* expression decreases with age suggesting a role for PKG in memory and learning behaviour in addition to a regulatory function in division of labour. In a supplementary experiment, the possible effect of the neonicotinoid insecticide imidacloprid and the JH analogue kinoprene, both known to be harmful for bumblebees, was tested. Imidacloprid had no effect on *Btfor* gene expression, while the lower expression in kinoprene treated workers concurs with a stimulation of the ovarian growth.

Oral presentation at Bee-together, Ghent, 21 December 2010

Vitellogenin, *Varroa destructor*, pollen flow and honey bee (*Apis mellifera* L.) colony development

J. van der Steen, B. Cornelissen, P. Hendrickx, T. Blacquièrè

Plant Research International, P.O. box 16, 6700 AA Wageningen, the Netherlands

The impact of varroa and pollen flow on hemolymph vitellogenin was studied on colony level. It was demonstrated that the number of mites in the August-September period, negatively impacts the fraction hemolymph vitellogenin. Colonies which had an impeded pollen flow from June till August also had a significantly reduced fraction hemolymph vitellogenin. The fraction vitellogenin of the total hemolymph protein appeared to be a more robust fitness parameter than hemolymph vitellogenin titers expressed as μg vitellogenin per μl hemolymph. In colonies which had, due to impeded or non impeded pollen flow, significant different fraction hemolymph vitellogenin in September, no quantitative differences expressed as number of bees and sealed brood cells, were recorded. It is conceivable that colonies that have a mean relatively low fraction hemolymph vitellogenin, have less chance to overwinter, particularly if poor pollen flow and inadequate varroa control are coupled.

Mass spectrometry as a powerful tool for Hymenoptera venom analysis

Matthias C. Van Vaerenbergh¹, Bart Devreese², Dirk C. de Graaf¹

¹ Department of Physiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium; matthias.vanvaerenbergh@ugent.be

² Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium, 9000 Ghent, Belgium.

Venoms are highly complex mixtures containing up to several hundred compounds including proteins, peptides or small organic compounds. The highly diverse biological functions of these venom compounds explain the often high toxicity and different functions (predation, defense or parasitism) of these venoms. An increasing number of venom screening programs is done to extend knowledge of the venom composition. These often rely on a combination of sample extraction, electrophoresis, reversed phase high performance liquid chromatography (RP-HPLC), mass spectrometry (MS), Edman degradation and/or molecular cloning. The Laboratory of Zoophysiology in cooperation with the Laboratory for Protein Biochemistry and Biomolecular Engineering (L-PROBE) already put effort in Hymenoptera venom research in the past and will continue this research. In 2005, 3 new honeybee venom compounds were discovered by separation of pure honeybee worker venom using 2-DE followed by MALDI-TOF/TOF MS of excised protein spots. Using the same technology, a proteomic analysis of the honeybee worker venom gland was accomplished, which revealed multiple components with a possible function in tissue damage protection. Several other components were discovered with no clear function in the honeybee venom gland. Recently, the availability of Fourier transform mass spectrometry (FTMS) gave us insights into the venom composition of the ectoparasitoid wasp *Nasonia vitripennis*: 79 proteins were identified and half of them were not yet associated with insect venoms. This high complexity might be assigned to the function of the venom which contributes to influencing the host's physiology.

As FT-MS provides the highest performance in mass resolution and mass accuracy, this technology is very useful in analyzing complex mixtures like venom. In present research we focus on gathering further information about honeybee and bumblebee worker venom using this technique. Major pitfalls in proteomic profiling such as the masking of low abundant proteins by high abundant proteins and high heterogeneity in proteomes due to isoforms will be tackled. Further research on the allergic potential or function of new discovered venom compounds can be important for allergy diagnosis/treatment or other biomedical applications.

Optimizing biocontrol and pollination services through informed landscape management

Felix Wäckers ^{1,2}

¹Biobest, Westerlo, Belgium; felix.wackers@biobest.be

²Centre for Sustainable Agriculture, Lancaster University, Lancaster, UK; f.wackers@lancaster.ac.uk

Insect predators and pollinators provide valuable ecosystem services that help secure the production of safe and healthy food. However, in agro-ecosystems the effectiveness of both pollinators and predators can be severely constrained by a range of factors including the lack of alternative prey, floral resources, and suitable overwintering sites.

In our work we seek to identify such bottlenecks and subsequently develop strategies to remove or minimize these impediments (Olson and Wäckers 2007). Recently, developed tools allow us to better investigate energetic reserves, feeding history, reproductive success and age structure of field collected insects (Steppuhn and Wäckers 2004; Konrad et al., 2009). On the basis of this information we can develop targeted landscape management tools that are effective in supporting specific groups of beneficials.

In my presentation I will touch upon the mechanistic studies that have provided the basis of our landscape management projects in the Netherlands and the UK. Subsequently, results from the large-scale field studies with commercial growers will be presented. While these field studies demonstrate that it is possible to support beneficial arthropods through targeted use of non-crop vegetation, possible pitfalls are addressed as well. Finally, I will argue that it is possible to combine and optimize joint biocontrol and pollination benefits through an informed selection of floral resources and a rational design of agro-ecosystems.

Konrad R, Wäckers FL, Romeis J, Babendreier D (2009) Honeydew feeding in the solitary bee *Osmia bicornis* is a function of aphid species and nectar availability. *Journal of Insect Physiology* 55:1158-1166

Olson D, Wäckers FL (2007) Management of field margins to maximize multiple ecological services. *Journal of Applied Ecology* 44:13-21

Steppuhn A, Wäckers FL (2004) HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. *Functional Ecology* 18:812-819

Wäckers FL, van Rijn PCJ, Bruin J (eds) (2005) *Plant-Provided Food for Carnivorous Insects: a Protective Mutualism and its Applications*. Cambridge University Press, Cambridge

Poster
abstracts
(alphabetically)

Bumblebee decline and parasite invasion: a survey of parasite prevalence in bumblebees

Marina Arbetman¹, Ivan Meeus², Hande Bayraktar², Carolina Morales¹, Marcelo Aizen¹, Guy Smagghe²

¹ Lab Ecotono, Universidad Nacional del Comahue, Quintral 1250, 8400 Bariloche, Argentina; marbetman@gmail.com

² Lab of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure 653, 9000 Ghent Belgium; ivan.meeus@ugent.be

Bombus dahlbomii experienced strong declines over the past 15 year in North West Patagonia (Argentina). Although agriculture intensification has been considered as the main driver for global bumblebee declines this can not explain the declines happening in the large national park Nahuel Huapi. Recently the European bumblebee *Bombus terrestris* has invaded the habitat of *B. dahlbomii* and is locally blamed for current losses. In this project we examined one of the possible drivers of these declines, transmission of pathogens from invaded toward native species. Although we can not prove spillover has occurred, the invaded species *B. terrestris* carries multiple parasites and is a potential risk for *B. dahlbomii*.

A survey of parasite prevalence in wild bees

Ivan Meeus¹, Hande Bayraktar¹, Dries Laget², Guy Smagghe¹

¹ Lab of Agrozoology, Ghent University, Coupure 653, 9000 Ghent Belgium; ivan.meeus@ugent.be

² Department of Physiology, Faculty of Sciences, Ghent University, B-9000 Ghent, Belgium; dries.laget@ugent.be

The importance of pollinators for ecosystem integrity and food security has been repeatedly stressed in recent years. While evidence for a global pollination crisis has been questioned, there is no doubt that pollinators themselves are in crisis. Honeybee mortality has been linked with different pathogens, like the invasive *Varroa destructor* and associated viruses and the newly emergent microsporidian *Nosema ceranae*. Furthermore different protozoan parasites of bumblebees have strong effects on colony fitness. However, other often neglected pollinators are also in danger. An exploratory survey of protozoan parasites in solitary bees revealed a new slowly evolving trypanosome infecting *Osmia cornuta* and that solitary bees could function as a reservoir species for *Apicystis bombi* a parasite which is believed to kill most infected bumblebee queens.

Identification of sublethal effects of crop protection products on honey bees (*Apis mellifera*) and their impact on bee behavior and colony health

T. Beliën¹, J. Kellers¹, K. Heylen², J. Billen², L. Arckens², R. Huybrechts², B. Gobin^{1,*}

¹ Zoology Department, PCFruit, Fruittuinweg 1, B-3800 Sint-Truiden, Belgium

² Zoological Institute, KULeuven, Naamsestraat 59, B-3000 Leuven, Belgium

* Present address: PCS Research Centre for Ornamental Plants, Schaessestraat 18, B-9070 Destelbergen, Belgium

Bee pollination is essential for the production of a variety of agricultural crops, including pome fruit. However, bee populations suffered significant declines in recent years, and the extensive use of crop protection products is considered by many beekeepers to be at least partially responsible for it. Although application of bee-toxic pesticides is not allowed during flowering, toxic products applied in the pre-flowering period and non-lethal products during flowering still could cause sublethal effects to occur. We have conducted a four-year study in which a multidimensional approach was followed to identify and evaluate such side effects. Herein we combined molecular (functional genomics/proteomics) and histological techniques, as well as behavioural studies, in order to identify molecular and/or physiological determinants associated with behavioural effects induced by sublethal intoxications. During the last phase of this project, we intensively monitored a set of bee hives that were contaminated with sublethal doses of commonly used crop protection agents (active ingredients fenoxycarb, imidacloprid and indoxacarb). Although no direct (short-term) toxic effects were observed (as could be expected from the experimental setup using sublethal intoxications), our data provide evidence for some medium-term detrimental effects, as significant differences for several colony vitality parameters were obtained a couple of weeks after treatment. Based on the outcomes of behavioural studies as well as molecular analyses we postulate that these effects are at least partially caused by the aberrant onset of foraging, i.e. abnormal timing of bees' shift from in-hive nursing activities to outside food gathering activities.

Host-plant specialisation in Colletidae (Hymenoptera, Apoidea): Does physiology interface with ecology?

Maryse Vanderplanck¹, Georges Lognay², Denis Michez¹

¹ Laboratory of Zoology, University of Mons - UMONS, Place du parc 20, B-7000 Mons, Belgium. maryse.vanderplanck@umons.ac.be

² Unit of Analytical Chemistry, University of Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, B-5030 Gembloux, Belgium.

Represented by more than 16,000 described species, bees are the major pollinators of angiosperms in most ecosystems. Since early Cretaceous, they share a long and intimate evolutionary history with flowering plants.

Bees forage on pollen and nectar as the exclusive food source. Interactions between flowering plants and wild bees are highly diverse. Different pollen foraging strategies have been described among bees. Some taxa display floral specificity, restricting their flower visits to closely related plant taxa (pollen specialists) while other bee species are more opportunistic, exploiting a wide range of different flowers (pollen generalists). To characterize different degrees of host-plant associations, different terms were progressively introduced by several authors until better reflect the reality of a continuum in bee host breadth, from extreme specialisation to extreme generalisation.

Ancestral host-plant and specialist behavior seem highly inherited both. However some rare “host-plant shifts” occurred during evolution inside clades. The origin and the mechanism of these host-plant shifts remain misunderstood. They can be based on morphological or phylogenetical similarity with ancestral host-plant but host switches to unrelated plant families are also common. The constraints of these switches are not well known. Because the proportions of nutrients can vary widely among pollens of different plant species, floral specialisations in bees could be constrained by the chemical composition of the pollen, reducing the range of suitable alternative hosts. In particular, sterol metabolism could constitute a dietetic constraint of host plant specialisation in bee evolution (Hymenoptera, Apoidea). Indeed, sterolic compounds are involved in some key metabolism pathways like syntheses of ecdysteroid hormones. As insects do not produce endogenous sterols, they must assimilate them from their food. The understanding of sterol requirements and metabolism in insects would shed light on how insect sterol physiology interfaces with ecology.

Preliminary results in Colletid bees show that in a group of closely related species, sterolic profiles of host plant pollen are similar, corroborating the hypothesis that host plant specialisation and evolutionary shifts could partly be explained by chemical composition of pollen.

The altruistic infertility of honeybees: a physiological approach

Dries Cardoen¹, Uli Ernst^{1,3}, Dirk de Graaf², Tom Wenseleers³, Peter Verleyen¹, Liliane Schoofs¹

¹ Research Group of Functional Genomics and Proteomics, Department of Biology, K.U.Leuven, Naamsestraat 59, B-3000 Leuven, Belgium; dries.cardoen@bio.kuleuven.be

² Laboratory of Zoophysiology, Department of Sciences, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

³ Laboratory of Entomology, Department of Biology, Department of Biology, K.U.Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

The success of a honeybee colony depends largely on the reproductive altruism of the worker bees, which usually remain sterile, and instead help their mother queen to reproduce. As yet, little is known about the genomic basis of this spectacular form of altruism. In this study, 16 sterile and 16 reproductive workers of two colonies were differentially analyzed with microarrays, using whole bodies. 1292 genes were found to be differentially expressed, of which 740 up-regulated in fertile workers and 552 up-regulated in sterile workers. GO-enrichment analysis demonstrated that more than 25% of the GO-terms enriched in reproductive workers are linked to mitosis, meiosis or oogenesis. But interestingly, the enrichment of GO-terms involved in muscle contraction, metabolism, flight behavior etc. in sterile bees indicates that 18-day old sterile workers are clearly foraging, whereas the fertile workers of the same age are not. This is in accord with theoretical predictions that reproductive workers should tend to carry out less work and less risky tasks inside the colony. Particular interesting genes are odorant receptor 156, which has a nearly 2-fold up-regulation in sterile workers, and farnesyl pyrophosphate synthase (juvenile hormone synthesis), odorant binding protein 9 and 7, chemosensory protein 5 which were up-regulated in fertile bees. Several of these candidate genes lie within the region of previously identified QTLs linked to differences in worker reproductive capacity in honeybees. These clues point at physiological pathways starting with chemosensory sensitivity modifying both reproductive and foraging behavior. Overall, our results provide unprecedented insight into the detailed physiology of non-reproductive honeybee workers.

Exploring the functionality of *Nasonia vitripennis*' unknown venom proteins

Ellen L. Danneels, Ellen Formesyn, Frans Jacobs, Dirk C. de Graaf

Department of Physiology, Faculty of Sciences, Ghent University, B-9000 Ghent, Belgium;
ellen.danneels@ugent.be; ellen.eormesyn@ugent.be

Nasonia vitripennis is an ectoparasitoid that uses host flies as a food source for its progeny. The venom of adult females is used to subdue the host and affects the immune responses, physiology and biochemical profile of parasitized flesh flies. Furthermore, venom assays with insect cells revealed that the venom influences the morphology and behavior and evokes apoptotic and/or non-apoptotic programmed cell death. Recently the genome of this wasp was sequenced and an in depth investigation of the venom composition was able to identify 79 venom proteins. The possible functions and interactions of these 79 venom proteins with host pathways remain largely speculative and a subset of 23 venom compounds does not display similarities to any known protein. In order to determine their function, these 23 unknown venom proteins will be recombinantly produced in *E.coli* BL21 (DE3) cells, using a pET 100/D-TOPO expression vector. The purified proteins will then be used in different bioassays including an MTT cell proliferation assay and an NF-kB assay.

Development of multiplex PCR technology with microsatellite primers to study genetic structure of bumblebee populations.

Kevin Maebe, Ivan Meeus, Guy Smagghe

Lab of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure 653, 9000 Ghent Belgium; kevin.maebe@ugent.be

Gene flow and dispersal are important biological parameters that determine how species cope with climatic change and habitat fragmentation. Reduced genetic diversity lowers the capacity of a population to respond to environmental change, and may lead to inbreeding depression caused by the expression of deleterious alleles. To estimate these effects in bumblebees, a group of key pollinators in decline, we developed a multiplex PCR of microsatellite DNA markers. Knowledge of gene flow isolation is essential toward predicting local needs for bee-friendly habitat for the conservation of endangered species of the genus *Bombus*.

The selection of microsatellite primers was based on: originating from different linkage groups, highest polymorphism degree, effectiveness in multiple species and primer compatibility. The development of a multiplex PCR, several separate PCR reactions combined in a single reaction, is a considerable saving in costs and time, making this technique suitable for large surveys.

A laboratory assay to evaluate sublethal effects on the foraging behaviour of the pollinator *Bombus terrestris*

Veerle Mommaerts¹, Guido Sterk², Guy Smagghe^{1,3}

¹ Department of Biology, Faculty of Sciences, Free University of Brussels, Pleinlaan 2, Belgium; veerle.mommaerts@vub.ac.be

² Biobest NV, Ilse Velden 18, Westerlo, Belgium

³ Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Belgium; guy.smagghe@ugent.be

Species belonging to the family of the Apidae such as the bumblebee *Bombus terrestris* are widely used for the pollination of greenhouse crops. However, to obtain satisfactory yields growers need to protect their crops against plant diseases and pests who are up until today still mainly controlled by chemical pesticides. Consequently exposure to pesticides during foraging is not unlikely. To assess detrimental effects (lethal and sublethal effects) on the vector following pesticide exposure the classic toxicity test using microcolonies can be applied. However, beside toxicity pesticides are known to induce sublethal effects on the foraging behaviour. In this context, a foraging behaviour test was developed allowed to assess the impact on the foraging activity by use of two endpoints drone production and nest development. Hereto workers were exposed during 9 weeks ad libitum to two sublethal concentrations of the neonicotinoid imidacloprid (10 ppb and 20 ppb) via the drinking of sugar water and to untreated pollen. To reach the food (present in the food compartment), bumblebee workers needed to walk through a tube of 20 cm connecting their nest compartment with the food compartment.

In the nests strong negative effects on the bumblebee reproduction were observed as for a concentration up to 20 ppb the drone production (5.6 ± 0.1) was significantly ($p < 0.05$) lower when compared with 28.4 ± 2.9 in the control group. Moreover observation of these nests revealed that workers had difficulties in finding their nest and in foraging for food.

In general, this laboratory bioassay demonstrated that concentrations that look safe for bumblebees can have an influence on the foraging behaviour. Therefore it is recommendable that behaviour tests should be included in risk assessments as impairment with the foraging behaviour could result in a decreased pollination, lower reproduction and finally in colony mortality due to a lack of food.

Side-effects of bio-fungicides and bio-insecticides on the pollinator *Bombus terrestris*

Veerle Mommaerts¹, Kris Jans², Guido Sterk², Lucien Hoffmann³, Sarah Labiese⁴, Guy Smagghe^{1,4}

¹ Department of Biology, Faculty of Sciences, Free University of Brussels, Pleinlaan 2, Belgium; veerle.mommaerts@vub.ac.be

² Biobest NV, Ilse Velden 18, Westerlo, Belgium

³ Département Environnement et Agro-biotechnologies (EVA), Centre de Recherche Public - Gabriel Lippmann, Rue du Brill 41, Luxembourg; hoffmann@lippmann.lu

⁴ Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Belgium; guy.smagghe@ugent.be

The study was initiated to search for potential adverse side-effects of a selection of seven microbiological control agents (MCAs) on the bumblebee *Bombus terrestris* L. in the frame of a combined use in IPM. We investigated AQ10 (*Ampelomyces quisqualis*), Binab-T-vector (*Trichoderma atroviride* and *Hypocrea parapilulifera*), Botanigard (*Beauveria bassiana* GHA), Prestop-Mix (*Gliocladium catenulatum* J1146), Trianum-P (*Trichoderma harzianum* T22), Serenade (*Bacillus subtilis* QST713) and Granupom (*Cydia pomonella* granulovirus), containing fungi, bacteria and viruses. Hereto, bumblebee workers were essentially exposed under laboratory conditions to each MCA at its respective maximum field recommended concentration (MFRC) via three different routes of exposure: dermal contact and orally via the drinking of treated sugar water and pollen.

The tested MCAs were found safe for workers of *B. terrestris*, with the exception of Botanigard and Serenade. Exposure to Botanigard via contact at its MFRC caused 92% mortality after 11 weeks and at 1/10 MFRC this was still 46%. For Serenade, topical contact and drinking sugar water resulted in 88% and 100% worker mortality, respectively. With lower concentrations (1/2, 1/5 and 1/10 MFRC) the toxicity decreased, but the effect depended on the route of exposure. Next to acute toxic effects, nests were also evaluated for sublethal effects after treatment with the seven MCAs at their respective MFRC. In these bioassays, only Botanigard and Serenade provoked a significant ($p < 0.05$) decrease of drone production during 11 weeks. Besides sublethal effects on foraging behaviour were evaluated, and here, only with Botanigard at its MFRC via drinking of treated sugar water there were negative effects.

The results demonstrated that most of the MCAs tested can be considered as safe to be combined with *B. terrestris*, but some can be harmful, and therefore it is recommendable that all should be tested before use in combination with pollinators.

Advancing Bee Taxonomy in Sub-Saharan Africa through *Abc Taxa* and Global Taxonomic Initiative on wild bees in Ethiopia and Burundi

Connal Eardley^{1,2}, Michael Kuhlmann³, Alain Pauly⁴, Yves Samyn⁵

- 1) Agricultural Research Council, Private Bag X134, Queenswood, 0121, Pretoria,
- 2) University of KwaZulu-Natal, P.O. Box X01, Scottsville, Pietermaritzburg, 3209, South Africa
Email: EardleyC@arc.agric.za
- 3) The Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom; Email: m.kuhlmann@nhm.ac.uk
- 4) Entomology Department, Royal Belgian Institute of Natural Sciences, Rue Vautier 29, B-1000 Brussels, Belgium; Email: alain.pauly@brutele.be
- 5) Belgian Focal Point to the Global Taxonomy Initiative (*Abc Taxa* Office) Rue Vautier 29, B-1000 Brussels, Belgium; Email: yves.samyn@naturalsciences.be

For the first time the full diversity of bee genera of Subsaharan Africa is summarized in a book published in the ABC Taxa serie. All genera are reproduced in color, significantly aiding their recognition. Keys are provided for each family. This guide will open the gates to new generations of bee research, improving efforts in documenting diversity, elucidating biologies and ecological associations, establishing conservation practices and policies, and informing the wise development of agriculture throughout Africa. The book is written in English and has now a French version.

In parallel, two projects on wild bee biodiversity are launched by Belgium for the Global Taxonomic Initiative. They are located in Ethiopia and Burundi. The first results of the Ethiopian project show a high diversity of endemic new species.

Do *Aconitum* plants protect their pollen against herbivory?

Dorothee Roelants¹, G. Glauser², Pierre Rasmont¹

¹ Laboratory of Zoology, University of Mons, Place du parc 20, B-7000 Mons, Belgium; dorothee.roelants@umons.ac.be

² Institute of Biology, University of Neuchâtel, CH-2000 Neuchâtel, Switzerland

Chemical protection is a widespread phenomenon in plants that develops under pathogen and herbivore pressures through plant evolution. The molecules involved are secondary metabolites that are found in key organs like roots, seeds and flowers. These compounds are highly diversified among plant groups. The *Aconitum* genus (Ranunculaceae) includes more than 250 toxic species also known as monkshoods with regards to their floral morphology. The toxicity derives from polycyclic diterpene alkaloids that are known to be neurotoxic. The most abundant compound is aconitine but a lot of other aconitine-type alkaloids are also present in plant tissues. Despite their high toxicity, the monkshoods are exclusively pollinated by bumblebees.

In this study, we focus on an oligolectic species highly specialized on *Aconitum* sp.: *Bombus consobrinus*. This long-tongued subalpine bee is able to use nectar and pollen of *Aconitum septentrionale* as main source of food for their colonies. We developed a UHPLC method to extract, identify and quantify the aconitine-type alkaloids from the pollen and nectar of *A. septentrionale*. For the first time, alkaloids were detected in these tissues and, surprisingly, pollen contained high levels of these potential toxic molecules. Chemical protection of the pollen could be the result of the herbivory pressure. Moreover, we hypothesize that these alkaloids could provide protection against micro-organism and/or predator attacks to the bumblebees. From the plant point of view, this intimate association could specialize pollinators to improve pollination efficiency.

Parameters of bumblebee foraging/feeding behaviour for sugar water in a two-way choice assay and relation with its nutrient value

Ellen Van Campenhout¹, Veerle Mommaerts¹, Kris Jans², Guy Smagghe^{1,3}

¹ Department of Biology, Faculty of Sciences, Free University of Brussels, Pleinlaan 2, Belgium; ellenvancampenhout@hotmail.com; veerle.mommaerts@vub.ac.be

² Biobest NV, Ilse Velden 18, Westerlo, Belgium

³ Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Belgium; guy.smagghe@ugent.be

It is a common knowledge that bumblebees depend on nectar (sugar source) as energy source, providing thermal energy which in turn allows to heat the brood and to regulate their own body temperature. The two main objectives of this study were (1) to determine different parameters to describe the foraging and feeding behaviour of individual bumblebees of *Bombus terrestris* for common sugar water in a laboratory two-way choice bioassay, and then (2) to evaluate whether there exists a correlation with the nutritive value of the sugar source.

In the laboratory, bumblebee workers were selected from a queen-right colony for a two-way choice experiment with a sugar water solution as commonly used for bumblebee rearing and that consists of fructose:glucose:saccharose (1:1:1). The setup consisted of a microcolony nest equipped with a T-tube with two falcon tubes. Under these conditions, we recorded different parameters involved in foraging and feeding behaviour as the time period to make the choice for foraging from the nest to the food, and the duration and frequency of drinking. Interestingly, the mean feeding period was 226 s. In a second series, the nutritional value of sugar waters was determined as the survival of bumblebee workers that were kept individually and allowed to feed on two 50 µl-droplets of a sugar water per day under optimal rearing conditions. With these experimental setups we want to further investigate the gustatory behaviour of pollinators and specifically the impact of different carbohydrates sources, qualities and combinations of sugars, as different nectar flowers, aphid honeydews, etc.

Analysis of the glycoprotein diversity between insect species

Gianni Vandenborre^{1,2}, Els J.M. Van Damme², Bart Ghesquière³, Gerben Menschaert⁴, Rameshwaram Nagender Rao^{1,2}, Kris Gevaert³, Guy Smagghe¹

¹ Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; gianni.vandenborre@ugent.be

² Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

³ Department of Medical Protein Research, VIB, Albert Baertsoenkaai 3, B-9000 Ghent, Belgium

⁴ Laboratory for Bioinformatics and Computational Genomics, Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure links 653, B-9000 Ghent, Belgium

The most occurring post-translational protein modification in multicellular organisms is the addition of carbohydrate structures to the peptide backbone called protein glycosylation. Although insects are without any doubt the largest animal taxon found on Earth, knowledge concerning protein glycosylation in insects is still very limited. Moreover, all information on protein glycosylation known in insects is mainly derived from studies using *Drosophila*.

Here, we present a study that compares the diversity in glycoproteins between insects belonging to several economically important insect orders. Using lectin affinity chromatography based on GNA (*Galanthus nivalis* agglutinin), different sets of glycoproteins with mannosyl-containing glycan structures were purified from the honeybee (*Apis mellifera*; Hymenoptera), the flour beetle (*Tribolium castaneum*; Coleoptera), the silkworm (*Bombyx mori*; Lepidoptera), the fruit fly (*Drosophila melanogaster*; Diptera) and the pea aphid (*Acyrtosiphon pisum*; Hemiptera). Purified glycoproteins were identified using LC-MS/MS analysis and annotated based on the presence of functional protein domains/motives in the peptide sequence using the InterProScan tool. Subsequently, WEGO analysis was performed to classify the different sets of glycoproteins according to biological process or molecular function.

It was clearly demonstrated that the majority of glycoproteins retained on the GNA-affinity column were unique to one particular insect species and only a few glycoproteins were present in the five different glycoprotein sets. In addition, the identified glycoproteins were related to a broad range of biological processes as well as molecular functions. To our knowledge, this is the first report that presents a comparative study of the glycoproteomes present in different insect species.

The abnormal bumblebees scarcity syndrom: Are heat waves leading to local extinctions of bumblebees?

Pierre Rasmont and Stéphanie Iserbyt

Laboratoire de Zoologie, Université de Mons (UMons), B-7000 Mons (Belgium);
pierre.rasmont@umons.ac.be, stephanie.iserbyt@umons.ac.be

It is now well known that a lot of bumblebees species are threatened in Europe and in N. America. Various hypotheses have been proposed to explain the regressions. Some of the hypothetical factors act at a continental level, as the general restructuration of the agriculture toward the use of synthetic nitrogen fertilisation, in place of leguminous. The landscape fragmentation is typically a local factor the spatial coalescence of which leads also to large-scale effects. Since 2002, we observed a great number of situations where local droughts and heatwaves occurred in France, UK, Scandinavia, Turkey, leading to very strong local reductions of the bumblebees fauna. We observed so many local cases in 2007-2009 that we could hypothesise that a coalescence of these local effects could lead to a new general thread. The species with the late phenology should be the most sensitive to this risk.

List of participants

(alphabetically, 15-12-2010)

Name (affiliation) e-mail

1. Baets, Dirk (Bayer) dirk.baets@bayer.com
2. Bayraktar, Hande (UGent) hande.bayraktar@ugent.be
3. Beliën, Tim (Proefcentrum fruitteelt) tim.belien@pcfruit.be
4. Benaets, Kristof (KULeuven) kristof.benaets@bio.kuleuven.be
5. Berx Peter (De Lieteberg) peterberx@telenet.be
6. Blacquièrè, Tjeerd (U.Wageningen, Nederland) tjeerd.blacquièrè@wur.nl
7. Bolckmans, Karel (Koppert) kbolckmans@koppert.nl
8. Bonte, Dries (UGent) dries.bonte@ugent.be
9. Brunain, Marleen (UGent) marleen.brunain@ugent.be
10. Bryon, Astrid (UGent) astrid.bryon@ugent.be
11. Cardoen, Dries (KULeuven) dries.cardoen@bio.kuleuven.be
12. Christiaens, Olivier (UGent) olchrist.christiaens@ugent.be
13. Coppée, Audrey (Université de Mons) audrey.coppee@umons.ac.be
14. Cornette, Vincent (KULeuven) vincent.cornette@student.kuleuven.be
15. Danneels, Ellen (UGent) ellen.danneels@ugent.be
16. De Clercq, Patrick (UGent) patrick.declercq@ugent.be
17. de Graaf, Dirk (UGent) dirk.degraaf@ugent.be
18. De Koker, Dieter (UGent) dieter.dekoker@ugent.be
19. De Loof, Arnold (KULeuven) arnold.delooft@bio.kuleuven.be
20. De Smet, Lina (UGent) lina.desmet@ugent.be
21. D'Haeseleer, Jens (Dienst Natuurpunt.Studie) jens.dhaeseleer@natuurpunt.be
22. Dierickx, leen (UGent) leen.dierickx@ugent.be
23. Dijkstra, Jelte Pieter (UAntwerpen) jeltapieter.dijkstra@ua.ac.be
24. Dubois, Cindy (Provinciale Hogeschool Limburg) cindy.dubois@mail.phl.be
25. Ernst, Uli (KULeuven) uli.ernst@bio.kuleuven.be
26. Formesyn, Ellen (UGent) ellen.formesyn@ugent.be
27. Hamshou, Mohamad (UGent) mohamad.hamshou@ugent.be
28. Huvenne, Hanneke (UGent) hanneke.huvenne@ugent.be
29. Huybrechts, Roger (KULeuven) roger.huybrechts@bio.kuleuven.be
30. Iserbyt, Stéphanie (Université de Mons) stephanie.iserbyt @umons.ac.be
31. Jacques, Yann (Biobest) yann@biobest.be
32. Jans, Kris (Biobest) kris.jans@biobest.be
33. Janssen, Tom (KULeuven) tom.janssen@bio.kuleuven.be
34. Kellers, Jeroen (Proefcentrum fruitteelt) jeroen.kellers@pcfruit.be
35. Keppens, Willem (Biobest) willem.keppens@biobest.be
36. Labiese, Sarah (UGent) sarah.labiese@ugent.be
37. Laget, Dries (UGent) dries.laget@ugent.be
38. Leistra, Paul (Koppert) pleistra@koppert.nl
39. Lhomme, Patrick (Université de Mons) patrick_lhomme@hotmail.fr
40. Liu, Jisheng (UGent) jisheng.liu@ugent.be
41. Lognay, Geroges (Université de Liège-Gembloux Agro Bio Tech) georges.lognay@ulg.ac.be
42. Maebe, Kevin (UGent) kevin.maebe@ugent.be
43. Meeus, Ivan (UGent) ivan.meeus@ugent.be
44. Michez, Denis (Université de Mons) denis.michez@umons.ac.be

45. Mommaerts, Veerle (VUBrussel) veerle.mommaerts@vub.ac.be
46. Nagaba, Yoshiaki (Kobe University, Japan; KULeuven)
47. Nieberding, Caroline (UCLouvain) caroline.nieberding@uclouvain.be
48. Pauly, Alain (Royal Belgian Institute of Natural Sciences) alain.pauly@brutele.be
49. Rasmont, Pierre (Université de Mons) pierre.rasmont@umons.ac.be
50. Roelants Dorothee (Université de Mons) dorothee.roelants@umons.ac.be
51. Roels, Astrid (Taminco) astrid.roels@taminco.com
52. Schoofs, Liliane (KULeuven) liliane.schoofs@bio.kuleuven.be
53. Smagghe, Guy (UGent) guy.smagghe@ugent.be
54. Staljanssens, Dorien (UGent) dorien.staljanssens@ugent.be
55. Sterk, Guido (IPM Impact) guido.sterk@skynet.be
56. Thys, Tom (Proefcentrum fruitteelt) tom.thys@pcfruit.be
57. Timmermans, Inge (KULeuven) inge.timmermans@student.kuleuven.be
58. Tobback, Julie (KULeuven) julie.tobback@bio.kuleuven.be
59. Toczko, Izabela (Biobest) izabela.toczko@biobest.be
60. Wackers, Felix (Biobest) felix.wackers@biobest.be
61. Van Campenhout, Ellen (VUBrussel) ellen.vancampenhout@vub.ac.be
62. Van de Velde, Didier (UGent) didier.vandavelde@ugent.be
63. Van de Wiele, Tom (UGent) tom.vandewiele@ugent.be
64. van der Steen, Sjef (U.Wageningen, Nederland) sjef.vandersteen@wur.nl
65. van der Veken, Lieselot (Biobest) lieselot@biobest.be
66. Van Dyck, Hilde (Bayer) hilde.vandyck@bayer.com
67. van Heesch, Nieke (U.Wageningen, Nederland) nienke.vanheesch@wur.nl
68. Van Huylenbrouck, Guido (UGent) guido.vanhuylenbrouck@ugent.be; decaan.fbw@ugent.be
69. Van Isacker, René (Koppert) vanisacker@skynet.be
70. Van Steenkiste, Danny (IWT) DVS@iwt.be
71. Van Vaerenbergh, Matthias (UGent) matthias.vanvaerenbergh@ugent.be
72. Vancaenegem, Hendrik (UGent) hendrik.vancaenegem@ugent.be
73. Vandekerkhove, Björn (UGent) bjorn.vandekerkhove@ugent.be
74. Vanden Broeck, Jozef (KULeuven) jozef.vandenbroeck@bio.kuleuven.be
75. Vandenborre, Gianni (UGent) gianni.vandenborre@ugent.be
76. Vanderplanck, Maryse (Université de Mons) maryse.vanderplanck@umons.ac.be
77. Vanreusel, Wouter (Dienst Natuurpunt.Studie) wouter.vanreusel@natuurpunt.be
78. Wenseleers, Tom (KULeuven) tom.wenseleers@bio.kuleuven.be
79. Verheyen, Kris (UGent) kris.verheyen@ugent.be
80. Verleyen, Peter (KULeuven) peter.verleyen@bio.kuleuven.be
81. Vermeulen, Jan (Biobest) jan.vermeulen@biobest.be
82. Wouters, Filip (KATHO - HIVB campus Roeselare) filip.wouters@katho.be
83. Yarden, Gal (Beeologics) gal@beeologics.com
84. Boot, Willem Jan (Inbuzz, U.Wageningen) willem.boot@wur.nl
85. Calis, Johan (Inbuzz, U.Wageningen) johan.calis@wur.nl

welcome !



Faculteit Bio-ingenieurswetenschappen
Faculty of Bioscience Engineering



Organizer:

Prof. Dr. ir. Guy Smagghe (guy.smagghe@ugent.be)
Faculty Bioscience Engineering, Ghent University, Coupure Links 653,
9000 Ghent, Belgium