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Association of Institutes for Bee Research
Report of the 56th Seminar in Schwerin
24–26 March 2009

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56. Jahrestagung in Schwerin
vom 24–26 März 2009

Association des Instituts de Recherche sur les abeilles
Comptes rendus du 56e Congrès à Schwerin
24–26 mars 2009

List of reports (*after the title indicates that no abstract of this report is published).
Verzeichnis der Referate (*bedeutet, dass zu diesem Titel keine Zusammenfassung aufgeführt ist).
Liste des communications (*après le titre indique que le résumé de la communication n'est pas publié dans ce numéro).

Invited talk
Einführungsvortrag
Conférence inaugurale

1. Cognition, behaviour and ion channels – complex processes in the honeybee brain. *B. Grünewald**
Kognition, Verhalten und Ionenkanäle – Komplexe Vorgänge im Bienengehirn.
Cognition, comportement et canaux ioniques – processus complexes dans le cerveau de l'abeille.

Physiology, behavior
Physiologie, Verhalten
Physiologie, comportement

2. Social parasitism of queens and workers in *Apis mellifera*: A major life history trait. *R.F.A. Moritz*, *H.R. Hepburn**
Sozialparasitismus von Königinnen und Arbeiterinnen bei *Apis mellifera*: ein wichtiges Merkmal der Naturgeschichte.
Le parasitisme social des reines et des ouvrières chez *Apis mellifera* : un caractère important du cycle biologique.

3. Influences of QMP on social structure in groups of queenless bees. *J. Wegener*, *K. Bienefeld**
Einflüsse von QMP auf die Sozialstruktur in Gruppen von weisellosen Arbeitsbienen.
Influences de la phéromone mandibulaire royale (QMP) sur la structure sociale dans les groupes d'abeilles orphelines.

4. Alternative splicing of gemini controls selfish behavior in the honeybee *Apis mellifera*. *A. Jarosch*, *R.F.A. Moritz**
Alternatives splicing des gemini Locus kontrolliert eigennütziges Verhalten der Honigbiene, *Apis mellifera.*
L'épissage alternatif du locus gemini contrôle le comportement égoïste chez l'abeille *Apis mellifera.*

5. Gaps, caps and thermal maps: brood heating in honey bees. *M.A. Becher*, *R.F.A. Moritz**
Lücken, Deckel und thermische Karten: Brutwärmen bei Honigbienen.
Lacunes, opercules et carte thermique : réchauffement du couvain chez les abeilles.

6. Flight weather: weather-dependent activity of bee colonies. *C. Otten**
Flugwetter: witterungsabhängige Aktivität von Bienenvölkern.
Activités de vol liées au climat dans les colonies d'abeilles.

7. Temporal dynamics of an *Apis mellifera scutellata* drone congregation area. *R. Jaffé*, *V. Dietemann*, *R.M. Crewe*, *R.F.A. Moritz**
Zeitliche Dynamik eines Drohnensammelplatzes von *Apis mellifera scutellata.*
Dynamique dans le temps d'un lieu de rassemblement des mâles d'*Apis mellifera scutellata.*

8. Effects of carbohydrate deficiency on the immune competence of bees. *R. Siede*, *M. Meixner*,

R. Büchler
Auswirkungen eines Kohlenhydratmangels auf die Immunkompetenz von Bienen.
Effets d'une carence en carbohydrate sur l'immunité des abeilles.

Pathology - viruses, AFB
Pathologie - Viren, AFB
Pathologie - virus, loque américaine

9. In vivo-model for overt DWV-infections in honeybees. *S. Gisder*, *E. Genersch**
Ein in vivo Modell für overte DWV-Infektionen bei der Honigbiene.
Modèle in vivo des infections dues aux virus des ailes déformées (DWV).

10. Survival thresholds of honey bee drone and worker larvae (*Apis mellifera*) infected with *Paenibacillus larvae*. *D. Behrens*, *E. Forsgren*, *I. Fries*, *R.F.A Moritz**
Überlebensschwellen von Drohnen- und Arbeiterinnenlarven (*Apis mellifera*) nach Infektionen mit *Paenibacillus larvae*.
Seuils de survie des larves de mâles et d'ouvrières d'abeilles (*Apis mellifera*) infectées par *Paenibacillus larvae*.

Pathology - varroosis, etc.
Pathologie - Varroose, u.a.
Pathologie - varroose, etc.

11. Regulation of reproduction of Varroa female by host odors. *R. Odemer*, *E. Frey*, *P. Rosenkranz**
Reproduktionssteuerung bei Varroa-Weibchen durch Wirtsduftstoff.
Régulation de la reproduction chez la femelle de varroa par les odeurs de l'hôte.

12. Is the sexual pheromone of *Varroa destructor* gender-unspecific? *T. Ziegelmann*, *P. Rosenkranz**
Ist das Sexualpheromon bei *Varroa destructor* geschlechtsunspezifisch?
La phéromone sexuelle de *Varroa destructor* est-elle non liée au sexe ?

13. Mapping of genes involved in Varroa resistance in the honeybee. *F.B. Kraus*, *D. Behrens*, *P. Rosenkranz*, *E. Frey*, *D. Kleber*, *R.F.A. Moritz**
Kartierung von Genen der Honigbiene, die die Resistenz gegen Varroa beeinflussen.
La cartographie des gènes impliqués dans la résistance au Varroa chez l'abeille.

14. Further experiments on efficacy of varroa treatment with rotenone in stripes, together with efficacy of alternative summer varroa treatment with preparative KAS-81 and sugar in powder (Istria and Rijeka, Croatia/August and September 2008). *D. Šekulja*
Weitere Experimente zur Wirksamkeit von Rotenone in Streifen und Wirksamkeit alternativer Behandlungen der Varroa mit KAS-81 und Puderzucker während des Sommers (Istrien und Rijeka, Kroatien/August und September 2008).
Expérimentations supplémentaires sur l'efficacité d'un traitement contre le varroa à l'aide de bandes imprégnées de roténone et efficacité du traitement alternatif d'été à base de KAS-81 et de sucre en poudre (Istrie et Rijeka, Croatie/août et septembre 2008).

15. Powerful colonies through the formation of artificial swarms – effect on population dynamic, bee diseases and honey yield. *R. Büchler*
Vitale Völker durch die Bildung von Fluglingen – Auswirkungen auf Volksentwicklung, Krankheitsbefall und Honigertrag.
Colonies fortes obtenues par la formation d'essaims artificiels – effet sur la dynamique des populations, les maladies des abeilles et la récolte de miel.

16. Comparison of beekeeping management techniques using estimates of the population dynamics – the BIV project (1st year). *O. Boecking*, *P. Aumeier*, *G. Liebig**
Betriebsweisenvergleich auf der Basis von Populationsschätzungen – das BIV- Projekt (1. Jahr).
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Pathologie – Nosemose
Pathologie – nosemose

17. Comparative virulence between *Nosema apis* and *Nosema ceranae*. *I. Fries*, *E. Forsgren*
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Comparaison de la virulence de *Nosema apis* et *Nosema ceranae.*

18. To be or not to be? Effects of *Nosema ceranae* infections on honeybees. *P. Aumeier*, *S. Sterner*, *D. de Craigher*, *K. Porbeck*, *C. Schmidt*, *E. Genersch*, *W.H. Kirchner*, *O. Boecking*, *G. Liebig**
Sein oder Nichtsein? Auswirkungen der *Nosema ceranae*-Infektion auf Honigbienen.
Être ou ne pas être ? Effets des infections par

Nosema ceranae sur les abeilles.

19. Brood behavior of bee colonies during fall and Nosema infections. *D. de Craigher*, *G. Liebig**
Brutverhalten von Bienenvölkern im Herbst und Nosemabefall bei Ein- und Auswinterung.
Comportement du couvain dans les colonies d'abeilles infectées par *Nosema* en automne.

20. Nosema infections in drones of DCAs (*Apis mellifera*). *A. Huth*, *R.F.A. Moritz*, *J. Settele**
Nosemainfektionen von Drohnen eines Drohnensammelplatzes.
Infections par *Nosema* chez les mâles d'*Apis mellifera* sur leurs lieux de rassemblement.

Other hymenopterans
Andere Hymenopteren
Autres hyménoptères

21. Microsatellites in three hymenopteran genomes. *E. Stolle*, *H.M.G. Lattorff*, *R.F.A. Moritz**
Microsatelliten von drei Hymenopterengenomen.
Microsatellites dans trois génomes d'hyménoptères.

22. In vitro cultivation of *Crithidia bombi* – a visceral parasite of bumblebees. *M. Popp*, *H.M.G. Lattorff**
Kultivierung von *Crithidia bombi* in vitro – ein Viszeralparasit von Hummeln (*B. terrestris*).
Culture in vitro de *Crithidia bombi* – un parasite intestinal de bourdons.

23. Temporal dynamics of the effective population size in bumblebees (*B.terrestris*). *S. Wolf*, *R.F.A. Moritz**
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Dynamique dans le temps de la taille réelle de la population chez les bourdons (*B. terrestris*).

24. Genetic structure of drone congregations in a neotropical stingless bee, *Scaptotrigona mexicana. M. Müller*, *F.B. Kraus*, *R.F.A. Moritz**
Genetische Struktur von Drohnenansammlungen einer neotropischen stachellosen Biene, *Scaptotrigona mexicana*.
Structure génétique des rassemblements de mâles chez l'abeille sans aiguillon néo-tropicale, *Scaptotrigona mexicana.*

25. Comparative population genetics of host- parasite interactions: bumblebees and their intestinal parasites. *H.M.G. Lattorff*, *S. Erler*, *M. Popp**
Vergleichende Populationsgenetik von Wirt-Parasiten-Wechselwirkungen: Hummeln und ihre Darmparasiten.
Génétique des populations comparative dans les interactions hôte-parasite : les bourdons et leurs parasites intestinaux.

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Ökologie, Bestäubung, Pflanzenschutz
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26. The use of colony splits as environmental indicators. *G. Liebig**
Die Eignung von Fluglingen als Umweltindikator.
L'utilisation des dédoublements de colonies comme indicateurs environnementaux.

27. Successful use of honey bees for grey mould biocontrol on strawberries and raspberries in Finland. *H.M.T. Hokkanen*, *I. Menzler-Hokkanen*
Erfolgreiche Nutzung von Honigbienen zur Biokontrolle von Grauschimmel an Erdbeeren und Himbeeren in Finnland.
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28. Development of bee colonies during and after the maize inflorescence 2009 in the Rhine valley. *T. Kustermann*, *G. Liebig**
Die Entwicklung von Bienenvölkern während und nach der Maisblüte 2009 im Rheintal.
Développement des colonies d'abeilles pendant et après l'inflorescence du maïs en 2009 dans la vallée du Rhin.

29. Effects of stacked Bt maize on longevity and hatching weight of honeybee workers.
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Auswirkungen von *stacked* Bt Mais auf Langlebigkeit und Schlupfgewicht von Arbeiterinnen der Honigbiene.
Effets du maïs Bt mis en gerbe sur la longévité et le poids à l'éclosion des ouvrières d'abeilles.

30. Brood-Ring-Test 2008: A method to test the effects of pesticides on bee brood (*Apis mellifera* L.) under laboratory conditions. *M. Janke*, *P. Aupinel*, *D. Fortini*, *B. Michaud*, *P. Medrzycki*, *E. Padonani*, *D. Przygoda*, *C. Maus*, *J.-D. Charrière*, *V. Kilchenmann*, *U. Riessberger-Galle*, *J.J. Vollmann*, *L. Jeker*, *J.-F. Odoux*, *J.-N. Tasei**
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31. Effects of insecticides on the foraging behaviour of flight bees: measurements using radio frequency identification. *C. Schneider*, *D. Bevk*, *B. Grünewald*, *J. Tautz*
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Effets des insecticides sur le comportement d'approvisionnement des abeilles en vol : mesures utilisant l'identification par fréquence radio.

32. From nectar to honey: fungicides in the cultivation of oilseed rape. *F. Schatz*, *K. Wallner**
Aus dem Nektar in den Honig: Fungizide im Rapsanbau.
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Bee products
Bienenprodukte
Produits de la ruche

33. Pyrrolizidine alkaloids in honey and bee pollen. *T. Beuerle*, *K. von der Ohe*, *M. Kempf*, *P. Schreier**
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36. Characterization of European honey dew honeys. *W. von der Ohe*, *K. von der Ohe*, *M. Janke**
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Posters
Poster
Posters

Biology, physiology, behavior
Bienenbiologie, Physiologie, Verhalten
Biologie, physiologie, comportement

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38. Do winter bees have a weaker immune system compared to summer bees? *K. Azzami*, *H. Gätschenberger*, *O. Gimple*, *H. Beier*, *J. Tautz*
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39. A hypothetical protein (HP) of 30 kDa: a bee specific immune factor. *S. Albert*, *K. Azzami*, *H. Gätschenberger*, *G. Grimmer*, *O. Gimple*, *M.J. Mueller*, *J. Tautz*
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40. The influence of different pollen diets on the lifespan and the immune system of honeybees (*Apis mellifera*). *S. Steigerwald*, *I. Illies*, *K. Azzami*, *H. Gätschenberger*, *J. Tautz*
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41. Does the retention time of the sperm within the spermatheca influence the mortality and embryonic development of the honeybee offspring? *H. Al-Lawati*, *K. Bienefeld**
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42. Protein uptake in honeybee colonies supplemented with two protein diets simultaneously. *R. Brodschneider*, *C. Haidmayer*, *U. Riessberger-Gallé*, *K. Crailsheim*
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43. Contact-free age determination of honeybee larvae (*Apis mellifera*). *J. Vollmann*, *R. Thenius*,

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46. Cooperative thermotaxis in honeybees: Group decisions in a complex temperature gradient. *M. Scopek*, *G. Radspieler*, *R. Thenius*, *T. Schmickl*, *K. Crailsheim*
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47. Synthetic larval diet produces lighter and smaller honeybees (*Apis mellifera*). *R. Brodschneider*, *D. Steiner*, *A. Moder*, *J. Vollmann*, *U. Rissberger-Galle*, *K. Crailsheim*
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Genetics, breeding
Genetik, Zucht
Génétique, élevage

48. An analysis of beekeeping in Syria as a basic for the development of a breeding program for native honeybees. *K. Zakour*, *K. Bienefeld**
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49. An estimate of genetic parameters for Varroa tolerance with consideration of direct and maternal effects. *K. Ehrhardt*, *R. Büchler*, *C. Garrido*, *K. Bienefeld **
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50. The behavior of artificially inseminated and natural mated honeybee queens during the introduction into the colony. *S. Masry*, *K. Bienefeld**
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51. New experimental observations on survivability and vitality of the queens kept for longer periods in transport cages. *D. Šekulja*, *H. Pechhacker*
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Pathology – varroosis, AFB, etc.
Pathologie – Varroose, AFB, u.a.
Pathologie – Varroose, loque américaine, etc.

52. A new PCR-based method for the analysis of the Nosema (*Nosema* sp.) infection of honeybees. *K. Bogenschütz*, *A. Schroeder*, *P. Rosenkranz**
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53. Epidemiological situation for *Nosema* sp. in Germany between 2004 and 2008. *K. Hedtke*, *S. Gisder*, *E. Genersch*
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54. Distribution and interaction of *Nosema ceranae* infected bees (*Apis mellifera*) within the colony. *D. Michelberger*, *S. Berg*, *J. Tautz*
Steck mich nicht an! Verteilung und Interaktionen

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55. Establishment of a method for manipulation of *Paenibacillus larvae. L. Poppinga*, *E. Genersch*
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56. Comparative genome analysis within the species *Paenibacillus larvae. A. Fünfhaus*, *E. Genersch*
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57. Regional differences in the occurrence of contamination by accompanying germs in honey sample analysis for *Paenibacillus larvae. A. Otto*, *C. Otten**
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58. Establishment of an in vitro-model for DWV-infections. *N. Möckel*, *S. Gisder*, *E. Genersch*
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59. The wintering behavior of honey bee colonies with special regard to their Varroa and virus loads. *G. Liebig*, *A. Woelk**
Das Überwinterungsverhalten von Bienenvölkern unter besonderer Berücksichtigung von Varroa- und Virenbefall.
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60. Experiments on the toxicity of oxalic acid for *Apis mellifera* – comparison of dermal vs. oral application. *E. Rademacher*, *M. Harz*
Untersuchungen zur Toxizität von Oxalsäure an *Apis mellifera* – dermale vs. orale Applikation.
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61. Varroa, viruses and the survival prognosis for bee colonies. *M. Meixner*, *E. Genersch**
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62. Comparative efficacy of 60% and 85% formic acid for Varroa control. *S. Berg*, *F. Schürzinger*
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Efficacité comparative de l'acide formique titré à 60 % ou 85 % dans la lutte contre Varroa.

63. The enemy of my enemy. Experiments on the influence of entomopathogenic fungi on *Apis mellifera* and *Varroa destructor. M. Holt*, *P. Aumeier*, *W.H. Kirchner*
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L'ennemi de mon ennemi. Expériences sur l'influence des champignons entomopathogènes sur *Apis mellifera* et *Varroa destructor*.

64. Mimicking natural selection for Varroosis tolerance without sacrificing bee colonies in the Marmara island population. *I. Cakmak*, *S. Cakmak*, *S. Fuchs*
Ein Versuch zur Nachahmung der natürlichen Selektion für Varroatoleranz ohne Völkerverluste auf der Marmarainsel.
Imitation de la sélection naturelle à la tolérance au varroa sans perte des colonies d'abeilles dans la population de l'île de Marmara.

65. Forensic microbiology of septicemia victims. *U. Hartmann*, *A. Roetschi*, *J.-D. Charrière*, *P. Neumann**
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Expertise microbiologique de victimes de septicémie.

Ecology, pollination, plant protection, bee products
Ökologie, Bestäubung, Pflanzenschutz, Bienenprodukte
Écologie, pollinisation, protection des plantes, produits de la ruche

66. Effects of honey bees on yields in oilseed rape cultivation. *J. Radtke*
Zum Einfluss der Honigbiene auf den Ertrag im Rapsanbau.
Influence des abeilles sur les récoltes de colza.

67. Benefits of the honey bee for the wild flora, using *Prunus spinosa* as an example. *J. Radtke*, *E. Etzold*
Nutzen der Honigbiene für Wildpflanzen am Beispiel von *Prunus spinosa.*
Bienfaits de l'abeille sur la flore sauvage : exemple de *Prunus spinosa.*

68. Evaluation of multiple insect resistant Bt maize pollen consumption in honeybee workers under semi- field conditions. *H.P. Hendriksma*, *S. Härtel*, *W. von der Ohe*, *I. Stefan-Dewenter**
Beurteilung des Konsums von Bt Maispollen mit mehrfacher Insektenresistenz durch Arbeiterinnen der Honigbiene unter Semi- Feldbedingungen.
Évaluation de la consommation de pollen de maïs Bt à résistance multiple contre les insectes chez les ouvrières d'abeilles en conditions semi-naturelles.

69. A laboratory method for detection and quantification of BT-corn Mon 810-pollen in honey. *S. Gisder*, *E. Genersch*, *B. Lichtenberg-Kraag*
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79. The utilization of *Apis dorsata* in the Mekong delta (Vietnam). *T. Kustermann*, *P.D. Hanh**
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Abstracts

8. Effects of a carbohydrate restriction on the immunocompetence of bees. *R. Siede*, *M.*

Meixner, *R. Büchler* (LLH, Bieneninstitut Kirchhain, 35274 Kirchhain, Germany)

Honey bee viability depends on the functionality of the immune system. Due to intensification in agriculture, bees can suffer from pollen or nectar dearth. Nutritional stress is supposed to weaken immunocompetence. We started this study with the goal to test this assumption. For identifying immune indicators we challenged bees by injuring them with a needle or by injecting Ringer, ABPV, lipopolysaccharide (LPS), lipoteichoic acid (LTA), *Paenibacillus larvae* or peptidoglycan (PG). Changes in the transcription of abaecin (Ab), apidaecin (Ap), defensin 1 (Def), hymenoptaecin (Hym), hopscotch, toll and prophenoloxidase (ProPO) were analysed by comparative real time PCRs (Evans et al. (2006), Insect Mol. Biol. 154, 645–56). Changes were most prominent for Hym (>100-fold), less distinct for Ab and Ap (between 10-and 100-fold), below 10-fold for Def and even lower for hopscotch, toll and ProPO (<6-fold). Among the elicitors LPS, LTA, *P. larvae* and PG had clear immunostimulatory effects. Additionally we used an inhibition zone assay (IZA, Randolt et al. (2008), Arch. Insect Biochem. 69, 155–167). For the nutritional experiment, workers in cages were supplemented with a 50% sucrose solution (w/v) or with a 5% solution (w/v). Records of consumption showed that the bees fed 5% solution starved, as their higher uptake did not compensate the lower concentration. After LPS injection, the mRNA-change of Ab, Ap, Def and Hym was quantified. Transcriptional changes were significant between challenged and non-challenged bees, but not between the two sugar diets (N = 5, randomisation test, Pfaffl 2001). The IZA corroborated exactly the findings of the gene expression analysis (N = 3, $P = 0.05$, Bonferroni test). Carbohydrate restriction did not lead to immunosuppression, at least with regard to the antimicrobial peptides considered here.

14. New experimental data on efficacy of rotenone in strips and efficacy of optional pretreatment with preparative KAS-81 and sugar in powder. *D. Sekulja* (Polytechnic of Rijeka, 51000 Rijeka, Croatia)

In the region of Rijeka most of the 450 registered beekeepers regularly use rotenone in strips for the last seven years. To avoid the effect of the reinfestation with mites from neighboring apiaries, additional treatments were done with KAS-81 and icing sugar. In 2008 experiments, rotenone in strips was placed in the middle of the brood nest and left there for 30 days. Altogether, 111 colonies were used on two locations. In Rijeka, the initial infestation level of 2.93%, fell to 0.48% after one day, with small weekly oscillations after 30 days to 0.24%. In Pazin, the infestation level dropped from initial 1.18% to 0.24% next day, and after 30 days to 0.06%. Control colonies were treated with Checkmite®, containing Coumaphos as the active ingredient. KAS–81 is an alternative Russian preparative for Varroa treatment. It is made from pine buds (*Pinus* sp.) and wormwood (*Artemisia absinthium* L.). KAS-81 experiments were conducted on 40 colonies on two different locations in Istria and Rijeka. No significant difference was detected in the development of Varroa population in colonies which received the KAS-81 treatment and test colonies which got a pure sugar syrup. Icing Sugar treatment was conducted on 40 colonies (20 on each location). Colonies were simply dusted between the combs (a tea cup of icing sugar per one-story hive). In Rijeka, the initial infestation level of 3.89% on 15th of August 2008 increased to 4.59% till 11th of September, while in Pazin on the same number of hives it dropped from an initial 1.42% to 1.18%. At both locations, a single dusting treatment with icing sugar permitted control of Varroa infestation levels prior the late summer treatment with rotenone strips.

15. Vital colonies due to brood withdrawal - effects on population development, disease infection and honey yield. *R. Büchler* (Landesbetrieb Landwirtschaft Hessen, Bee institute, 35274 Kirchhain, Germany)

To imitate effects of natural swarming on colony development and health status in a modern bee management system, all brood combs except one were removed with just some adhering bees. The brood chamber was reduced to one box and the removed brood combs were replaced with wax foundation and/or newly drawn combs. The remaining comb with mainly young brood acted as a Varroa trap and was destroyed after the brood was sealed. The removed brood was collected in separate hives till complete emergence and afterwards the bees were used to establish additional colonies. In 2007 and 2008, 24 colonies in two institute bee yards and 30–53 colonies in 6–7 private bee yards were treated as described. In each apiary, control colonies without brood removal were treated with formic acid or Thymovar® at the end of July. In September, bee samples were taken from the edge of the nest to measure the average Varroa infestation, to check for Nosema spores and to analyze for ABPV, CPBV and DWV in head extracts of 10 bees by end time PCR. The complete brood removal didn't affect the

wintering size (10.3 versus 10.1 combs, $P_{(F-value)}$ = 0.845) nor the average honey yield (26.1 versus 25.8 kg, $P_{(F-value)}$ = 0.901), although there were highly significant differences between the seasons and test locations. An early brood withdrawal (20th May) resulted in a significantly lower honey yield compared to the control (Tukey, $P < 0.05$) while a late brood withdrawal (3rd July, 14 days before the final harvest) slightly increased the honey yield compared to the control. Varroa infestation in September was slightly but not significantly higher in the untreated test group (4.3 versus 3.6 mites/10 g bees, F-values 0.777, $P = 0.379$). The test colonies had lower infection rates for Nosema, ABPV and CBPV while DWV infections were less prevalent in the control group.

17. Comparative virulence between *Nosema apis* and *Nosema ceranae*. *I. Fries*, *E. Forsgren* (Dept. of Ecology, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden)

Dramatic losses of honey bee colonies in the field following infections with *Nosema ceranae* have been reported from Spain. To compare the effects from *N. ceranae* and *Nosema apis* in individual bees we have used cage experiments. Different number of *N. apis* and *N. ceranae* spores have been fed individually to honeybees to study the infectious dose and the course of infection in individual bees. Furthermore, different mixtures of spores of the two parasites have been fed to study within host competition between the parasites. In addition, the effects on spore viability from storing spores refrigerated or frozen for one week were tested. Throughout all tests, cage mortality was monitored. Nosema species were verified using specific PCR. All tests were made with fresh spore suspensions except for the storage test. The results of three replicates demonstrate that *N. ceranae* may have a lower average infectious dose (ID50 = 85), compared to *N. apis* (ID50 = 389). The course of infection differs slightly between the two parasites. Mature spores are produced earlier in *N. apis* compared to *N. ceranae*, but after 12 days the same level of infection measured as spore load occur with both parasites. Within host competition between species could not be demonstrated using RT-qPCR to measure amounts of parasite DNA produced from different mixtures of spores. The storage test demonstrated little effect on spore viability from refrigeration, but the effect from freezing was dramatic. Viability of *N. apis* may actually be enhanced from exposure to freezing temperatures, whereas the viability of *N. ceranae* is significantly reduced from one week in a deep freezer. This difference in temperature sensitivity probably has epidemiological implications. No differences in cage mortality could be documented between the two parasites although no real longevity tests were conducted.

27. Successful use of honey bees for grey mould biocontrol on strawberries and raspberries in Finland. *H.M.T. Hokkanen*, *I. Menzler-Hokkanen* (Applied Biology, University of Helsinki, Finland)

Honeybees were used as vectors to spread a commercial formulation of *Gliocladium catenulatum* (Prestop Mix, Verdera Oy, Finland) to control grey mould (*Botrytis cinerea*) on commercial strawberry and raspberry farms in Finland during three seasons (2006, n = 3; 2007, n = 10; 2008, n = 3). Three farms grew organic berries, and seven used conventional growing methods. At the conventional farms, 3-4 fungicide sprays were applied in addition to the bee-vectored biocontrol. Two strong beehives with inoculum dispensers were placed close to the berry fields, and about 5 g of the product ($>10^7$ cfu/g) was filled into the dispenser daily during the main flowering (about three weeks). Bees visited the strawberry flowers on the average 10 times every day during flowering. Four treatments were included: untreated control, chemical control, bee-vectored biocontrol, and chemical + biocontrol combined. Consistently, all control treatments were highly effective: bee-vectored biocontrol alone decreased disease incidence on average by 50%, chemical control by 65%, and both methods together by 80%. However, these differences between the control methods disappeared when the marketable yield was measured: biocontrol was as effective as either of the two other methods. Furthermore, biocontrol increased the storage durability of the berries more than chemical control. Bee-vectored biocontrol saved the growers time, work, equipment, environment, and money: costs of chemical control were 500–1000 €/ha, biocontrol alone about 300 €/ha.

31. Radiofrequency Identification. *C. Schneider*[1], *D. Bevk*[2], *B. Grünewald*[1], *J. Tautz*[3], *S. Fuchs*[1] ([1] Institut für Bienenkunde, Polytechnische Gesellschaft, J.W. Goethe-Universität Frankfurt a.M., 61440 Oberursel, Germany; [2] National Institute of Biology, 1000 Ljubljana, Slovenia; [3] BEEgroup, Biozentrum Universität Würzburg, 97074, Germany)

We developed an experimental design that allows us to detect the effects of acute sub-lethal

doses of pesticides under field conditions. The tested chemicals included the systemic neonicotinoid Imidacloprid, used for plant protection e.g. by seed dressing and the organophosphate Coumaphos, used as an acaricide for the control of the parasitic mite *Varroa destructor*. To record the foraging activity, bees from a test-hive were trained to an artificial feeder filled with a 2 M sucrose solution. Trained bees were marked with RFID (radiofrequency identification) transponders for individual recognition during the experiment through reading devices at the food source and at the hive entrance. At the following day, RFID marked bees were treated with one of the pesticides and monitored over 48 h. Both substances were applied orally by feeding individual bees once with 10 μL of 33% honey solution. The dose used for Imidacloprid was 3 ng per bee, for Coumaphos doses of 5, 2 and 1 μg /bee were used. Bees treated with 3 ng/bee Imidacloprid visited the feeder less frequently ($P < 0.05$, Mann-Whitney U test) and needed significantly longer for a visit ($P < 0.001$, Mann-Whitney U test) compared to an untreated control group. The effect could be observed immediately after treatment in two observation trials over 4 h, but was no longer detectable after 24 h and 48 h. Bees treated with 5 μg of Coumaphos also visited the feeder less frequently ($P < 0.001$, Mann-Whitney U test) and needed longer time for a single visit ($P < 0,001$, Mann- Whitney U test) compared to untreated bees. In contrast to Imidacloprid, these effects were still present 48 h after the treatment. The results show that pesticides affect foraging at a feeder, which indicates that the method is sufficiently sensitive to detect the sub-lethal effects of pesticides on the foraging behavior of honey bees.

37. Drones respond with a humoral immune reaction after septic injury. *H. Gätschenberger*, *O. Gimple*, *J. Tautz* (BEEgroup, University of Würzburg, Am Hubland, 97074 Würzburg, Germany)

As social insects with a huge population density in their hives, honey bees are especially vulnerable to infections. Like all insects, honey bees lack an adaptive immune system, but instead possess an innate immune system, consisting of humoral and cellular components and the phenoloxidase activating system to combat pathogens and diseases. Adult workers and larvae react to septic injury with activation of their humoral immune response. Drones are present in the hive only for a short period of time and have limited duties, but their immune status is important for reproductive performance. Therefore we studied reactions of drone larvae and adults after bacterial infection, initiated by injection of 10^4 *E. coli* cells per drone. The established in vitro cultivation of worker larvae was adapted to drone larvae by altering the composition of the feeding solution beginning at the fifth instar developmental stage. Drone larvae were artificially infected seven days after hatching. Haemolymph of adult drones and larvae was collected 24 hours post infection (p.i.) and analysed by one and two dimensional gel electrophoresis. After bacterial challenge, drone larvae produced the antimicrobial peptides (AMPs) hymenoptaecin and defensin1 indicating the induction of humoral response. In the haemolymph of non-infected adult drones, the major proteins throughout all life stages are ApoLp-I/ApoLp-II and transferrin, while small amounts of vitellogenin and α-glucosidase II are expressed only in older drones. As observed in drone larvae, the known bee AMPs are induced after bacterial infection as well as the immune factors carboxylesterase and HP30. Prophenoloxidase, a key enzyme mediating humoral and cellular immune reactions, is present at increasing amounts in adult drones as demonstrated by immunoblots. We conclude that the immune response of drones resembles that of workers.

38. Do winter bees have an immune system that is weaker than that of summer bees? *K. Azzami*, *H. Gätschenberger*, *O. Gimple*, *H. Beier*, *J. Tautz* (BEEgroup, University of Würzburg, Am Hubland, 97074 Würzburg, Germany)

Winter bees differ from summer bees in many ways. In particular, winter and summer bees differ markedly in longevity, due mainly to differences in the development of the fat bodies and the amounts of storage proteins and lipids. Because newly emerged summer bees counter bacterial infections with strong humoral and cellular immune responses, we were interested if this applies to winter bees, too. Therefore we injected living *E. coli* cells or the cell wall component lipopolysaccharide in the haemocoel of bees removed from the hive during the winter season (Nov to Feb). We analysed the haemolymph proteins 24 h post-injection (p. i.) via gel electrophoresis and subsequent mass spectrometry of interesting protein bands. The major haemolymph proteins of winter bees are provitellogenin (~200 kDa) and transferrin. Additionally, a specific N-terminal processing product of vitellogenin (~128 kDa) was detected whose function remains unclear. The antimicrobial peptides hymenoptaecin and defensin, which are part of the

humoral immune response, are inducible in winter bees as strongly as in summer bees. Interestingly, the two immune-related factors HP30 and carboxylesterase that are expressed strongly upon septic injury in young summer bees are constitutively present at a low level in the haemolymph of non-challenged winter bees. Another striking difference between the immune reactions of summer and winter bees occurs at the level of the cellular immune response. Nodulation is a predominant cellular defence reaction in insects. A high yield of melanized nodules is observed in newly emerged summer bees, whereas no nodules are formed in any winter bees challenged with bacteria. Therefore we suspect that the defence strategies of winter bees rely in part on components other than those associated with the innate immune response.

39. Hypothetical protein of 30 kDa (HP30), a bee-specific immune factor. *S. Albert*[1,2], *K. Azzami*[2], *H. Gätschenberger*[2], *G. Grimmer*[1], *O. Gimple*[2], *M.J. Mueller*[1], *J. Tautz*[2] ([1]Pharmazeutische Biologie, Universität Würzburg, Julius-von-Sachs-Platz, 97082 Würzburg, Germany; [2]BEEgroup, Biozentrum, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany)

Similar to other insects, infection of honey bees (*Apis mellifera*) with bacteria, or mock infection with lipopolysaccharides or laminarin results in the expression of antimicrobial peptides (AMPs) and activation of phenoloxidase in their haemolymph. Besides AMPs, induction of two additional immune-related proteins in the bee haemolymph has been observed after bacterial challenge (Randolt et al. (2008), Arch. Insect Biochem. 69, 155–167). One of these proteins with a calculated mass of about 30 kDa (HP30) shows no significant similarity with any proteins in other organisms including fully characterized genomes of 12 *Drosophila* species, *Nasonia vitripennis*, *Tribolium castaneum* and *Bombyx mori.* We generated the molecular characterization of the HP30 gene and found that the genomes of related Asian honeybees (i.e., *A. florea*, *A. dorsata* and *A. cerana*) encode highly similar proteins. Using antibodies produced against recombinant HP30 we have shown that: (i) HP30 is a glycoprotein with a molecular mass of 37 kDa, (ii) HP30 appears 6 hours after artificial infection in the haemolymph of newly emerged worker bees, (iii) non-infected winter bees constitutively express small amounts of HP30, whose amount is up-regulated after aseptic and septic injury, (iv) a protein induced after bacterial infection in the haemolymph of bumble bees (*Bombus terrestris*) is recognized by anti-HP30 antibodies. In conclusion, the immune factor HP30, although not present in other insects, seems broadly distributed at least in the subfamily *Apinae*. Further experiments will be performed to elucidate the phylogeny and function of this protein in bee immunity.

40. The influence of different pollen diets on the life span and the immune system of the honey bee. *S. Steigerwald*[1,2], *I. Illies*[1], *K. Azzami*[2], *H. Gätschenberger*[2], *J. Tautz*[2] ([1]LWG, Fachzentrum Bienen, 97209 Veitshöchheim, Germany; [2]BEEGroup Biozentrum Universität Würzburg, 97074 Würzburg, Germany)

Honey bees meet their protein need through the consumption of pollen of many different flowering plants. Possible influences on the lifespan of bees in cages and on the development of storage and immune-related proteins in the haemolymph were investigated by comparing a diet of mixed pollen with maize pollen and an artificial lysine-free diet. Freshly emerged bees were supplied with different protein diets and Api-Invert® ad libitum in cages containing 50 bees. They were provided mixed pollen, maize pollen and an artificial amino acid compound (GA1 Milupa). One group was fed with a pure carbohydrate diet. The mortality was observed daily over a period of 5 weeks. Haemolymph samples were taken from bees at the age of 6 to 8 days and analysed for its protein composition by SDS-PAGE. Additionally, bees were infested with *E. coli* at the age of 6 days. After 24 h haemolymph samples were taken and analysed. Furthermore, artificial swarms (each 200 g of bees) in flight tents were fed with different protein diets (mixed pollen, maize pollen and artificial diet). After broodrearing the freshly emerged bees were provided the corresponding pollen diets in cages and observed for 32 days. A diet consisting only of maize pollen had no negative effects on the lifespan compared to mixed pollen. This applies to bees fed with the diets after hatching, as well as to bees which were supplied with maize or mixed pollen during larval development. However, there were considerably smaller amounts of storage proteins (vitellogenin) and immune-related proteins in the haemolymph of bees fed with pure maize pollen. Presumably this is related to the low histidine content of maize pollen, which amounts to less than 50% compared to mixed pollen. The minor quantity of this essential proteinogenic amino acid could have caused the observed differences in generating storage and immune-related proteins. The artificial lysine-free

diet seems to be abhorrent to bees as the mortality is increased compared to bees supplied with a pure carbohydrate diet.

42. Protein uptake in honeybee colonies supplemented with two protein diets simultaneously. *R. Brodschneider*, *C. Haidmayer*, *U. Riessberger-Gallé*, *K. Crailsheim* (Department of Zoology, Karl-Franzens University Graz, Universitätsplatz 2, 8010, Graz, Austria)

Honeybees need protein-rich pollen to synthesize jelly that is fed to larvae. Additional protein diets may be fed to honeybee colonies to enhance colony growth. We investigated the acceptance and consumption of two supplemental protein diets presented to the bees simultaneously. Both diets (pollen and Feedbee, a commercial pollen substitute) were applied inside two 3-frame observation hives as patties over a period of 5 consecutive days in May 2007 in a standardized form. Every day the patties were renewed and the quantity of each diet consumed in 24 hours, the number of feeding bees (every 15 minutes) and the duration of feeding acts was recorded. During the last two days of our experiments the amount of supplemental food was doubled to evaluate the effect of the quantity of food applied. On average more pollen (16.0 +/– 1.1 g) than Feedbee (7.5 +/– 0.7 g) was consumed in 24 hours (Mann-Whitney test, $P < 0.05$). We attribute this to phagostimulants in pollen. When we fed doubled amounts, more bees were feeding on the patties ($P < 0.05$) and the consumption was higher (pollen: 22.7 +/– 4.3 g, Feedbee: 14.8 +/– 1.4 g, $P < 0.05$). The amount of consumed protein diet correlated with the respective number of bees feeding on both patties. Single feeding events were longer on Feedbee than on pollen ($P < 0.05$).

Food is extensively exchanged within a honeybee colony through trophallaxis. We estimated the average amount of protein a single bee obtained in our experiments (each colony consisted of ~6000 bees and the protein content of pollen was assumed to be 10%): 0.229 mg/bee/24 h for pollen and 0.189 mg/bee/24 h for Feedbee, respectively. This increased when the amount of applied food was doubled: 0.326 mg for pollen and 0.375 mg for Feedbee, respectively. Our results show that the consumption of protein diets depends on the amount of food applied and suggests that beekeepers may increase the surface area of fed diets to increase the protein uptake in times of protein need.

43. Contact-free age determination of honeybee larvae (*Apis mellifera*). *J. Vollmann*, *R. Thenius*, *T. Schmickl*, *K. Crailsheim* (Department of Zoology, Karl-Franzens-University-Graz, Universitätsplatz 2, 8010 Graz, Austria)

All known methods of age determination of honeybee larvae (e.g. determination of egg laying time and time of eclosion or morphological investigations) are subject to a lot of effort and reiterated disturbances of colonies. We developed a novel, easy to handle contact-free method for age determination of larvae based on standard photographical and morphometric methods. For our investigations high resolution images of brood combs were taken. On these images the coordinates of 17 easy definable points at the inner contour of bent larvae were collected. The distances between these morphometrical reference points were calculated and analysed using standard statistical techniques. A significant increase of the length of the inner contour could be found until the age of 96 ± 2 h. Larvae older than 96 ± 2 h showed no significant increase. From an age of 108 ± 2 h a significant decrease of the length could be found (Mann-Whitney U-test; $P < 0.05$). The distances of the opposite corresponding coordinates (except the innermost 2 coordinates) decreased with increasing age. The distance of the two innermost coordinates significantly increased till the age of 72±2 h. A non significant increase until the age of 84±2 h is followed by a significant decrease until the age of 96 ± 2 h. Using the collected data, a calibration curve can be created and age determination of larvae is possible without physically interfering with the larvae. The morphometrical analysis of images of larvae is highly adequate for contact-free age determination. For age determination of small larvae, the distances between the coordinates close to the caput and the anus are most appropriate, whereas for bigger larvae the distances between the coordinates close to the center of larvae are most accurate. The age of larvae in the period before capping is best determined by a combination of length of the inner contour and the distances between all opposite coordinates.

44. Cooperative thermotaxis in honey bees: How robust are group decisions? *S. Hahshold*, *G. Radspieler*, *R. Thenius*, *T. Schmickl*, *K. Crailsheim* (Department of Zoology, Karl-Franzens University, 8010 Graz, Austria)

Young honey bees (<24 h) show thermotaxis. They prefer temperatures near 36 °C. In our experiments we tested the ability of honeybees to find their temperature optimum in a circular arena. We investigated bees with different thermotactic abilities. We amputated their antennae to be sure that

all thermosensors were eliminated. First we tested single bees either not manipulated (AA) or manipulated, with one (AA^-) or both antennae amputated (A^-A^-). Secondly we investigated group behaviour of homogenous groups and heterogenous groups. Homogenous groups consisted of 15 bees (AA, AA^- or A^-A^-). Heterogenous groups consisted of 15 bees: 10 AA + 5 A^-A^-; 7 AA + 8 A^-A^-; 5 AA + 10 A^-A^- and 3 AA + 12 A^-A^-. We counted the number of bees in a defined target zone (under the heat lamp; 36 °C) after 30 minutes. The target zone represented 25% of the area of the arena. Our results show that 70% of the AA and the AA^- found the target area, but only 30% of the A^-A^- (Chi^2-test: $P < 0.001$). Groups of AA-bees have a higher success rate to find the target area, than AA^--groups. AA^--groups have a higher success rate than A^-A^- (Mann-Whitney U-test $P < 0.05$). In presence of A^-A^- the AA bees are reaching the target area. There was no significant difference between AA-groups and mixed groups (Kruskal-Wallis: n.s.). This is an important adaptation because young honeybees should stay in the brood area to take over the cell cleaning task and to build cluster to isolate the brood against cooling. They should not let themselves be disturbed by bees engaged in other jobs within the brood area, like pollen storers or nurse bees.

46. Cooperative thermotaxis in honeybees: Group decisions in a complex temperature gradient. *M. Szopek*, *G. Radspieler*, *R. Thenius*, *T. Schmickl*, *K. Crailsheim* (Department of Zoology, Karl-Franzens University, 8010 Graz, Austria)

Young honeybees show thermotaxis and locate their temperature preferendum (approx. 36 °C) in a temperature organ. In a two-dimensional temperature gradient only a minority of single bees stops at the optimum whereas groups of bees, exposed to the same gradient, can aggregate collectively at the optimum. In this work we investigated this cooperative thermotactic behaviour of groups of young honeybees (2–30 h). We tested their ability to distinguish between a local and a global optimum and the influence of different group sizes. We used a circular temperature arena where we generated a complex two-dimensional gradient by using two heat lamps. The basic temperature was 30 °C, the local optimum was 32 °C and the global optimum 36 °C. We tested groups of 6, 24, 64 and 128 bees (N = 8/group size). For validation purposes we tested single bees under the same conditions (N = 10). We defined a target zone for each optimum and counted the bees in the target zones and outsides those zones in the 30th minute. We found that the median fraction of bees in the 36 °C target is significantly higher than in the 32 °C target within each group size (Kruskal-Wallis $P < 0.01$). There was no significant difference between the median fraction of the different group sizes in the 36 °C zone (Kruskal-Wallis $P > 0.05$). The median fraction of bees in the 32 °C zone increases with increasing group size (Kendall's tau 0.3995; $P = 0.0068$). Groups of young honeybees are able to discriminate the local optimum and aggregate successfully in the global optimum, independent of the group size. Higher median fractions at higher group sizes at 32 °C could be a result of overcrowding of the 36 °C zone and the consequential switching of some bees to the 32 °C zone. In overcrowded brood nests this behaviour could ensure that young bees move to the cooler edge or other brood nest areas.

47. Synthetic larval diet produces lighter and smaller honeybees (*Apis mellifera*). *R. Brodschneider*, *D. Steiner*, *A. Moder*, *J. Vollmann*, *U. Riessberger-Gallé*, *K. Crailsheim* (Department of Zoology, Karl-Franzens University Graz, Universitätsplatz 2, 8010, Graz, Austria)

During its 5-day larval development, a honeybee larva is inspected continuously and fed numerous times by its adult sisters with proteinaceous brood-food. During the pupal stage, in which its body undergoes massive changes from the wormlike larva to the adult honeybee, no food is consumed. Protocols have been developed to rear honeybee larvae in the laboratory in vitro for standardized toxicity testing and experiments with brood diseases. In these procedures, the brood-food produced by specialized nurse-bees is replaced by a partly artificial diet which is fed to the larvae only 5 times during their larval development (Aupinel et al. (2005), Bull. Insectol. 58, 107–111). In our experiments we measured body weight and various body size parameters of adult honeybees (younger than two days) exclusively fed artificial diet as larvae and compared them to their sister honeybees that were reared in a colony. In total 49 artificially reared bees derived from two different colonies were compared to 96 naturally reared bees from the same colonies. Width, length and area of fore- and hind wings, the number of hamuli on the hind wing, and the length of the femur and tibia of the hind leg were measured. Fresh and dry weight of head, thorax and abdomen were weighed to the nearest 0.01 mg. Our results show that the artificial diet used in in-vitro rearing produces healthy honeybees, but all measured body size parameters of artificially reared

honeybees were slightly smaller (Mann-Whitney test, $P < 0.05$) than in their sisters reared in the colony. Artificially reared honeybees also had lower dry weight of head and thorax (Mann-Whitney test, $P < 0.05$). Since both groups of bees emerged in an incubator, all observed differences must be due to larval nutrition. To obtain more insight into the quality of honeybees produced with artificial larval food, further parameters of viability other than body weight and size have to be investigated.

51. New experimental observations on survivability and vitality of honeybee queens kept for longer periods in transport cages. *D. Sekulja*[1], *H. Pechhacker*[2] ([1] Polytechnic of Rijeka, 51000 Rijeka, Croatia; [2] Austrian Carnica Association, 3293 Lunz am See, Austria)

Queens are usually taken out of mating hives and kept in transport cages until they are used by beekeeper. The aim of this work was to find out if the period the queen is caged affects their acceptance and/or supersedure rate in their host colonies. In a pilot test at Lunz am See (Austria) 26 queens and Rijeka (Croatia) 30 queens were kept in transport cages from the middle of August over the winter, with replacement of bees and food usually after 10–14 days, depending on conditions in the cages. From March on these queens were successfully used in queenless colonies. All colonies used in experiments in 2007 and 2008, were more or less uniform at the moment of queen introduction: 10 frames covered by bees, 4–5 combs of brood and 4–5 frames of honey. In 2007, a total number of 177 queens was split in 6 groups with 1, 7, 15, 21, 30 and 35 days of life in transport cages. There was no significant difference in the acceptance rate of the queens or in the supersedure rate in the spring, regardless of the duration of the caging of queens prior the introduction to the colony. Throughout the season, colonies exhibited similar brood development and similar honey collection, regardless of the group of queens used. In 2008, a total number of 178 queens was split in two groups, 1 day freshly collected queens and queens left in cages over 30 days before their introduction to the colonies. Again, there was no significant difference in acceptance rate of the queens regardless of the duration of the cage life of queens prior to the introduction to the colony. The supersedure rate and colony development in spring 2009 was similar to in the year 2007. In all experiments, there was no difference in the activity of the queens regardless of the group that was used.

53. Epidemiological situation of *Nosema* spec. in Germany between 2004 and 2008. *K. Hedtke*, *S. Gisder*, *E. Genersch* (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

The microsporidia are a group of obligate intracellular parasites derived from fungi, infecting at least 1200 species of animals from every major evolutionary lineage, and with a large proportion of them infecting insects. Two species of microsporidia have been detected in honeybees, *Nosema apis* (*N. apis*) and *Nosema ceranae* (*N. ceranae*), both causing dysentery (nosemosis) in highly infected animals. *N. apis* and *N. ceranae* were described as specific parasites for the European honeybee, *Apis mellifera* (*A. mellifera*), and the Asian honeybee, *Apis ceranae*, respectively. Quite recently, *N. ceranae* has also been detected infecting *A. mellifera* both in Asia and in Europe. Further studies suggested a host jump of *N. ceranae* from the Asian to the European honeybee around a decade ago. It was proposed that since then *N. ceranae* may have been displacing *N. apis* in the *A. mellifera* population in certain regions of Europe. Moreover, studies from Spain suggested an association between *N. ceranae* infection and unusually severe honeybee colony losses. To assess the epidemiological situation in Germany, we collected 1980 honeybee samples from 220 colonies in spring and autumn between 2004 and 2008. We microscopically analyzed all samples for the presence of *N.* sp. and used a highly specific molecular technique to differentiate between *N. apis* and *N. ceranae*. Our results show that in Germany *N. ceranae* is widely distributed but did not replace *N. apis* completely. No correlation between *N. ceranae* and colony losses could be demonstrated so far.

54. Distribution and interaction of *Nosema ceranae* infected bees (*Apis mellifera*) within the colony. *D. Michelberger*[1], S. Berg[2], *J. Tautz*[1] ([1] BEEgroup, University of Würzburg, Am Hubland, 97074 Würzburg, Germany; [2] Fachzentrum Bienen, LWG Veitshöchheim, An der Steige15, 97209 Veitshöchheim, Germany)

Trophallaxis events and their distribution within the nest influence the horizontal transmission of pathogens in a bee colony. We investigated whether infection of bees with *Nosema ceranae* results in differences in trophallaxis frequency and spatial distribution compared to uninfected bees. For this purpose, young bees were marked in a group-specific manner, fed with different *Nosema*-spore-solutions (type A 1 000 000; B 160 000;

C 0 spores/bee), introduced into test colonies, and observed in two day intervals. In trial A (50 bees/type A and B), the life-span and the trophallaxis events (feeding contacts in 2 minutes/bee) were recorded. In trial B (150 bees/type A, B, C), the positions of the test bees in the colony were identified by evaluation of photographs (trial B1+2).The lifespan of infested bees (20.6 days +/– 4.7 days) was significantly shorter than the lifespan of the control group (26.2 days +/– 6.7 days; Wilcoxon, $P < 0.001$). In general, the trophallaxis events increased with the age of the bees. Although there were no significant differences between the infested and the control groups (Mann-Whitney-U-Test $P > 0.285$), there was a tendency for older infested bees to reduce participation in trophallaxis events. Regarding the distribution of the bees within the colony (trial B), the brood nest area was the preferred area of congregation, irrespective of an infection. In general, the distribution in the colony did not differ significantly between the test groups (Wilcoxon $P = 1.0$). The tendency of older infested bees to participate less in trophallaxis might indicate their exclusion from social-feeding only after massive multiplication of the pathogen. The survey on the distribution of infested bees does not suggest a separation of diseased bees from healthy bees within the colony.

55. Establishment of a method for manipulation of *Paenibacillus larvae*. *L. Poppinga*, *E. Genersch* (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Genetic analysis of *Paenibacillus larvae*, the causative agent of American foulbrood (AFB), identified four different genotypes (ERIC I-IV), which clearly differ in terms of their virulence. Using Suppression Subtractive Hybridization (SSH), several potential virulence factors were detected in the genome of ERIC I. In order to understand their role in pathogenesis, the generation of knock-out mutations in the corresponding genes has been established to further compare the co-mutants with the corresponding wildtypes in various assays. By means of the integration vector pCP115, the corresponding genes in the *P. larvae* genome can be disrupted. Therefore, insertion of a gene fragment into the vector pCP115 was carried out. Selection and reproduction of the manipulated integration vector in *E. coli* was followed by transformation of *P. larvae* by electroporation. By transforming *P. larvae*, the plasmid integrates into the chromosomal locus in a single, homology-driven recombination event. Integration splits the targeted gene into two incomplete parts, interrupted by the inserted plasmid sequence. As the application of integration vectors into *P. larvae* is a novel method, different recombinant vectors were constructed in order to increase the chances of a successful knock-out mutation. Gel electrophoresis of restriction- and PCR-analyses yielded clear bands of correct size. In addition, DNA sequencing and growth curve analysis of the *E. coli* clones showed the cloning strategy to be successful. Due to the loss of tetracyclin resistance resulting from the insertion of the fragment in the vector, *E. coli*-clones exhibiting recombinant plasmid showed no growth in media containing tetracycline. Currently, the transformation of *P. larvae* by electroporation with the constructed vectors is being optimized.

56. Comparative genome analysis within the species *Paenibacillus larvae*. *A. Fünfhaus*, *E. Genersch* (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

The four common different genotypes ERIC I - ERIC IV of *Paenibacillus larvae* (*P. larvae*), the causative agent of American Foulbrood (AFB), differ from each other in features like virulence, metabolism, and also in the morphology of their colonies and spores. In order to identify the underlying molecular factors of these phenotypical characteristics, the PCR based method of Suppression Subtractive Hybridization (SSH) was applied. This method enables the isolation and identification of genetically variable sequences. The obtained sequences were compared to the EMBL/GenBank database using the BLASTx algorithm. Thereby we obtained evidence for the homology of the SSH sequences to other proteins. Based on this information, the SSH sequences were classified in the Cluster of Orthologous Groups of proteins (COG). The main groups of COG are: information storage and processing, cellular processes and signalling, metabolism, and poorly characterized proteins. The classification comprised every COG main group. Several sequences showed homologies to proteins of the metabolism. These results correlated with the outcome of the recent biochemical characterisation of different genotypes of *P. larvae*. Furthermore, SSH sequences were detected which showed homology to different toxins, as well as nonribosomal peptide synthases (NRPS) and polyketide synthases (PKS). These proteins constitute putative virulence factors of *P. larvae*. However, most of the SSH sequences were assigned to the COG main group of the poorly characterized proteins. These

results domonstarte how little *P. larvae* and comparable organisms have been characterised.

58. Establishment of an *in vitro*-model for DWV-infections. *N. Möckel**, *S. Gisder**, *E. Genersch* (* These authors contributed equally) (Institute for Bee Research, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Deformed wing virus (DWV) is a viral pathogen of the European honeybee (*Apis mellifera*). As is typical for honeybee viruses, DWV normally causes covert infections. However, when transmitted by the ectoparasitic mite *Varroa destructor* (*V. destructor*) to pupae DWV is able to cause overt infections characterized by adult bees emerging with malformed appendages, shortened abdomen and discolorations. Recent studies demonstrated that overt infections are also characterized by viral RNA detection in total RNA isolated from bees' heads and that DWV infection can lead to learning deficiencies. Taken together this indicates the possibility that DWV is able to infect the brain and the nervous system. To analyze the interaction between DWV and its target cells we established an in vitro-cell culture model for DWV using primary neuronal cells of honeybees isolated from mushroom bodies. We showed that these neuronal cells were indeed permissive for DWV. Successful infection of non-infected cells with purified viral particles was verified by RT-PCR-analysis, in situ-hybridisation, and Western blot analysis. This in vitro-model will now be used to investigate host cell-pathogen interactions in more detail.

60. Oxalic acid: Toxicology on *Apis mellifera.* *E. Rademacher*, *M. Harz* (Free University, Institute of Biology/Neurobiology, 14195 Berlin, Germany)

Oxalic acid is the most important organic acid used for the control of *Varroa destructor*. The trickling method combines high efficacy against *V. destructor* with good bee tolerability. So far, no toxicology data on individual bees in the laboratory have been available to establish a dose-response-relationship for oxalic acid. Additionally, its mode of action in mite control is still unclear. In laboratory trials, oxalic acid dehydrate (OA) solution dissolved in sugar-syrup (50%) was applied to bees in different dosages using two application forms: trickling on the abdomen (175, 250 or 345 μg OA/bee) or individual feeding (10, 50, 75 or 100 μg OA/bee). Every dosage was tested on n = 60 bees (3 × 10 bees per cage, one replicate). The control groups were treated with sugar-syrup (50%) and kept under the same laboratory conditions (22 °C and 62% relative humidity). The bee mortality was determined in intervals of 24 hours for a three day period. The statistical analysis was conducted using the Mann-Whitney *U*-Test ($P \geqslant 0.05$) on the total bee mortality after 72 hours. After topical application of OA the toxicity increased slowly during the intervals of the observation time. After 72 hrs the dosage of 175 μg/bee, corresponding to the 3.5% solution used in beekeeping practice, did not cause mortality different from controls. Application of 250 μg led to increased mortality. After application of 345 μg OA the bee mortality was significantly higher (MWU, $P \geqslant 0.05$). Bees reacted much more to the oral application of OA and the maximal toxicity was reached 48 hrs after treatment. After 72 hrs, 10 and 50 μg did not cause mortality different from the control group, while 75 μg caused significantly higher bee mortality (MWU, $P \geqslant 0.05$). 100 μg killed 55% of the treated honeybees. When bee colonies in the field are treated with OA we can assume that bees do not ingest OA in large amounts as the tolerability under beekeeping conditions is well proven. With high probability, OA acts as a contact poison and not systemically against the mite *V. destructor*.

62. Comparison of 60% and 85% formic acid treatments against *Varroa destructor.* *S. Berg*, *F. Schürzinger* (LWG, Fachzentrum Bienen, 97209 Veitshöchheim, Germany)

We compared the efficacy and compatibility of 60% and 85% formic acid treatment, both individually and in combination as summer treatments for the control of *V. destructor*. A total number of 57 colonies, divided in three groups (1–3), was treated after the last honey harvest in 2008. Group 1 (n = 20) was treated three times with 60% formic acid on foam tissue in 3 days intervals (2 mL per comb from above). Group 2 (n = 17) was accordingly treated with 85% formic acid. In group 3 (n = 20), 85% formic acid was applied with the Liebig-Dispenser (liquid dispenser with paper wick, two treatments with 100 mL and 200 mL, feeding of colonies in between). In December, oxalic acid (trickling method) was used to determine the efficacy of the treatments. In terms of mite mortality the treatment in group 1 was significantly (Kruskal-Wallis, $P = 0.003$) less effective (86.1% ± 7.8%) than the treatments in group 2 (91.5% ± 4.0%) and group 3 (92.3% ± 2.9%). Those of group 2 and 3 did not differ significantly. Both 85% formic acid treatments were independent of the number of *V. destructor* (ranging from 290 to 1700 per colony) while the efficacy of 60% formic acid was reduced with higher *V. destructor* loads (ANOVA, $P = 0.002$). During

the experiment the temperature raised up to 30 °C, resulting in brood mortality of about 20% in the upper brood chamber only in group 2 and 3. In group 2, one queen loss and in group 3 two queen losses were recorded at the end of the treatments. The treatments with 85% formic acid confirmed in general a higher and consistent efficacy compared to the treatment with 60% formic acid. The brood mortality and queen losses due to high temperature during treatments with the 85% formic acid might be reducible by placing the brood combs in the brood chamber beneath. Our experiments reveal 85% formic acid as an appropriate treatment against Varroosis.

63. The enemy of my enemy...the impact of entomopathogenic fungi on *Apis mellifera* and *Varroa destructor.* *M. Holt*, *P. Aumeier*, *W.H. Kirchner* (Ruhr-University Bochum, Faculty of Biology and Biotechnology, 44780 Bochum, Germany)

Previous research on antagonistic fungi as a promising approach for controlling *Varroa destructor* produced contradictory results. We, therefore, evaluated four strains belonging to entomopathogenic fungi species which are supposed to be highly effective against this honeybee parasite. Strains of *Paecilomyces fumosoroseus*, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* were cultivated on malt-extract-agar. Application dose was 1×10^6 conidia per larva and bee, respectively. Larvae were maintained according to Peng (1995) except the addition of yeast extract. Adult bees were kept in standard boxes with 50 bees and 25 mites each. Larval or bee mortality, mite mortality and growth of fungi from fallen mites were recorded every day. In the *Beauveria bassiana* group and in the *Verticillium lecanii* group, bee mortality was significantly increased (compared to the control) at days four and six ($P < 0.05$, t-test, two-sided), and at days four and seven ($P < 0.05$), respectively. However, neither in the larval nor in the adult honeybee assay was a significant mite-specific impact observed. Increased growth of fungi from mite carcasses could only be observed at day seven in *Beauveria bassiana* group ($P < 0.05$, Fishers exact test, two-sided). We conclude that the weak effect of the investigated fungi is caused by mycotoxins ingested together with the conidia. However, we could not confirm a lethal growth of fungi in mites. Therefore, the analyzed fungi do not provide a basis for the control of *Varroa destructor.*

64. Mimicking natural selection for Varroosis tolerance without sacrificing colonies in the Marmara island bee population. *I. Cakmak*[1], *S. Cakmak*[1], *S. Fuchs*[2] ([1]Uludag Universitesi, Mustafakemalpasa MYO, M. Kemalpasa-Bursa,Turkey; [2]Institut fuer Bienenkunde, Polytechnische Gesellschaft, Faculty of Biological Sciences, Goethe-Universitaet Frankfurt am Main, 61440 Oberursel, Germany)

Natural selection and experiments with honey bee populations untreated for Varroa have been reported to eventually result in honey bee colonies surviving or showing higher tolerance. We here propose an approach mimicking natural "live or die" selection compatible with current beekeeping by avoiding massive colony losses. Colonies are treated only if their infestation exceeds a critical threshold, which exerts selection pressure against *V. destructor* mites with high population increase. Then highly infested colonies are requeened, inducing a "genetical death" of the susceptible colony but preserving the worker bees. We explored the practicability of the method on Marmara Island, Turkey, in July 2008. Honeybee infestation was determined in 217 colonies owned by 5 beekeepers. 50 g bee samples were washed in detergent water (0 to 18 mites/100 bees, median = 0.43). 39 colonies with no mites in the samples were left untreated, all other colonies were treated (Bayvarol© strips). Dead mites on bottom inserts after treatment (0 to 1098 mites, median 39) were highly correlated with bee infestation ($r = 0.82$, $P < 0.0005$). In 34 highly infested colonies (infestation > 1.3) queens were caged and removed after 6 days, and colonies received brood combs from untreated, low-infested colonies to produce a new queen from these. 23 out of 26 requeened colonies had produced a new queen in September. We conclude that a differential treatment of high-infested colonies combined with requeening can be realistically performed even under comparatively undeveloped bee keeping conditions. Though measurable changes in average colony infestation may develop only over years, such a low-level broad-scale selection, integrated into common beekeeping practice, could become an important component in efforts to increase bee tolerance to Varroosis.

66. The impact of honeybees (*Apis mellifera*) on the seed production of rape (*Brassica napus*). *J. Radtke*[1], *T. Pfannenstill*[2] ([1]Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany; [2]Landesamt für Verbraucherschutz, Landwirtschaft und Flurneuordnung des Landes Brandenburg, Referat Acker- und Pflanzenbau, Berliner Str., 14532 Güterfelde, Germany)

Despite the decline of beekeeping, the yield of rape crops increased considerably during the last decades. Thus, farmers claim that modern varieties of rape do not require pollination by honeybees. Is this the case? In 2007 we conducted an analysis in areas (agricultural land grade 40 = loamy soil) cultivated with the winter-rape hybrid cultivars "Titan" and "Taurus" and in 2008 we repeated the analyses in areas with "Taurus". The adjacent rape fields were at least 500 m long and covered an area of about 30 ha each. Bee colonies were installed on one side of the fields in different apiaries. Populations of wild bees (solitary bees and bumblebees) were located in distant fallows separated by further cultivated areas. Due to the low stocking rate of only 0.3 hives per ha, we expected a considerable shortage of honeybees in the canola fields. Consequently, the bees were expected to forage mainly in the vicinity of their apiaries. Along harvested aisles, we took samples from areas of one square meter at different distances to the apiaries and threshed them separately. In 2008 we conducted additional observations on bee visitation to rape flowers. The highest number of honeybees visiting flowers per area and hour was found within a distance of 60 m to an apiary (23 visits/m^2/h). The number decreased significantly with increasing distance (R^2 = 0.8, b = −0.045). Wild bees were evenly distributed over the area with 0.4 ± 0.15 visits/m^2/h. The value for the seed production was corrected by the number of plants per square meter and biomass per square meter. In 2007 the yield at a distance of 15 to 60 meters to the apiaries averaged 4820 ± 220 kg/ha and was 39% higher compared to the yield at a distance of 300 to 500 m (3480 ± 190 kg/ha; $P < 0.001$; t-test; n = 54). However, in relation to the very high overall yield in 2008, the increase close to the apiaries amounted to 16% overall, compared to areas at a distance of 300 to 500 m to the apiaries (6900 kg/ha ± 224 and 6000 kg/ha ± 210; respectively, $P < 0.01$; t-test, n = 54).

67. The benefit of honeybees (*A. mellifera*) to wild plants using the example of *Prunus spinosa*. *J. Radtke*, *E. Etzold* (Länderinstitut für Bienenkunde, Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

The benefit to cultivated plants which are pollinated by honeybees (*Apis mellifera*) has been shown in numerous studies. Here, we studied the effect of honeybees on a wild plant, *Prunus spinosa* L. We covered three shrubs with five cages each (mesh inner width 2.5 mm) on 29.03.2007 before the start of the blooming period, thereby excluding honeybees and other large pollinating insects from 3400 blossoms. Additionally, we examined untreated branches with a total of 4498 blossoms. The shrubs were located at a distance of 50 m from an apiary in an area with a manifold plant cover. Honeybees, bumblebees, solitary bees, beetles and sporadic butterflies were observed on the blossoms of the untreated branches. All blossoms of the untreated branches were withered after 18 days on 04/16/2007, while the blossoms of the covered branches flowered six days longer. While counting the number of fruits on 05/10/2007, we found a high percentage of extremely small fruits with a diameter less than 2 mm (28% of all fruits on untreated branches, 99% on caged branches). These fruits were soon discarded by the plant. On 07/24/2007, the average fruit load per blossom of the untreated branches was 14.9%. The load of different shrubs varied between 2.5 and 23.8%. Hardly any fruit was found on the caged branches (0.1%). However, these few ones developed as well as the ones on the untreated branches. We assume that these blossoms were in direct contact with the cages and could be pollinated from outside. In 2008, the untreated branches had less blossoms compared to the caged ones, pointing out the well known alternation of cultured fruit trees. Moreover, only few fruits were found on the branches of all shrubs.

69. A laboratory method for detection and quantification of BT-corn Mon 810-pollen in honey. *S. Gisder*, *E. Genersch*, *B. Lichtenberg-Kraag* (Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Friedrich-Engels-Str. 32, 16540 Hohen Neuendorf, Germany)

Since the registration of genetically modified (GM) plants like Bt-corn for cultivation, many consumers are unsettled. Beekeepers are confronted with the problem whether their natural product honey is free of material from GMO. Together with the nectar, pollen is taken up by the bees. The amount of pollen found in honey is on average 0.05% and has been defined as a product-typical contamination: the percentage of pollen in honey is below 0.9% and must be understood as an accidental, technically unavoidable admixture for which labeling is unnecessary. Honey containing pollen or nectar from GM plants is legally unaffected by EU Directive 1829/2003 on genetically modified food and animal feeds, since it neither consists of GMOs nor is produced from GMOs. However, some producers of organic honey ask for a certificate, such as, for instance, "GMO-free-honey". The threshold for contamination by GMO in organic production

of agricultural products is 0.1%. A qualitative and quantitative PCR-method was developed to detect pollen of the genetically modified Bt-corn Mon 810 (Monsanto) in honey. Bt-corn is a variant of maize, genetically altered to express the bacterial Bt toxin, which is poisonous to the European Corn Borer. Maize is not a nectar plant, and bees only utilize it as a pollen source. However, like other pollen transported by wind, it can also be detected in honey. Using this method, the DNA specific for corn and for the GM- Mon810 can be qualitatively detected, independently of the source of the pollen: plant, honey, or bee bread. The quantification procedure of the GM-fraction still needs to be improved, since the DNA of the small amount of pollen in honey is close to the detection limit. The results of the quantification of a commercial sample with a defined fraction of Bt-corn Mon 810 are in accordance with the manufacturer's data. This molecular biological laboratory approach can be used to analyze honey samples from local beekeepers to certify them as "free of GMO".

72. Pesticide use in rapeseed culture - are residues in honey unavoidable? *M.D. Meixner*[1], *I. Illies*[2], *R. Büchler*[1], *K. Wallner*[3] ([1]LLH Bieneninstitut Kirchhain, 35274 Kirchhain, Germany; [2]Fachzentrum Bienen, LWG, 97209 Veitshöchheim, Germany; [3]Landesanstalt für Bienenkunde, Universität Hohenheim, 70599 Stuttgart, Germany)

In rapeseed (*Brassica napus* L.), one of the most important nectar and pollen sources in apiculture, several fungicides are used during the flowering period. After initial detection in 2005, the active component Boscalid has been frequently detected in honey in considerable concentrations. The objective of our experiments in the years 2006–2008 was to minimize pesticide residues in honey by temporary closing of the flight entrances or by positioning the beehives at greater distances from the field. Experimental colonies were set up in six groups, where groups A, B, and C were positioned directly at the field, and groups D, E and F in distances of 200 m, 400 m and 800 m, respectively. Each group consisted of 3–4 colonies. Prior to application of the fungicide, the flight entrances of group B and C were closed for 24 h and 48 h, respectively. The colonies were provided with ample water and additional shading to prevent overheating. Groups B and C were omitted in 2008. The honey samples for residue analysis were collected immediately before and two and ten days after application of the fungicide. The weight gain of the colonies ranged from 1.1 ± 0.25 kg to 20.1 ± 8.1 kg, with temporarily closed colonies gaining less. However, the difference was only significant in group C (closed for 48 h) in 2006 (ANOVA, $F = 62.6$, $P < 0.001$). No Boscalid residue was detected in honey of group C (closed for 48 h) in 2006. In all other honey samples, residues were detected in variable quantities, ranging from 1.5 ± 0.5 to 91.2 ± 37.5 ppb. There were no significant differences between groups in each year. Although the application rate of the pesticide was the same in all three years, residues found in the honey samples differed considerably with higher differences occurring between years than between experimental groups. Presumably, weather conditions during the flowering period and the availability of other nectar and pollen sources play a significant role in determining overall residue levels in honey.

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