Bee Colony collapse disorder (CCD), Nosema and Varroa mite Infections (and many other suspects - except GM crops).

Klaus Ammann, ASK-FORCE, AF-1 Version 4. December 2016 open-source, 124 pages.
With 368 full text references, klaus.ammann@ips.unibe.ch

Contents

1. The Issue: false rumors about the Colony Collapse Disorder of Bees

1.1. Alarmist press releases:
The colony collapse disorder (CCD) is allegedly caused by GM crops

Read the article from the German weekly magazine DER SPIEGEL, the magazine maintains also a website in English: Der Spiegel Online International http://www.spiegel.de/ as a typical example of a sensational, unsubstantiated European press feature: In the headlines they ask: "Are GM Crops Killing Bees"? The German Contribution talks in the juicy headline about *"Aids in the Bee Hive"*. And instead of making their own allegations that GM crops are the real culprit, they interview a well-known beekeeper and fanatic opponent of GM crops. (the Spiegel has a well-known negative view on GMOs, recently continued by numerous articles

Latsch, G. (2007)
Aids im Bienenstock, Der Spiegel 12 pp 58-59 (Der Spiegel Article)
English: http://www.ask-force.org/web/Bees/Latsch-CCD-Spiegel-2007.pdf

Latsch, G. (2007)
Electronic Source: Are GM Crops Killing Bees? , Der Spiegel Online International, published by: Der Spiegel Online March 22, 2007
Spiegel Online for free: http://www.spiegel.de/international/world/0,1518,473166,00.html

"Walter Haefeker, the German beekeeping official, speculates that "besides a number of other factors," the fact that genetically modified, insect-resistant plants are now used in 40 percent of cornfields in the United States could be playing a role. The figure is much lower in Germany—only 0.06 percent—and most of that occurs in the eastern states of Mecklenburg-Western Pomerania and Brandenburg. Haefeker recently sent a researcher at the CCD Working Group some data from a bee study that he has long felt shows a possible connection between genetic engineering and diseases in bees.

The study in question is a small research project conducted at the University of Jena from 2001 to 2004. The researchers examined the effects of pollen from a genetically modified maize variant called "Bt corn" on bees. A gene from a soil bacterium had been inserted into the corn that enabled the plant to produce an agent that is toxic to insect pests. The study concluded that there was no evidence of a "toxic effect of Bt corn on healthy honeybee populations." But when, by sheer chance, the bees used in the experiments were infested with a parasite, something eerie happened. According to the Jena study, a "significantly stronger decline in the number of bees" occurred among the insects that had been fed a highly concentrated Ask-force.org/web-poison feed.
According to Hans-Hinrich Kaatz, a professor at the University of Halle in eastern Germany and the director of the study, the bacterial toxin in the genetically modified corn may have "altered the surface of the bee's intestines, sufficiently weakening the bees to allow the parasites to gain entry—or perhaps it was the other way around. We don't know." Of course, the concentration of the toxin was ten times higher in the experiments than in normal Bt-corn pollen. In addition, the bee feed was administered over a relatively lengthy six-week period.
Kaatz would have preferred to continue studying the phenomenon but lacked the necessary funding. "Those who have the money are not interested in this sort of research," says the professor, "and those who are interested don't have the money." Latsch (2007)

The irony is, that Prof. Kaatz got an additional hefty sum of 300'000 Euro to finish up his studies, his report was not available for many years to the Monitor experts (the author of this report was a member of the expert group). Prof. Kaatz was very unhappy about this illegally broken embargo with the preliminary alarm despite clear promises by the Spiegel journalist. The author of this report tried to help him, but it was simply too late and Prof. Kaatz himself did not want to exacerbate the dispute further on – unfortunately. The final report Kaatz (2004) then stated without any doubt that negative impacts could be excluded completely. All details in the chapter 3.3 of this report, ruling out GMOs as a reason for the CCD. It is astonishing, that the report has never been published. Citations from the report in chapter 3.3. In such disputes, a new phenomenon appears to work with success: the denial and automatic rejection of scientific work and expertise, nowhere in this unfortunate Spiegel-triggered controversy there was mention of the well-known and well published expertise of Prof. Hand Hinrich Kaatz, here just two examples of his earlier scientific work on bees: Kaatz, Hagedorn, et al. (1985, Kaatz, Hildebrandt, et al. (1992).

Nevertheless, bad news always finds the way into the journals, whether proven or not, is not important, a typical alarmist publication by the GMO journal: Gitlin Boris (20090924)

This kind of alarmist news was (and still is) usually exacerbated by a bogus Einstein citation:

"If the bee disappears from the surface of the earth, man would have no more than four years to live."

Although a juicy sentence from a world authority and often used, it nevertheless is a bogus citation after Shapley Dan (20110702, Snopes (2007).

1.2. False negative lobby reports of (organic) Bee Keeper Associations

Two papers are available from W. Haefeker, dealing with unproven allegations against GM crops and GM trees Haefeker (2000, Haefeker (2008), and his latest attempt to make up a story on “honey contaminated with Bt crop pollen did not really impress regulators: There are no facts presented, just lobby statements of beekeepers, see the critical comment of Mueller-Jung (2008) and Winter (2007) in a controversial case of organic farmer lobby against scientific risk assessment research at the University of Giessen, Germany. German PM Christel Happach-Kasan, speaker of the Liberal Party on agriculture and biotechnology and member of the parliamentary expert group on genetic engineering pointed to another absurdity of GM regulation related to honey as early as 2009: She made it clear, that there is no legal requirement to destroy honey ‘contaminated’ with pollen from Bt-maize Happach-Kasan (2009). Self-imposed rules established by organic farmers require destruction of Bt contaminated organic farmers, but there is no legal requirement for financial compensation. And interestingly enough, organic lobbyists claim to be forced to destroy this kind of contaminated honey although the legislators and regulators did first not require this. In an utterly absurd movement the anti-GMO honey lobbyists went through all the courts and actually succeeded even on the highest level of the EU courts to treat honey as GM product because of possible spurious contents of Bt-pollen could be found. More about this debate between German organic farmers and GM crop promoters under the website Bienen & Agrogentechnik Mellifera (20131008), again with lots of incorrect statements. The controversy whether organic honey contaminated by Bt-maize pollen was then successfully promoted in local, national and finally international courts with some contradicting sentences. Winter (2007) and finally the highest court decided to label Honey containing Bt maize pollen – frankly – an absurdity not based on science and hurting the honey producers in a deeply unfair way. Fortunately, the judgement has been overturned later Davison John and Kershen Drew (2014, Hoefer Eberhard and Jany Klaus (2014, Jany (2012). See for details in chapter 4.19 on legal aspects.

1.3. More cheap propaganda blaming GM crops for CCD, without scientific evidence

Donovan (2009)
Electronic Source: Genetically Modified Crops Implicated in Honeybee Colony Collapse Disorder Global News about Mother Nature http://globalnewsaboutmothernature.blogspot.com/
http://globalnewsaboutmothernature.blogspot.com/2009/01/genetically-modified-crops-implicated.html AND
http://www.ask-force.org/web/Bees/Donovan-GM-crops-Implicated-in-CCD-20090122.pdf

The Institute of Science in Society also did not miss to imply GM crops as a cause, although they do not cite (typically enough) a shred of scientific evidence.

van Ho and Cummings (2007)
Electronic Source: Mystery of Disappearing Honeybees, published by: Institute of Science in Society
http://www.i-sis.org.uk/MysteryOfDisappearingHoneybees.php AND
http://www.ask-force.org/web/Bees/VanHo-Mystery-Disappearing-Honeybees-20070407.pdf

"The possibility that GM crops in North America is contributing to the decline in honeybees was given little consideration by the NRC Committee even though the timing of the honeybee decline appears to coincide with the widespread deployment of GM crops. GM crops are engineered to tolerate herbicides, especially glyphosate, or to contain bio-pesticides (the Bt Cry toxins from Bacillus thuringiensis), or both. The bio-pesticide toxins produced in Bt crops are not highly or acutely toxic to bees, but are toxic to butterflies, moths and beetles. Nevertheless, in some instances, the toxins can kill bees or modify their behavior."

But the top hoax of the GM-opponents thesis comes from a very particular blog which involves as an absolute authority "the Great Spirit":

Whitedove (2009)
Electronic Source: Psychic Answers: Where have the Honeybees gone?
http://www.michellewhitedove.com/Blog-HoneybeeGMplants.php AND
http://www.ask-force.org/web/Bees/Whitedove-Blog-HoneybeeGMplants-2009.pdf

The "number one psychic" of the US got a message from the *Great Spirit*: it must be the GM crops causing the CCD:

"Decided that the bee mystery needs to be solved and so I asked Great Spirit.. Spirit told me that the major contributing factor to the loss of our Honeybee population is due to the genetically engineered crops that are now being processed on a massive scale, not just the plants, but even the seeds are now being genetically mutated".

It is rewarding to see, that GM crops have fallen out of the CCD debate, mainly because there was not a shred of evidence making the connection. Even one of the most ardent GM opposition website Natural News is now conceding indirectly, that CCD must have other causes like parasites etc. Huff Ethan A. (20121003). Anything like a direct confession of wrongdoing would be a great surprise.

But wait, there are still some die-hearts resisting to the facts, still blaming GMOs as a threat to bees and one of the reasons for CCD: Mellifera (20131008).

Summary

The assumption, that GM crops could be the cause of Colony Collapse Disorder (CCD) is not substantiated in any scientific documentation of peer-reviewed journals. CCD happened decades before ever GM crops showed up. CCD has also recently been reported from many regions in Europe, where the acreage of GM crops up to now (2009) remains zero to small.

There are a number of different hypothesis named as the cause of the CCD documented in the scientific literature, but GM crops per se can be ruled out as a cause and therefore the false claims can be labeled as anti-GM-crop scare propaganda. The possible causes – still not really nailed down as the one main factor – which it might never be. The extensive enumeration below names infections of parasites, fungi, viral infection etc., as a sum multiple stress factors have also to be taken into account, as we will see below. Recent report for EFSA confirms this summary Hendrikx, Chauzat, et al. (2009). Recently the COLOSS study has been finalized including extensive chapters on standardization of CCD research. See the extended summary of this most comprehensive report published in 2012-2013 Williams, Dietemann, et al. (2012): The Varroa mite is in the center of all concerns, its aggressive colonization of bee hives, in many cases combined with a lower resistance of the bees due to environmental stress of various kinds makes the situation rather complex.

As an excellent summary the FORBES piece of Jon Entine is recommended: Entine Jon (20130430), it demonstrates the politics of GMO-scaremongering of Greenpeace with no scientific background – in short – a very weak justification which actually can be denominated as a crime against humanity: Moore Patrick and Morano Marc (20120910).

2. Introduction to CCD: General comments and reviews

2.1. Reports of CCD from times when GM crops did not exist yet

As a further proof (besides the fact that CCD occurs in Europe too, which is practically GMO-free) that GM crops cannot play a role in CCD, see also some scientific publications from times, when GM crops did not exist yet, at that time the researchers from the Ohio State University Agricultural Research and Development Center called the phenomenon 'Disappearing Disease': in a comprehensive overview, Kulincevic et al published a report for the Research center in Ohio with extensive historic references on the CCD:

Kulincevic, J. M., Rothenbuhler, W. C., & Rinderer, T. E. (1984). Disappearing Disease .3. a Comparison of 7 Different Stocks of the Honey Bee (Apis-Mellifera). *Ohio Agricultural Research and Development Center Research Bulletin*(1160), pp. 1-21. <Go to ISI>://A1984TD14900001 AND http://www.ask-force.org/web/Bees/Kulincevic-Disappearing-1984.pdf AND https://kb.osu.edu/dspace/handle/1811/24687

"Reports of disappearing bees have come from a number of countries over more than 100 years"

Disappearing disease of the Honeybee is a mysterious phenomenon known for many decades. Strong colonies suddenly become weak. Few or no dead bees are seen; bees simply disappear over a few week's time. Many colonies are lost completely.
Reports of disappearing bees have come from a number of countries over more than 100 years. An early example is "the disease of 1868" which struck in Indiana, Kentucky, and Tennessee in anonymous reports: Anonymous (1869, Anonymous (1874).
In the report on Agriculture to the US commissioner 1969 for 1968 the enigmatic and serious disease is already given with precise accounts on p. 278-280:

"During the past season a disease suddenly appeared in Indiana, Kentucky, and Tennessee, sweeping away whole apiaries. So quiet were its operations that the beekeepers became aware of its existence only by the disappearance of their bees. The hives were left, in most cases full of honey, but with no brood and little pollen; the whole appearance of the hives causing the casual observer to suppose that the bees have emigrated."

Other examples have come from Australia Beuhne (1910, Beuhne (1916) from Louisiana and Texas in 1963-64 Oertel (1965, Williams and Kauffeld (1974), from California in 1964-65 Foote (1966), from Mexico in 1977 (Mraz, personal communitation), from the Rio Grande Valley in Texas in 1974 Kauffeld, Everitt, et al. (1976), since at least 1996 Nosema-infections exist in Italy Ferroglio, Zanet, et al. (2013). Judging from these reports, there can be no doubt that many beekeepers have suffered devastating losses of bees for many decades, long before GM crops have been introduced. An extensive early review of literature of disappearing disease (DD) is given by Wilson and Menapace Wilson and Menapace (1979). Various explanations of the cause of the losses have been advanced but most have had little support. Furthermore, there is no reason to believe that all losses gathered under the umbrella of DD are due to the same cause. In fact, many of the earlier cases seem to have occurred In the fall or early winter, whereas some of the later cases Wilson and Menapace (1979) have occurred in the spring. A prominent hypothesis over the last few years has involved some sort of stock deterioration Wilson and Menapace (1979). It has been suggested that such deterioration may have resulted:

- *From the admixture of African bee genes to the gene pool of North American bees,*
- *From excessive inbreeding of bee stocks, or*
- *From the mal-adaptation to northern climates of bees reared over many generations in the South.*

If such genetic weaknesses exist, it should be possible to obtain evidence of them by a careful comparison of DD with non-DD stocks in the same location. Furthermore, such an investigation should reveal something about the range of variation in North American bees. Is there sufficient variation to insure success for a program of genetic selection, or are our bees reduced to a uniform genetic mediocrity? Do we have the genetic variation to deal success-fully with Africanized bees?
This investigation was designed to compare several stocks of bees with respect to colony population amount of brood, honey, and pollen presence of common bee diseases; and the possible presence and causes of Disappearing Disease." Kulincevic, Rothenbuhler, et al. (1984), see also other papers of the same authors group: Kulincevic, Ball, et al. (1990, Kulincevic and Mladjan (1988, Kulincevic, Stairs, et al. (1969, Kulincevic, Rinderer, et al. (1991A, Kulincevic, Rinderer, et al. (1991B, Kulincevic, Rinderer, et al. (1992, Kulincevic, Rinderer, et al. (1988, Kulincevic, Rothenbuehler, et al. (1973, Kulincevic and Rothenbuhler (1975, Kulincevic and Rothenbuhler (1982, Kulincevic and Rothenbuhler (1989A, Kulincevic and Rothenbuhler (1989B, Kulincevic, Rothenbuhler, et al. (1982, Kulincevic, Rothenbuhler, et al. (1983).

2.2. Selection of important recent scientific reviews on CCD

Recent reviews on the CCD worldwide emphasize all *the multifactorial character of the CCD syndrome*: the EFSA report reviews this perspective properly: EFSA-Report (20140224)

Another positive review for neonicotinoides (not for fibronil!) comes from Gibbons et a. Gibbons, Morrissey, et al. (2014)

Gibbons, D., Morrissey, C. and Mineau, P. (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife Environmental Science and Pollution Research 1-16 pp ISBN/0944-1344 http://www.ask-force.org/web/Bees/Gibbons-Review-Direct-Indirect-Effects-Neonics-Vertebrates-2014.pdf

"Concerns over the role of pesticides affecting vertebrate wildlife populations have recently focused on systemic products which exert broad-spectrum toxicity. Given that the neonicotinoids have become the fastest-growing class of insecticides globally ,we review here 150 studies of their direct (toxic) and indirect (e.g. food chain) effects on vertebrate wildlife—mammals, birds, fish, amphibians and reptiles. We focus on two neonicotinoids, imidacloprid and clothianidin, and a third insecticide, fipronil, which also acts in the same systemic manner. Imidacloprid and fipronil were found to be toxic to many birds and most fish, respectively. All three insecticides exert sub-lethal effects, ranging from genotoxic and cytotoxic effects, and impaired immune function, to reduced growth and reproductive success, often at concentrations well below those associated with mortality. ***Use of imidacloprid and clothianidin as seed treatments on some crops poses risks to small birds, and ingestion of even a few treated seeds could cause mortality or reproductive impairment to sensitive bird species. In contrast, environmental concentrations of imidacloprid and clothianidin appear to be at levels below those which will cause mortality to freshwater vertebrates, although sub-lethal effects may occur****. Some recorded environmental concentrations of fipronil, however, may be sufficiently high to harm fish. Indirect effects are rarely considered in risk assessment processes and there is a paucity of data, despite the potential to exert population-level-effects. Our research revealed two field case studies of indirect effects. In one, reductions in invertebrate prey from both imidacloprid and fipronil uses led to impaired growth in a fish species, and in another , reductions in populations in two lizard species were linked to effects of fipronil on termite prey. Evidence presented here suggests that the systemic insecticides, neonicotinoids and fipronil, are capable of exerting direct and indirect effects on terrestrial and aquatic vertebrate wildlife, thus warranting further review of their environmental safety." From Gibbons, Morrissey, et al. (2014)*

EFSA-Report (20140224), Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations, EFSA Journal, 12, 3, 3594, pp. 102, doi:10.2903/j.efsa.2014.3594 AND www.efsa.europa.eu/efsajournal AND http://www.ask-force.org/web/EFSA/EFSA-Report-Integrated-Environmental-Risk-Bees-2014.pdf

"This report reviews recent work on bee health carried out by EFSA, Member States (MSs) and the European Commission (EC). It identifies data and knowledge gaps and provides research recommendations that may facilitate the transition towards an integrated environmental risk assessment of multiple stressors on bees. The report was produced by the EFSA Bee Task Force (TF), involved representatives from six different Scientific Units, and was coordinated by the Scientific Committee and Emerging Risks Unit (SCER). The TF consulted experts from MSs and the Bee Interservice Group of the EC. Additional scientific exchanges with experts were promoted by SCER through the organization of a scientific colloquium on bee health in May 2013. The review identified a total of 220 research projects on bee health at EU level (EFSA, 19; MSs, 181; EC, 20), and 33 additional projects from other international organizations dealing with general aspects, non-research-focused, of bee issues. A quantitative assessment of the retrieved projects revealed that research projects on multiple stressors on bees and projects on bees other than honeybees were missing, especially with regard to monitoring and testing. EFSA projects were predominantly in the area of risk assessments of pesticides on bees. Research projects on in-hive treatments and bee exposure to chemicals funded at the EC level were scarce, as were those focusing on protection goals, bee diversity and pollination services at the MS level. ***The qualitative assessment of the retrieved projects revealed knowledge gaps at each step of the risk assessment, which led to several recommendations for future scientific work at EFSA and research to be undertaken in the framework of Horizon 2020.*** *Additional recommendations are given for research coordination, planning and knowledge sharing with MSs and the EC. At EFSA level, further communication, internal collaborations and training on bee health are suggested."*
Acknowledgement: EFSA wishes to thank Edith Authié, Ryszard Laskowski and Robert Luttik for the reviewing of this report and the hearing expert: Gérard Arnold and EFSA staff: Domenica Auteri, Yann Devos, Jean-Lou Dorne, Diane Lefebvre, Tobin Robinson, Agnès Rortais, Franz Streissl, Csaba Szentes, Simon Terry, Frank Verdonck and Sybren Vos for the support provided to this scientific report. EFSA-Report (20140224)

Summary:
In accordance with the strategy of the European Food Safety Authority (EFSA) to consider risk assessments in a wider integrated manner, the Scientific Committee and Emerging Risks (SCER) Unit set up an internal Bee Task Force (TF) to review the work carried out by EFSA, Member States (MSs) and the European Commission (EC) in the area of bee health, and to identify knowledge gaps and provide recommendations facilitating the transition towards an integrated environmental risk assessment of multiple stressors on bees.
The EFSA Bee TF was composed of (scientific) officers from four EFSA Directorates (i.e. the Science Strategy and Coordination, the Scientific Evaluation of Regulated Products, the Risk Assessment and Scientific Assistance and the Communications Directorates) and six scientific EFSA Units (SCER, Animal Health and Welfare, Genetically Modified Organisms, Pesticides, Plant Health and Scientific Assessment Support Units 4).
The multi-disciplinary composition of the Bee TF fostered an open dialogue on risk assessment approaches between Units and exchanges of information across scientific fields. This enabled the reinforcement of internal collaborations and the use of internal scientific expertise in the area of bee health.

To review research work produced in the area of bee health, the Bee TF scrutinized its own work and conducted a series of consultations with MSs and the EC in 2012/13, in order to collect information on recent and ongoing research on bees. For MSs, information was requested through several networks of experts: the internal EFSA networks, Panels and Focal points and the international network Honeybee Colony Losses (COLOSS). To collect information from the EC, the Bee TF liaised with the EC Bee Interservice, which is composed of the five Directorates General (DGs) involved in bee issues (i.e. Agriculture and Rural Development (DG AGRI), Enterprise and Industry (DG-ENTR), Environment (DG ENV), Research and Innovation (DG RTD), and Health and Consumers (DG SANCO)). In addition, the SCER Unit organized a scientific colloquium on risk assessments of multiple stressors on bees in May 2013, in order to discuss and gather views from a wide range of stakeholders. Finally, to complete this inventory, the Bee TF compiled information related to bee issues mainly from the European Environment Agency (EEA), the European Medicines Agency (EMA), the European and Mediterranean Plant Protection Organization (EPPO), the Food and Agriculture Organization (FAO), the Organization for Economic Co-operation and Development (OECD), the World Organization for Animal Health (OIE) and the United Nations for Environmental Programs (UNEP). Most of this information (i.e. guidelines, standards, manuals and/or general facts and communication items on bees) was not analyzed by the Bee TF which focused its assessment on research data related to risk assessments of single and multiple stressors on bees.

The review of EFSA's work led to the identification of 16 published outputs (Appendix A) and three ongoing activities (Appendix B). Consultations with the EC and MSs identified 201 projects, of which 20 were from the EC (Appendix C) and 181 were from MSs (Appendix D). Fifty-seven per cent of these projects were finalized at the time of the completion of the consultation. An additional 33 projects, mainly from EEA, EMA, EPPO, FAO, OECD and UNEP, were retrieved (Appendix E).

The Bee TF performed quantitative and qualitative assessments of the retrieved projects in order to identify data and knowledge gaps, crosscutting issues in risk assessment and research needs. The data gap analysis was performed by making a quantitative assessment of the projects, which were categorized according to scientific area(s) (eight pre-defined areas), type of bee(s) investigated (i.e. honeybees, bumblebees and/or solitary bees), level of coordination (i.e. EC or MSs), and status in terms of project completion (i.e. still ongoing or completed in June 2013). This assessment was conducted on EFSA scientific outputs and projects retrieved from the EC and MSs. To identify knowledge gaps and research needs for the environmental risk assessment of multiple stressors on bees, the Bee TF also performed a qualitative assessment at each step of the risk assessment scheme. This assessment was conducted mostly on EFSA scientific outputs because the information retrieved from the EC and MSs could not be thoroughly assessed (e.g. most projects were still ongoing with no final or published results).

A pilot bibliometric analysis was conducted on a small set of EFSA scientific outputs in the area of risk assessment of plant protection products (PPPs) for bees. The objective of this analysis was to illustrate the usefulness of such an approach for the identification of experts and missing/required expertise.

The analysis of EFSA scientific outputs revealed that EFSA has initiated work on bee health since 2008 and that, since this date, its involvement and workload in this area has increased continuously, especially in 2013. The EFSA scientific outputs cover seven of the eight pre-defined areas (i.e. no project on in-hive treatments which is an area that does not fall under EFSA's remit). Most EFSA outputs are on the risk assessment of PPPs on bees and they were mostly produced by the Pesticides Unit. The least covered areas are in the areas of —protection goals/bee diversity/pollination services|| and —bee pathogens/pests/predators||. Finally, most of these studies tend to focus on honeybee species (Apis mellifera spp.).

The number of projects collected from the EC and MSs was quite large. However, the list was not exhaustive and sometimes the information provided was incomplete or not publicly accessible. Most EC-funded projects (16/20) were received from DG RTD. However, the number of projects funded partly by DG AGRI (and MSs) is underestimated since such projects are mostly reported by MSs. Projects from the EC dealing with in-hive treatments and bee exposure to PPPs are not well represented or are absent, and projects on protection goals/bee diversity/pollination services coordinated by MSs are rare. Overall, whether at the EC or MS level, the number of projects on the risk assessment of multiple stressors on bees was low.

At the EC and MS levels, research on bees other than honeybees (i.e. bumblebees and solitary bees) is generally missing, in particular at the MS level and with regard to the fields of monitoring and testing. In addition, although there is a wide diversity of honeybee subspecies and ecotypes, with specific environmental adaptations, in Europe, research on honeybees usually focused on a few subspecies. Finally, too little research is conducted on honeybee reproduction to provide explanations on the troubles observed by beekeepers on queens and drones (e.g. abnormal laying behavior and shorter longevity in queens, sterility in drones, etc.).

To consolidate the transition towards an integrated environmental risk assessment of multiple stressors on bees, the Bee TF made a set of recommendations: recommendations for future scientific work to be undertaken by EFSA and the EC (DG RTD) through the framework of Horizon 2020; recommendations on how to tighten coordination and planning of research in Europe and enhance knowledge sharing with MSs and the EC; and finally, recommendations to strengthen communication, promote internal collaborations and to develop training on bee health at EFSA. For various aspects of the environmental risk assessment, specific recommendations are given, focusing on: problem formulation and protection goals for bees and pollination services (e.g. harmonization of risk assessment approaches to set protection goals, assessment of changes in pollination services with bee diversity); monitoring and exposure to bees (e.g. long term EU-wide monitoring of all types of bees; applied research for the development of calibrated tools and validated methods to assess bee mortality, colony development and sub-lethal effects in bees in field conditions; occurrence data of residues from several classes of chemicals including PPPs, veterinary medicines and contaminants in various matrices relevant for bees such as pollen, bee bread nectar, beeswax, honeydew, water, guttation (etc.); data on foraging and food intakes by honeybees, bumble bees and solitary bees; data on the nutritive value of different pollen types and on the sugar content in nectar; metabolism of xenobiotic in bee midgut; development of single- and multi-residual analysis methods with low limits of detection and quantification); hazard identification for different classes of chemicals (and their metabolites), including PPP and contaminants (e.g. dose–response relationships and species sensitivity distributions for bees; toxicokinetics and toxico-dynamics for the different chemicals and bee species; toxicity data for bees under different temperature ranges and types of diet; standardized laboratory tests for acute and chronic toxicity of lethal/sublethal endpoints to multiple chemicals and contaminants; standardized laboratory tests for toxicokinetics of single and multiple doses; critical review of behavioral and physiological protocols to assess sub-lethal and chronic effects in bees; population dynamics-based models to predict effects at the colony level; modelling techniques to extrapolate observations from individual to population level and to test multiple stressors and co-exposures; molecular markers for bees with omic techniques); risk assessment (e.g. case studies for risk

characterization, uncertainty analysis using deterministic and probabilistic models for single and multiple stressors; quantitative weight of evidence approach).
To tighten coordination of research in Europe, the Bee TF advocates the establishment of a group of experts or a network composed of the various stakeholders identified in this review (e.g. EFSA, the EC Bee Inter-service Group, the European Reference laboratory on bee health, EMA and experts from MSs), in order to develop methodologies for the risk assessment of multiple stressors on bees and, when needed, to develop action plans on new and emerging bee health issues in Europe. To consolidate forward research planning, the Bee TF recommends that EC-funded projects, which represent a large volume of information, are reviewed to assess those results and findings which could contribute to a better understanding of bee losses and colony weakening, with a particular attention to results dealing with co-exposure, (synergistic and cumulative) interactions of multiple stressors on bees.
Knowledge sharing with MSs and the EC could be enhanced by making EC-funded reports and relevant data publicly available and by developing an open-access bee health database containing relevant scientific information for the risk assessment of single and multiple stressors on bees. Finally, the Bee TF recommended that the development of horizontal projects on bee health be further explored with the continuation of internal collaborations and communications across Units on this topic. It is also suggested increasing external communications with MSs and the EC, on EFSA's work on bee health, through regular liaison with the EFSA Advisory Forum and the Bee Inter-service Group on the progress made by EFSA on this topic. EFSA-Report (20140224)

The EFSA report demonstrates that progress in tackling the multifactorial complexity of CCD should be based on intensified and well-planned coordinated research efforts over many years to come and with no respect to borders of political and scientific limits.

vanEngelsdorp, Caron, et al. (2012).

vanEngelsdorp, D., D. Caron, J. Hayes, R. Underwood, M. Henson, K. Rennich, A. Spleen, M. Andree, R. Snyder, K. Lee, K. Roccasecca, M. Wilson, J. Wilkes, E. Lengerich, J. Pettis and P. Bee Informed (2012), A national survey of managed Honeybee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership, Journal of Apicultural Research, 51, 1, pp. 115-124, <Go to ISI>://WOS:000299995700013 AND http://www.ask-force.org/web/Bees/vanEngelsdorp-National-Survey-US-2012.pdf

"This study records the fifth consecutive year that winter losses of managed Honeybee (Apis mellifera) colonies in the USA have been around 30%. In April 2011, a total of 5,441 US beekeepers (an estimated 11% of total US beekeepers) responded to a survey conducted by the Bee Informed Partnership. Survey respondents reported that they had lost an average of 38.4% of their colonies, for a total US colony loss of 29.9% over the winter of 2010-11. One-third of respondents (all classified as backyard beekeepers, i.e. keeping fewer than 50 colonies) reported no winter loss. There was considerable variation in both the average and total loss by state. On average, beekeepers consider acceptable losses to be 13.2%, but 68% of all responding beekeepers suffered actual losses in excess of what they considered acceptable. Of beekeepers who reported losing at least one colony, manageable conditions, such as starvation and a weak condition in the fall, were the leading self-identified causes of mortality. Respondents who indicated that varroa mites (Varroa destructor), small hive beetles (Aethina tumida), poor wintering conditions, and/or Colony Collapse Disorder (CCD) conditions were a leading cause of mortality in their operations suffered a higher average loss than beekeepers who did not list any of these as potential causes. In a separate question, beekeepers who reported the symptom "no dead bees in hive or apiary" had significantly higher losses than those who did not report this symptom. In addition, commercial beekeepers were significantly more likely to indicate that colonies died with this symptom than either backyard or sideliner beekeepers." vanEngelsdorp, Caron, et al. (2012).

In 2013 the final COLOSS project review is published: Spleen, Lengerich, et al. (2013)

Spleen, A. M., E. J. Lengerich, K. Rennich, D. Caron, R. Rose, J. S. Pettis, M. Henson, J. T. Wilkes, M. Wilson, J. Stitzinger, K. Lee, M. Andree, R. Snyder, D. vanEngelsdorp and P. Bee Informed (2013), A national survey of managed Honeybee 2011-12 winter colony losses in the United States: results from the Bee Informed Partnership, Journal of Apicultural Research, 52, 2, pp. <Go to ISI>://WOS:000317041800007 AND http://www.ask-force.org/web/Bees/Spleen-National-Survey-Managed-Honeybees-2013.pdf

"Estimates of winter loss for managed Honeybee (Apis mellifera) colonies are an important measure of Honeybee health and productivity. We used data from 5,500 US beekeepers (5,244 backyard, 189 sideline and 67 commercial beekeepers) who responded to the April 2012 Bee Informed Partnership Winter Colony Loss Survey and calculated loss as the difference in the number of colonies between October 1, 2011 and April 1, 2012, adjusting for increases and decreases over that period. In the US, the total colony loss was 22.5% for the 2011-12 winter; 45.1% (n = 2,482) of respondents reported no colony loss. Total loss during 2011-12 was substantially lower than loss during 2010-11 (29.9%). Of the 4,484 respondents who kept bees in 2010-11 and 2011-12, 72.0% reported that the loss during 2011-12 was smaller or similar to the loss during 2010-11. There was substantial variation in total loss by state (range 6.2% to 47.7%). The average loss per beekeeping operation was 25.4%, but the average loss was not significantly different by operation type (backyard, sideline, commercial). The average self-reported acceptable loss per respondent was 13.7%; 46.8% (n = 2,259) of respondents experienced winter colony losses in excess of the average acceptable loss. Of beekeepers who reported losing at least one colony during 2011-12, ***the leading self-identified causes of mortality were weak condition in the fall and queen failure. Respondents who indicated poor wintering conditions, CCD, or pesticides as a leading cause of mortality suffered a***

***higher average loss when compared to beekeepers who did not list these as potential causes**." Spleen, Lengerich, et al. (2013)*

Two important summaries on standardized methods in epidemiology in researching diseases of Apis mellifera comes from the same research group: vanEngelsdorp, Lengerich, et al. (2013)

vanEngelsdorp, D., E. Lengerich, A. Spleen, B. Dainat, J. Cresswell, K. Baylis, B. K. Nguyen, V. Soroker, R. Underwood, H. Human, Y. Le Conte and C. Saegerman (2013), Standard epidemiological methods to understand and improve Apis mellifera health, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300008 AND
http://www.ask-force.org/web/Bees/vanEngelsdorp-Standard-Epidemiological-Methods-corr-20130901.pdf

In this paper, we describe the use of epidemiological methods to understand and reduce Honeybee morbidity and mortality. Essential terms are presented and defined and we also give examples for their use. Defining such terms as disease, population, sensitivity, and specificity, provides a framework for epidemiological comparisons. The term population, in particular, is quite complex for an organism like the Honeybee because one can view "epidemiological unit" as individual bees, colonies, apiaries, or operations. The population of interest must, therefore, be clearly defined. Equations and explanations of how to calculate measures of disease rates in a population are provided. There are two types of study design; observational and experimental. The advantages and limitations of both are discussed. Approaches to calculate and interpret results are detailed. Methods for calculating epidemiological measures such as detection of rare events, associating exposure and disease (Odds Ratio and Relative Risk), and comparing prevalence and incidence are discussed. Naturally, for beekeepers, the adoption of any management system must have economic advantage. We present a means to determine the cost and benefit of the treatment in order determine its net benefit. Lastly, this paper presents a discussion of the use of Hill's criteria for inferring causal relationships. This framework for judging cause-effect relationships supports a repeatable and quantitative evaluation process at the population or landscape level. Hill's criteria disaggregate the different kinds of evidence, allowing the scientist to consider each type of evidence individually and objectively, using a quantitative scoring method for drawing conclusions. It is hoped that the epidemiological approach will be more broadly used to study and negate Honeybee disease." vanEngelsdorp, Lengerich, et al. (2013)

Another important review was published by T. Blacquiere: Blacquiere, Smagghe, et al. (2012) The second summary of standardized methods to study the toxicology of Apis mellifera: Medrzycki, Giffard, et al. (2013). A short glance on the long list of contents demonstrates the thoroughness of the study with a main emphasis to proper methodology related to statistical results which cannot be disputed:

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L. P., Chauzat, M. P., Classen, C., Colin, M. E., Dupont, T., Girolami, V., Johnson, R., Le Conte, Y., Luckmann, J., Marzaro, M., Pistorius, J., Porrini, C., Schur, A., Sgolastra, F., Delso, N. S., van der Steen, J. J. M., Wallner, K., Alaux, C., Biron, D. G., Blot, N., Bogo, G., Brunet, J. L., Delbac, F., Diogon, M., El Alaoui, H., Provost, B., Tosi, S. and Vidau, C. (2013) Standard methods for toxicology research in Apis mellifera Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800012 AND http://www.ask-force.org/web/Bees/Medrzycki-Standard-Methods-toxicology-research-Apis-mellifera-2013.pdf

"Modern agriculture often involves the use of pesticides to protect crops. These substances are harmful to target organisms (pests and pathogens). Nevertheless, they can also damage non-target animals, such as pollinators and entomophagous arthropods. It is obvious that the undesirable side effects of pesticides on the environment should be reduced to a minimum. Western honey bees (Apis mellifera) are very important organisms from an agricultural perspective and are vulnerable to pesticide-induced impacts. They contribute actively to the pollination of cultivated crops and wild vegetation, making food production possible. Of course, since Apis mellifera occupies the same ecological niche as many other species of pollinators, the loss of honey bees caused by environmental pollutants suggests that other insects may experience a similar outcome. Because pesticides can harm honey bees and other pollinators, it is important to register pesticides that are as selective as possible. In this manuscript, we describe a selection of methods used for studying pesticide toxicity/selectiveness towards Apis mellifera . These methods may be used in risk assessment schemes and in scientific research aimed to explain acute and chronic effects of any target compound on Apis mellifera." From Medrzycki, Giffard, et al. (2013)

As a further exerpt: a few tables and figures:

Effect	Looks like	To be recorded as:
Dead	Immobile, no reaction to stimuli such as touching with forceps	Mortality, number of bees
No effect	Bees having normal behaviour	NE, number of bees observed
Freeze	Motionless bees caught in action and looking active such as attached to feeder, standing on the floor but actually completely inactive.	F, number of bees observed
Paralysis	Motionless on the floor of the test cage, responding to stimuli by moving leg, antenna etc.	P, number of bees observed
Spasm	Crawling bees, movement uncoordinated	S, number of bees observed

Fig. 1 (From Table 1: Possible honey bee behavioural effects due to exposure to pesticides in individual tests. Note: "freeze" and "paralysis" bees may be recorded as dead bees at a certain point and later as living bees.

Also in the latest updates in peer reviewed journal articles from several of the most renowned bee scientists: *GM crops are again not mentioned as a cause of CCD*.

Stokstad, E. (2007)

ENTOMOLOGY: The Case of the Empty Hives. Science %R 10.1126/science.316.5827.970, 316, 5827, pp 970-972
http://www.sciencemag.org/cgi/content/summary/316/5827/970 AND http://www.ask-force.org/web/Bees/Stockstad-Empty-2007.pdf

"With the recent flap about CCD, insecticides have inevitably been identified as one of the possible causes of larger-than-normal bee loss. The history of the relationship between beekeeping and insecticide application goes back a long way. In the 1950s it took some sleuthing to finally figure out that arsenic dust was being collected by bees in the field as pollen to both their and their colony's detriment. Given the advantages of hindsight, who now could possibly argue that dusting with this extremely toxic substance does not affect Honeybees. This even includes the active material in treated wood.1 Another situation arose with the use of microencapsulated pesticides in the 1970s, especially a product called PennCap-M®.2 The capsules were like pollen-grain-size and were a time bomb in colonies because they could be brought back without harm to the forager and only became a problem when consumed by young bees in an effort to feed larvae.
Insecticides were such a problem to beekeepers in the late1970s that congress authorized the beekeeper indemnity program, which provided payments to beekeepers from colonies lost to chemical application in both agricultural and urban (mosquito control) situations.3 However, this program became unwieldy because it was difficult to tell the difference between legitimate and falsely reported claims, and was finally discontinued. This era brought into use the current information on the effects of pesticides on Honeybees, pioneered by Dr. Larry Atkins at the University of California, Riverside for which most extension publications continue to draw their information.4 This was based on topical exposure to workers in small cages (LD50), however, there is evidence that bees may be exposed through other routes, including contaminated nectar, and that measurement of toxicity (LC50) might be significantly different.5 In Florida, this became a hot issue with a material called Temik® used in citrus groves.6 The active ingredient in this material, aldicarb, is a systemic insecticide and was thought to trans-locate into the blooms contaminating nectar. And although the active ingredient is certainly harmful to Honeybees, there is evidence that the metabolites (break down products) of this material are even more toxic than the parent substance." Stokstad (2007A, Stokstad (2007B)

Johnson, R. (2007)

Recent Honeybee Colony Declines, CSR Report for Congress, Order Code RL33938. CSR Report for Congress, Order Code RL33938 pp 13 CSR Report for Congress, Order Code RL33938 Washington (Report)
http://www.ask-force.org/web/Bees/Johnson-CCD-CRS-2007.pdf

"This report examines the recent sharp decline in U.S. Honeybee colonies, which scientists are now calling the Colony Collapse Disorder (CCD). This phenomenon first became apparent among commercial migratory beekeepers along the East Coast during the last few months of 2006, and has since been reported nationwide. Honeybees are the most economically valuable pollinators of agricultural crops worldwide. Many scientists at universities and the U.S. Department of Agriculture (USDA) assert that bee pollination is involved in about one-third of the U.S. diet, and contributes to the production of a wide range of fruits, vegetables, tree nuts, forage crops, some field crops, and other specialty crops. The monetary value of Honeybees as commercial pollinators in the United States is estimated at about $15 billion annually.

Honeybee colony losses are not uncommon. However, current losses seem to differ from past situations in that ! colony losses are occurring mostly because bees are failing to return to the hive (which is largely uncharacteristic of bee behavior), ! bee colony losses have been rapid, colony losses are occurring in large numbers, and the reason why these losses are occurring remains still largely unknown.
To date, the potential causes of CCD, as reported by the scientists who are researching this phenomenon, include but may not be limited to parasites, mites, and disease loads in the bees and brood;

- *known/unknown pathogens;*
- *poor nutrition among adult bees;*
- *level of stress in adult bees (e.g., transportation and confinement of bees, or other environmental or biological stressors);*
- *chemical residue/contamination in the wax, food stores and/or bees;*
- *lack of genetic diversity and lineage of bees; and*
- *a combination of several factors.*

On March 29, 2007, the House Subcommittee on Horticulture and Organic Agriculture held a hearing to review the recent Honeybee colony declines reported throughout the United States. Based on information presented to Congress, both by scientists researching recent bee colony declines and by agricultural producers who may be potentially affected by these losses, Congress could consider options for subsequent action in this area."
Johnson (2007, Johnson, Pollock, et al. (2009)

van Engelsdorp, D., Foster, D.C., Frazier, M., Ostiguy, N., & Hayes, J. (2006)
Electronic Source: "Fall-Dwindle Disease": Investigations into the causes of sudden and alarming colony losses experienced by beekeepers in the fall of 2006. Preliminary Report: First Revision, CCD Working Group Preliminary Report
published by: Bee Alert Inc., Florida Dept. of Agriculture, Pennsylvania State University, Pennsylvania Dept. of Agriculture, USDA/ARS, 22pp
http://www.ask-force.org/web/Bees/vanEngelsdorp-CCD-working-group-Update-2007.pdf AND
http://www.doacs.state.fl.us/pi/plantinsp/apiary/fall_dwindle_report.pdf
vanEngelsdorp, Foster, et al. (2006)

van Engelsdorp, D., Hayes, J., Jr., Underwood, R.M., & Pettis, J. (2008)
A Survey of Honeybee Colony Losses in the US, Fall 2007 to Spring 2008. PLoS ONE, 3, 12, pp Article No.: e4071
<Go to ISI>://BIOSIS:PREV200900336218 AND
http://www.ask-force.org/web/Bees/vanEngelsdorp-Survey-Colony-Losses-2008.pdf

"Background: Honeybees are an essential component of modern agriculture. A recently recognized ailment, Colony Collapse Disorder (CCD), devastates colonies, leaving hives with a complete lack of bees, dead or alive. Up to now, estimates of Honeybee population decline have not included losses occurring during the wintering period, thus underestimating actual colony mortality. Our survey quantifies the extent of colony losses in the United States over the winter of 2007–2008.
Methodology/Principal Findings: Surveys were conducted to quantify and identify management factors (e.g. operation size, hive migration) that contribute to high colony losses in general and CCD symptoms in particular. Over 19% of the country's estimated 2.44 million colonies were surveyed. A total loss of 35.8% of colonies was recorded; an increase of 11.4% compared to last year. Operations that pollinated almonds lost, on average, the same number of colonies as those that did not. The 37.9% of operations that reported having at least some of their colonies die with a complete lack of bees had a total loss of 40.8% of colonies compared to the 17.1% loss reported by beekeepers without this symptom. Large operations were more likely to have this symptom suggesting that a contagious condition may be a causal factor. Sixty percent of all colonies that were reported dead in this survey died without dead bees, and thus possibly suffered from CCD. In PA, losses varied with region, indicating that ambient temperature over winter may be an important factor.
Conclusions/Significance: Of utmost importance to understanding the recent losses and CCD is keeping track of losses over time and on a large geographic scale. Given that our surveys are representative of the losses across all beekeeping operations, between 0.75 and 1.00 million Honeybee colonies are estimated to have died in the United States over the winter of 2007–2008. This article is an extensive survey of U.S. beekeepers across the continent, serving as a reference for comparison with future losses as well as providing guidance to future hypothesis-driven research on the causes of colony mortality". vanEngelsdorp, Foster, et al. (2006, vanEngelsdorp, Hayes, et al. (2008)

Some urgent calls for action: more research and monitoring necessary, the case not solved. Also a call for more research beyond the Colony Collapse Disorder. Cox-Foster and Vanengelsdorp (2009, VanEngelsdorp, Hayes, et al. (2009):

VanEngelsdorp, D., Hayes, J., & Pettis, J. (2009b)
Electronic Source: Preliminary Results: A Survey of Honeybee Colonies Losses in the U.S. between September 2008 and April 2009
http://maarec.cas.psu.edu/pdfs/PrelimLosses2009.pdf AND

http://www.ask-force.org/web/Bees/VanEngelsdorp-PrelimLosses2009.pdf

Cox-Foster, D. & Vanengelsdorp, D. (2009)
Saving the HONEYBEE, Solving the Mystery of the Vanishing Bees. Scientific American, 300, 4, pp 40-+
<Go to ISI>://WOS:000264456700029 AND http://www.scientificamerican.com/article.cfm?id=saving-the-oneybee&print=true AND http://www.ask-force.org/web/Bees/Cox-Foster-Solving-the-Mystery1-2009.pdf AND http://www.scientificamerican.com/article.cfm?id=breakfast-without-bees
Cox-Foster and Vanengelsdorp (2009)

This article from the Scientific American sums up the latest state of error, but one thing seems to become clear: The virus is an important element, but not the only one: Energetic stress, too many monocultures without hedgerows, negligence of the beekeepers in fighting off varroa infections and not optimizing diets might add to the syndrome.

Another good website with recent information is offered by Mid-Atlantic Apiculture http://maarec.cas.psu.edu/ColonyCollapseDisorder.html

The most recent national survey of managed Honeybee 2011-12 winter colony losses in the United States shows results from the Bee Informed Partnership, the problem still exists: Spleen, Lengerich, et al. (2013)

"Estimates of winter loss for managed Honeybee (Apis mellifera) colonies are an important measure of Honeybee health and productivity. We used data from 5,500 US beekeepers (5,244 backyard, 189 sideline and 67 commercial beekeepers) who responded to the April 2012 Bee Informed Partnership Winter Colony Loss Survey and calculated loss as the difference in the number of colonies between October 1, 2011 and April 1, 2012, adjusting for increases and decreases over that period. In the US, the total colony loss was 22.5% for the 2011-12 winter; 45.1% (n = 2,482) of respondents reported no colony loss. Total loss during 2011-12 was substantially lower than loss during 2010-11 (29.9%). Of the 4,484 respondents who kept bees in 2010-11 and 2011-12, 72.0% reported that the loss during 2011-12 was smaller or similar to the loss during 2010-11. There was substantial variation in total loss by state (range 6.2% to 47.7%). The average loss per beekeeping operation was 25.4%, but the average loss was not significantly different by operation type (backyard, sideline, commercial). The average self-reported acceptable loss per respondent was 13.7%; 46.8% (n = 2,259) of respondents experienced winter colony losses in excess of the average acceptable loss. Of beekeepers who reported losing at least one colony during 2011-12, the leading self-identified causes of mortality were weak condition in the fall and queen failure. Respondents who indicated poor wintering conditions, CCD, or pesticides as a leading cause of mortality suffered a higher average loss when compared to beekeepers who did not list these as potential causes." Spleen, Lengerich, et al. (2013)

The same picture in another recent review van der Zee, Pisa, et al. (2012) : the CCD problem persists:

van der Zee, R., L. Pisa, S. Andonov, R. Brodschneider, J.-D. Charriere, R. Chlebo, M. F. Coffey, K. Crailsheim, B. Dahle, A. Gajda, A. Gray, M. M. Drazic, M. Higes, L. Kauko, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, J. Kralj, P. Kristiansen, R. Martin Hernandez, F. Mutinelli, N. Bach Kim, C. Otten, A. Ozkirim, S. F. Pernal, M. Peterson, G. Ramsay, V. Santrac, V. Soroker, G. Topolska, A. Uzunov, F. Vejsnaes, S. Wei and S. Wilkins (2012), Managed Honeybee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10, Journal of Apicultural Research, 51, 1, pp. 91-114, <Go to ISI>://WOS:000299995700012 AND http://www.ask-force.org/web/Bees/vanderZee-Managed-Honeybee-Losses-2012.pdf

"In 2008 the COLOSS network was formed by Honeybee experts from Europe and the USA. The primary objectives set by this scientific network were to explain and to prevent large scale losses of Honeybee (Apis mellifera) colonies. In June 2008 COLOSS obtained four years support from the European Union from COST and was designated as COST Action FA0803 – COLOSS (Prevention of Honeybee Colony LOSSes). To enable the comparison of loss data between participating countries, a standardized COLOSS questionnaire was developed. Using this questionnaire information on Honeybee losses has been collected over two years. Survey data presented in this study were gathered in 2009 from 12 countries and in 2010 from 24 countries. Mean Honeybee losses in Europe varied widely, between 7-22% over the 2008-9 winter and between 7-30% over the 2009-10 winter. An important finding is that for all countries which participated in 2008-9, winter losses in 2009-10 were found to be substantially higher. In 2009-10, winter losses in South East Europe were at such a low level that the factors causing the losses in other parts of Europe were absent, or at a level which did not affect colony survival. The five provinces of China, which were included in 2009-10, showed very low mean (4%) A. mellifera winter losses. In six Canadian provinces, mean winter losses in 2010 varied between 16-25%, losses in Nova Scotia

(40%) being exceptionally high. In most countries and in both monitoring years, hobbyist beekeepers (1-50 colonies) experienced higher losses than practitioners with intermediate beekeeping operations (51-500 colonies). This relationship between scale of beekeeping and extent of losses effect was also observed in 2009-10, but was less pronounced. In Belgium, Italy, the Netherlands and Poland, 2008-9 mean winter losses for beekeepers who reported 'disappeared' colonies were significantly higher compared to mean winter losses of beekeepers who did not report 'disappeared' colonies. Mean 2008-9 winter losses for those beekeepers in the Netherlands who reported symptoms similar to "Colony Collapse Disorder" (CCD), namely: 1. no dead bees in or surrounding the hive while; 2. capped brood was present, were significantly higher than mean winter losses for those beekeepers who reported 'disappeared' colonies without the presence of capped brood in the empty hives. In the winter of 2009-10 in the majority of participating countries, beekeepers who reported 'disappeared' colonies experienced higher winter losses compared with beekeepers, who experienced winter losses but did not report 'disappeared' colonies." van der Zee, Pisa, et al. (2012)

The CCD problem seems to get worse according to another international review available: van der Zee, Pisa, et al. (2012)

van der Zee, R., L. Pisa, S. Andonov, R. Brodschneider, J.-D. Charriere, R. Chlebo, M. F. Coffey, K. Crailsheim, B. Dahle, A. Gajda, A. Gray, M. M. Drazic, M. Higes, L. Kauko, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, J. Kralj, P. Kristiansen, R. Martin Hernandez, F. Mutinelli, N. Bach Kim, C. Otten, A. Ozkirim, S. F. Pernal, M. Peterson, G. Ramsay, V. Santrac, V. Soroker, G. Topolska, A. Uzunov, F. Vejsnaes, S. Wei and S. Wilkins (2012), Managed Honeybee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10, Journal of Apicultural Research, 51, 1, pp. 91-114, <Go to ISI>://WOS:000299995700012 AND http://www.ask-force.org/web/Bees/vanderZee-Managed-Honeybee-Losses-2012.pdf

"In 2008 the COLOSS network was formed by Honeybee experts from Europe and the USA. The primary objectives set by this scientific network were to explain and to prevent large scale losses of Honeybee (Apis mellifera) colonies. In June 2008 COLOSS obtained four years support from the European Union from COST and was designated as COST Action FA0803 - COLOSS (Prevention of Honeybee COlony LOSSes). To enable the comparison of loss data between participating countries, a standardized COLOSS questionnaire was developed. Using this questionnaire information on Honeybee losses has been collected over two years. Survey data presented in this study were gathered in 2009 from 12 countries and in 2010 from 24 countries. Mean Honeybee losses in Europe varied widely, between 7-22% over the 2008-9 winter and between 7-30% over the 2009-10 winter. An important finding is that for all countries which participated in 2008-9, winter losses in 2009-10 were found to be substantially higher. In 2009-10, winter losses in South East Europe were at such a low level that the factors causing the losses in other parts of Europe were absent, or at a level which did not affect colony survival. The five provinces of China, which were included in 2009-10, showed very low mean (4%) A. mellifera winter losses. In six Canadian provinces, mean winter losses in 2010 varied between 16-25%, losses in Nova Scotia (40%) being exceptionally high. In most countries and in both monitoring years, hobbyist beekeepers (1-50 colonies) experienced higher losses than practitioners with intermediate beekeeping operations (51-500 colonies). This relationship between scale of beekeeping and extent of losses effect was also observed in 2009-10, but was less pronounced. In Belgium, Italy, the Netherlands and Poland, 2008-9 mean winter losses for beekeepers who reported 'disappeared' colonies were significantly higher compared to mean winter losses of beekeepers who did not report 'disappeared' colonies. Mean 2008-9 winter losses for those beekeepers in the Netherlands who reported symptoms similar to "Colony Collapse Disorder" (CCD), namely: 1. no dead bees in or surrounding the hive while; 2. capped brood was present, were significantly higher than mean winter losses for those beekeepers who reported 'disappeared' colonies without the presence of capped brood in the empty hives. In the winter of 2009-10 in the majority of participating countries, beekeepers who reported 'disappeared' colonies experienced higher winter losses compared with beekeepers, who experienced winter losses but did not report 'disappeared' colonies." van der Zee, Pisa, et al. (2012)

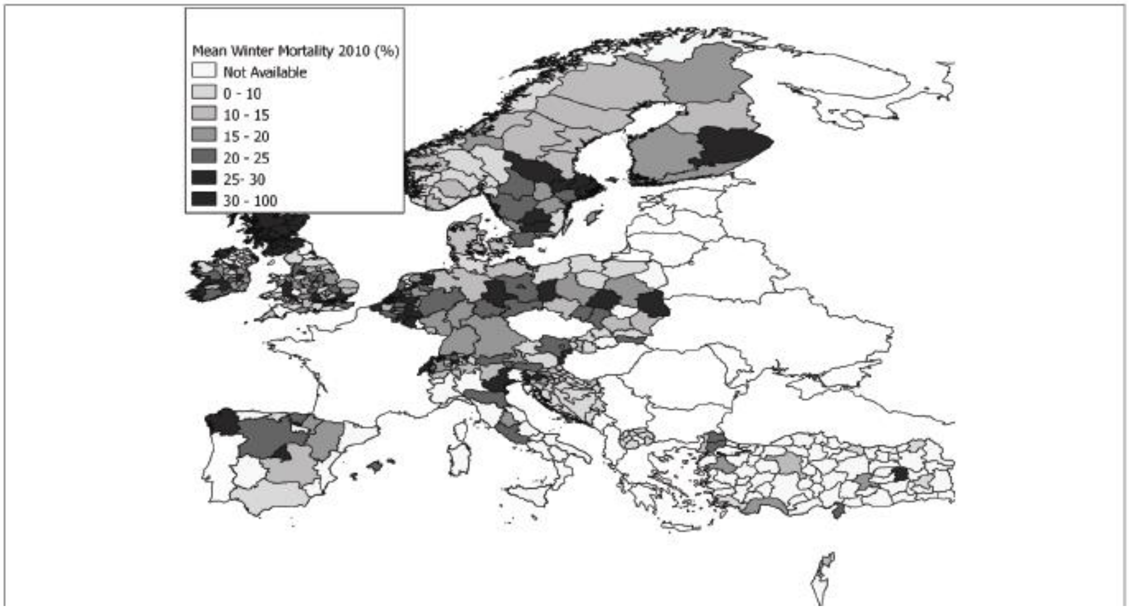

Fig. 2 Mean winter mortality 2009-2010 in Europe, Turkey and Israel. Comment from the text: Changing the level of aggregation of colony losses at higher than country resolution (Fig. 1) provides more detailed information about the spatial distribution. Only information at regional level was available. The administrative regional boundaries that correspond with the collected information differ in scale between the participating countries, thus complicating regional comparisons between countries.
The variation in regional losses is substantial within all countries with losses higher than 10%. Between the Canadian provinces (British Columbia, Manitoba, New Brunswick, Prince Edward Island and Québec) losses varied between 16–25%, with the exception of Nova Scotia (40%). The Chinese provinces of Sichuan, Zhejiang, Shanxi, Gansu and Jilin present in this study had very low losses (<10%). The relation between operation size and overwintering mortality for the hobbyist and intermediate size classes (1-50 colonies and 51-500 colonies respectively), based on the 95% confidence interval, was significantly different for Austria, Bosnia and Herzegovina, England and Wales, and for the total set. From van der Zee, Pisa, et al. (2012)

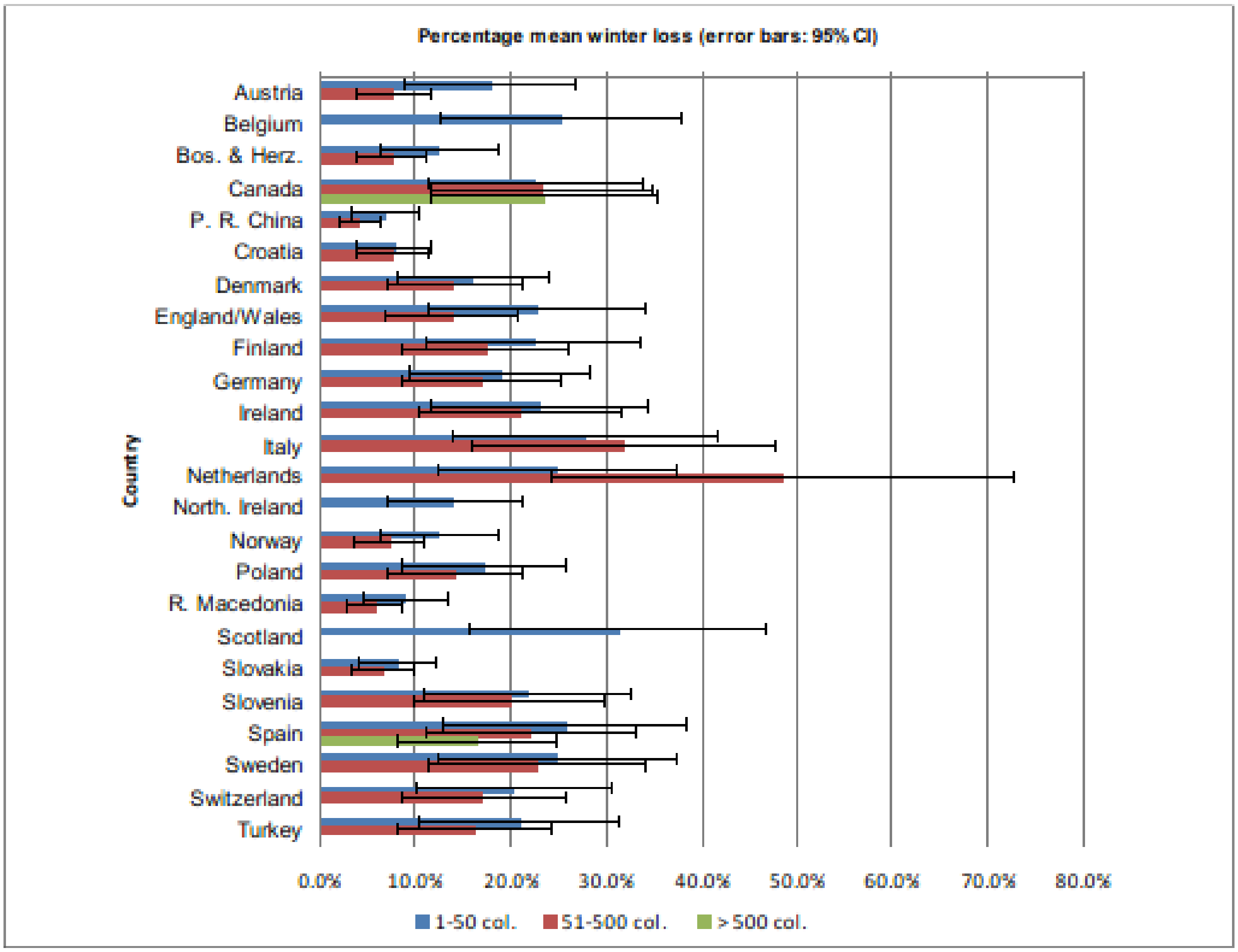

Fig. 3 No significant effects were found for the remaining individual countries, but for some countries a trend, but less pronounced compared to losses in 2009, can be observed for intermediate beekeepers reporting lower losses than hobbyist beekeepers, see fig.2 in van der Zee, Pisa, et al. (2012)

The final COLOSS *standard* approach reports from 2013 will be cited appropriately in the chapters below: Anonymous (2011, Buchler, Andonov, et al. (2013, Crailsheim, Brodschneider, et al. (2013, de Graaf, Alippi, et al. (2013, Delaplane, van der Steen, et al. (2013, Dietemann, Nazzi, et al. (2013, Ellis, Graham, et al. (2013, Forsgren, Budge, et al. (2013, Fries, Chauzat, et al. (2013, Genersch, Gisder, et al. (2013, Hartfelder, Bitondi, et al. (2013, Jensen, Aronstein, et al. (2013, vanEngelsdorp, Lengerich, et al. (2013, Williams, Alaux, et al. (2013)

Another summary of the CCD situation has been published earlier in 2010 by the Journal of Apicultural Research, here fully cited as an open source publication:

Neuman, P. and N. L. Carreck (2010). "Honeybee colony losses, guest editorial, open source." Journal of Apicultural Research **49**(1): 1-6. DOI 10.3896/IBRA.1.49.1.01, open source AND http://www.ask-force.org/web/Bees/Neumann-Honeybee-Colony-Losses-2010.pdf including all citations (as far as already available on WOS)

Honeybee colony losses, guest editorial, (from Neuman and Carreck (2010)
"Apiculture has been in decline in both Europe and the USA over recent decades, as is shown by the decreasing numbers of managed Honeybee (Apis mellifera L.) colonies (Ellis et al., 2010; Potts et al., 2010). It therefore is crucial to make beekeeping a more attractive hobby and a less laborious profession, in order to encourage local apiculture and pollination. Apart from socio-economic factors, which can only be addressed by politicians, sudden losses of Honeybee colonies have occurred, and have received considerable public attention. Indeed, in the last few years, the world's press has been full of eye catching but often uninformative headlines proclaiming the dramatic demise of the Honeybee, a world pollinator crisis and the spectre of mass human starvation. "Colony Collapse Disorder" (CCD) in the USA has attracted great attention, and scientists there and in Europe are working hard to provide explanations for these extensive colony losses. Colony losses have also occurred elsewhere (Figs 1 and 2), but examination of the historical record shows that such extensive losses are not unusual vanEngelsdorp and Meixner (2010).
Almost exactly a century ago, in 1906, beekeepers on the Isle of Wight, a small island off the south coast of England, noticed that many of their Honeybee colonies were dying, with numerous bees crawling from the hive, unable to fly. Despite some skeptical beekeepers suggesting that this was "paralysis", a condition which had long been known, the colony losses were widely reported in the media, and beekeepers became convinced that the cause was a novel and highly infectious disease, and the condition was soon reported from all parts of Britain. Within a few years, all losses of bees in Britain, from whatever cause, were ascribed to "Isle of Wight Disease" Bailey (2002, Bailey and Ball (1991)

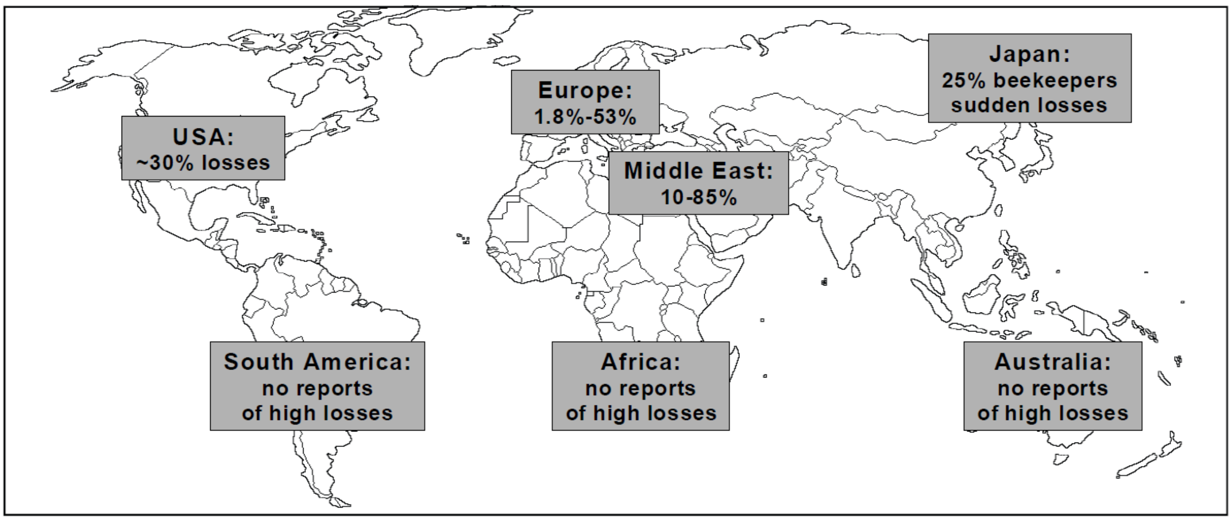

Fig. 4 The Varroa destructor equator of global colony losses. So far, elevated colony losses have recently been reported from Europe Crailsheim, Brodschneider, et al. (2009), the USA (vanEngelsdorp, Evans, et al. (2009B) and 2010), the Middle East Haddad, Bataeneh, et al. (2009, Soroker, Hetzroni, et al. (2009) and Japan Guttierrez (2009) but not from South America, Africa and Australia. Colonies of African Honeybees and Africanized Honeybees in South America survive without V. destructor treatment, whilst the mite has not yet been introduced into Australia. This global picture indicates a central role of this particular ectoparasitic mite for colony losses. From Neuman and Carreck (2010)

The response of the scientific community was instructive. Initially, the UK Government sent the eminent entomologist A D Imms to the Isle of Wight, but being unfamiliar with bees, he was unable to throw much light on the problem Bailey and Ball (1991). Other scientists soon made suggestions. By 1912, Fantham and Porter (1912) became convinced that the cause was the microsporidium Nosema apis, but this view was overshadowed by the discovery in 1919 of the tracheal mite Acarapis woodi Rennie, White, et al. (1921). Conventional wisdom and beekeeping text books soon accepted that this impressive mite was the cause of the "Isle of Wight Disease", yet close examination of the original paper shows that this could not be so. Rennie et al.'s experimental results clearly demonstrated that some bees heavily infested with the mite were able to fly normally, yet other crawling bees, exhibiting the symptoms of the disease, contained no mites. One can only conclude that carried away by the excitement of their new discovery, they had failed to test Koch's Postulates, and had jumped to conclusions.
Sober reassessment of the "Isle of Wight Disease" many years later Bailey (2002, Bailey and Ball (1991) led to the conclusion that the disease had been due to a combination of factors, in particular, infection by chronic bee paralysis virus (completely unknown at the time), together with poor weather which inhibited foraging, and an excess of bee colonies being kept for the amount of forage available.

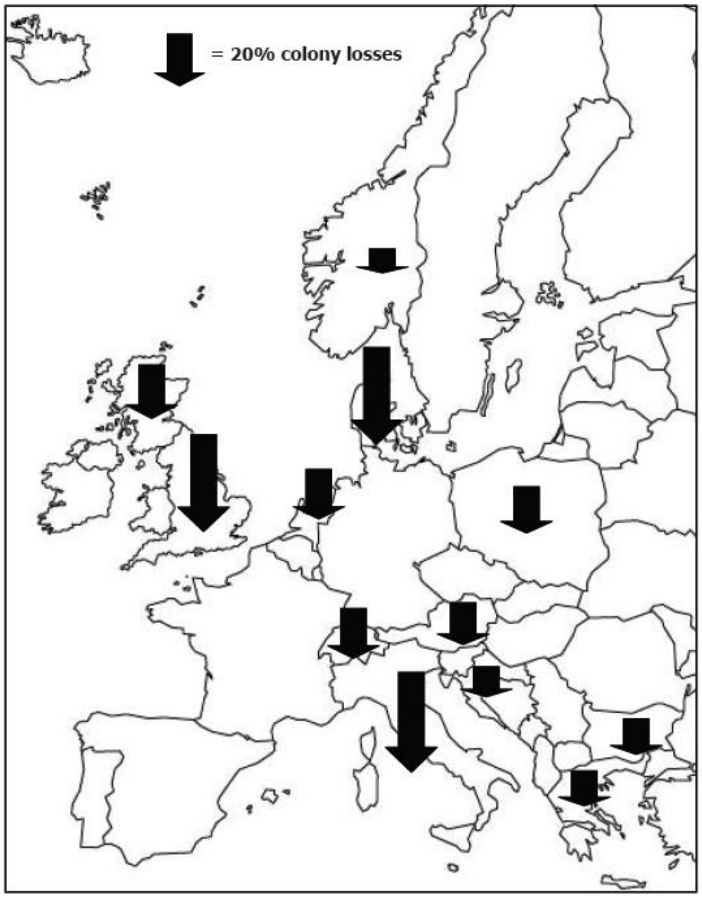

Fig. 5 Overview of recent colony losses in Europe. For details on individual countries please refer to papers in this Special Issue: Austria (Brodschneider et al., 2010); Bulgaria (Ivanova and Petrov, 2010); Croatia (Gajger et al., 2010); Denmark Vejsnæs and Kryger, 2010); England (Aston, 2010); Greece (Hatjina et al., 2010); Italy (Mutinelli et al., 2010); Norway (Dahle, 2010); Scotland (Gray et al., 2010); Switzerland (Charrière and Neumann, 2010). From Neuman and Carreck (2010)

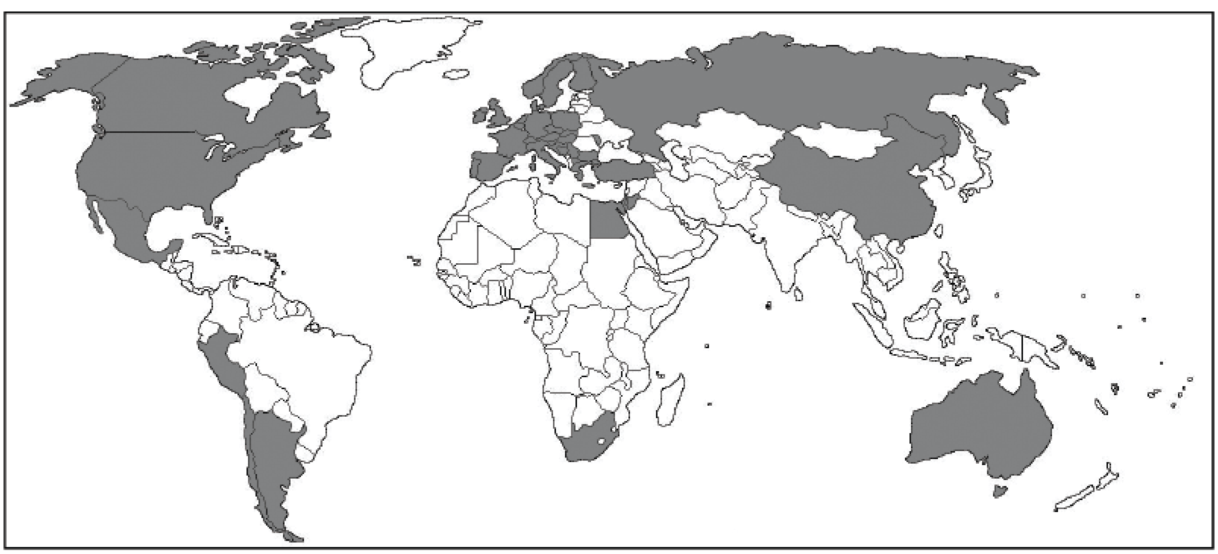

Fig. 6 The global COLOSS network ("Prevention of Honeybee COLony LOSSes", consisting of 161 individual members from 40 countries (= grey areas). From Neuman and Carreck (2010)

The recent concern over CCD has much in common with the historical "Isle of Wight Disease" episode, and many lessons can be learned. Initial concern about colony losses in one particular area, the USA, has led to global media attention. Moreover, colony losses throughout the world are being ascribed to CCD, yet that term was specifically coined to describe a precisely defined set of symptoms VanEngelsdorp, Evans, et al. (2009A, vanEngelsdorp, Evans, et al. (2009B, VanEngelsdorp, Hayes, et al. (2009) and not colony losses per se. Indeed, Honeybee colonies can die in many ways, and CCD is just one of them vanEngelsdorp and Meixner (2010). Finally, since both Honeybee host and pathogens are genetically diverse, the symptoms and causes of colony losses may well be different in different regions. Many well intentioned suggestions as to the possible causes of colony losses, including such improbable ideas as mobile telephones, genetically modified crops and nanotechnology, have perhaps overshadowed much more likely explanations such as pests and diseases, pesticides, loss of forage and beekeeping practices. For example, the long known major pest of A. mellifera apiculture, the ectoparasitic mite Varroa destructor has recently received comparatively little attention, but is certainly involved. Indeed, the broad patterns of CCD coincide with continents with different pressures from V. destructor (Fig. 1). Since African and Africanized Honeybees survive without treatment for V. destructor Martin and Medina (2004) and the mite has not yet been discovered in Australia, this supports a central role of V. destructor for the current colony losses. In fact, data by Dahle (2010) strongly support this view, showing that regions with established mite populations had consistently higher losses than those without. After the development and dissemination of adequate mite control methods, however, losses due to V. destructor remained at tolerable limits until recently, suggesting that the mite alone cannot explain all of the recent losses.

Despite comprehensive recent research efforts on these colony losses, no single driver has yet emerged as the definitive cause of the phenomenon. Instead, interactions between multiple drivers are the most probable explanation for elevated over-wintering mortality, similar to the conclusions for the Isle of Wight disease Bailey (2002).

At a global scale, most managed A. mellifera colonies are infested by V. destructor, facilitating the potential interaction between this factor and multiple other potential drivers almost anywhere in the world. Moreover, many other prominent Honeybee pathogens are now also almost globally distributed, for example Nosema spp. and several viruses Allen and Ball (1996, Ellis and Munn (2005, Fries , Maori, Lavi, et al. (2007). Multiple infections with pathogens and also interactions between pathogens and other suspected drivers of Honeybee loss are therefore almost inevitable, at least in areas with established mite populations. Whilst the list of these other potential drivers is not novel, the evidence of such interactive effects, although limited, is important and growing. These interactions are particularly worrying, as sub-lethal effects of one driver could make another one more lethal; for example a combination of pesticides and pathogens.

Ascribing a definitive cause to losses has also been made much more difficult because of differing pathogen virulence and different host susceptibility in different regions, and different methods used by scientists in previous surveys and experiments. In order to eliminate this latter variability, an international standardization of methods is urgently required (Nguyen et al., 2010). Moreover, the complex interactions between individual drivers of colony mortality and the high number of interacting factors easily exceed the research facilities of individual bee laboratories or even entire countries. Thus, efforts by individual countries to reveal the drivers of colony losses are probably doomed. The international COLOSS network (Prevention of Honeybee COlony LOSSes http://www.coloss.org/) has therefore been created to coordinate efforts to explain and prevent large scale losses of Honeybee colonies at a global scale (Figs 3 and 4). For that purpose, international standards will be developed for monitoring and research in the form of an online BEE BOOK, analogous to the RED BOOK of the Drosophila community Lindsley and Zimm (1992). Only this will enable collaborative large scale international research efforts to identify the underlying factors and mechanisms, such as global ring tests conducted to ensure common practices across diagnostic laboratories. These efforts appear critical for the development of adequate emergency measures and sustainable management strategies.

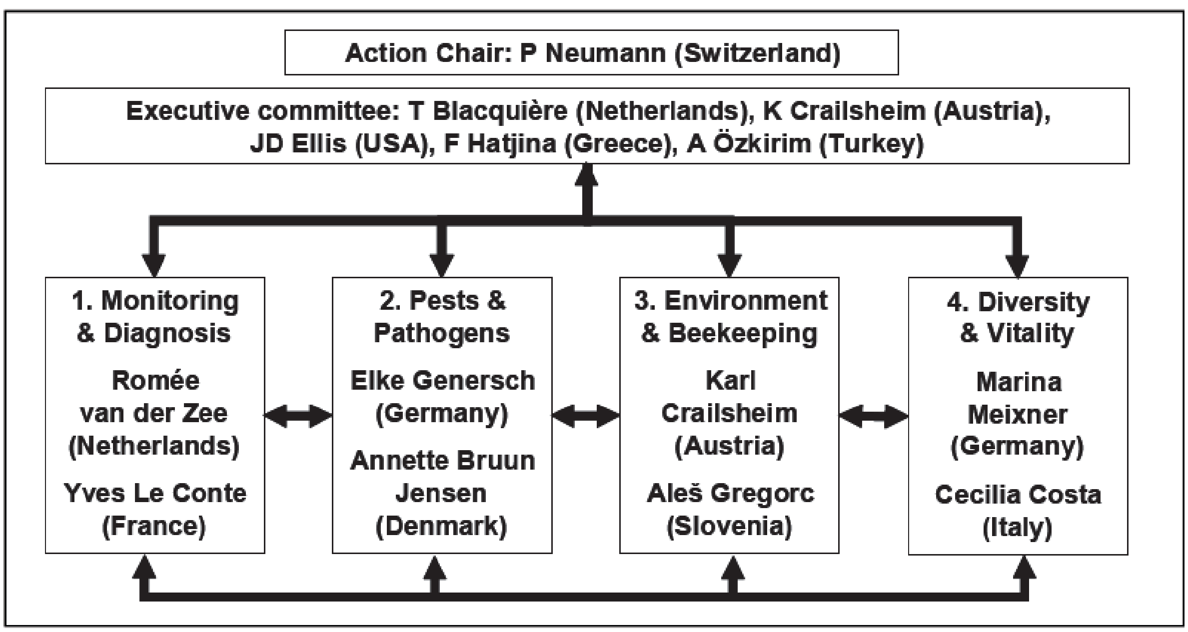

Fig. 7 Structure of the COLOSS network. Organizational matters are addressed by an executive core group. The four working groups (WG) concentrate on different aspects relevant for Honeybee colony losses. WG 1 focuses on monitoring and diagnosis which are crucial to obtain reliable field data on losses, comparable between countries and years (Nguyen et al., 2010). WGs 2-4 address in detail factors governing Honeybee health at both individual and colony level (see Meixner et al., 2010 for WG4). Co-operation across working groups is fundamental

to address the interactions between factors driving mortality (e.g. between pathogens and pesticides for WGs 2 and 3). From Neuman and Carreck (2010)

The COLOSS network does not directly fund research, but aims to coordinate national research activities across Europe and worldwide (Fig. 4). COLOSS comprises all three groups of stakeholders; scientists, beekeepers and industry with the aim of complementing rather than duplicating research approaches, and to create transnational synergies. Initiatives to obtain sustainable support for the network are in preparation. Networking is facilitated through conferences and scientific exchange programmes, but more importantly also through a large series of workshops for extension specialists and apiculturists. Only if we succeed in bridging the gap between bee science and apiculture will we achieve sustainable progress in the prevention of colony losses at a global scale.

For these reasons, this Special Issue of the Journal of Apicultural Research addresses the subject of colony losses. A mixture of Original Research Articles, Review Articles and Notes and Comments address the possible causes of Honeybee colony losses: viruses Berthoud, Imdorf, et al. (2010, Carreck, Ball, et al. (2010, Carreck, Bell, et al. (2010, Martin, Ball, et al. (2010) open access: Nosema ceranae Paxton (2010); Santrac, Granato, et al. (2010) Varroa destructor (Carreck, Bell, et al. (2010, Dahle (2010, Martin, Ball, et al. (2010) pesticides Chauzat, Carpentier, et al. (2010, Medrzycki, Sgolastra, et al. (2010) the effects of acaricides Harz, Mueller, et al. (2010) the loss of genetic diversity Meixner, Costa, et al. (2010); and loss of habitats Potts, Biesmeijer, et al. (2010, Potts, Roberts, et al. (2010). In addition, gathered together for the first time in one place, a group of papers report on colony losses and possible causes in sixteen individual countries: Austria Brodschneider, Moosbeckhofer, et al. (2010, Bromenshenk, Henderson, et al. (2010); Bosnia and Herzegovia Santrac, Granato, et al. (2010) Bulgaria Ivanova and Petrov (2010); Canada Currie, Pernal, et al. (2010) Croatia Gajger, Tomljanovic, et al. (2010, Gajger, Vugrek, et al. (2010) Denmark Vejsnaes, Nielsen, et al. (2010) England Aston (2010) France Chauzat, Carpentier, et al. (2010, Chauzat, Martel, et al. (2010A, Chauzat, Martel, et al. (2010B) Greece Hatjina, Bouga, et al. (2010) Italy Mutinelli, Costa, et al. (2010); the Netherlands van der Zee (2010) Norway Dahle (2010) Poland Topolska, Gajda, et al. (2010) Scotland (Gray, Peterson, et al. (2010) Switzerland Charriere and Neumann (2010) and the USA vanEngelsdorp, Caron, et al. (2012, vanEngelsdorp, Hayes, et al. (2010, vanEngelsdorp and Meixner (2010). Potts, Biesmeijer, et al. (2010, Potts, Roberts, et al. (2010, Roberts and Potts (2010)Finally, two further papers consider the general status of both managed Honeybees (Potts et al., 2010) and non-Apis bees (Roberts and Potts, 2010) in Europe. (all citations 2010 stem from the special issue of , see http://www.ibra.org.uk/articles/specialissue2010.

Another account on the CCD has been released in 2009 by a committee of specialists in this field Hendrikx, Chauzat, et al. (2009). It is the most comprehensive science report over 217 pages on CCD, based on a thorough analysis of the literature (hundreds of references with priorities 1 to 3 of science and apiculture reports. It also includes the screening of ca. 100 specialized websites on apiculture. The results of this study deserve to be summarized extensively:

Hendrikx, P., M. Chauzat, et al. (2009). Bee Mortality and Bee Surveillance in Europe, SCIENTIFIC REPORT submitted to EFSA. CFP/EFSA/AMU/2008/02. Accepted for Publication on 03 December 2009**:** 217. http://www.efsa.europa.eu/cs/BlobServer/External_Rep/027e.pdf?ssbinary=true AND http://www.ask-force.org/web/Bees/Hendrikx-Bee-Mortality-Surveillance-Europe-2009.pdf

"The bee surveillance project sought information on both the prevalence of Honeybee colony losses, and the surveillance systems found in 27 European countries. Through a standardized questionnaire, data was obtained from 24 countries, relating to 25 systems. Each of the surveillance systems collecting these data was evaluated. In addition, a thorough literature search of the existing databases, as well as relevant grey literature about causes of colony losses was completed, and the literature evaluated.

The main conclusions from project activities can be summarized as follows:

General weakness of most of the surveillance systems in the 24 countries investigated;

Lack of representative data at country level and comparable data at EU level for colony losses;

General lack of standardization and harmonization at EU level (systems, case definitions and data collected);

Consensus of the scientific community about the multi-factorial origin of colony losses in Europe and in the United States and insufficient knowledge of causative and risk factors for colony losses.

The project makes recommendations, in the following areas:

Establishment of a sustainable European network for coordination and follow-up of surveillance on colony losses to underpin monitoring programs;

Strengthen standardization at European level by harmonization of surveillance systems, data collected and by developing common performance indicators.

Build on the examples of best practice found in existing surveillance systems for communicable and notifiable diseases already present in some countries;.

Undertake specific studies that build on the existing work in progress to improve the knowledge and understanding of factors that affect bee health (for example stress caused by pathogens, pesticides, environmental and technological factors and their interactions) using appropriate epidemiological studies (case control and longitudinal studies).

The set up of the coordination team at European level. This is a crucial issue and the coordination team should be organized in such a way so as to ensure its sustainability and to enable effective surveillance program activities at the European level." Hendrikx, Chauzat, et al. (2009)

Some useful illustrations from this open source report:

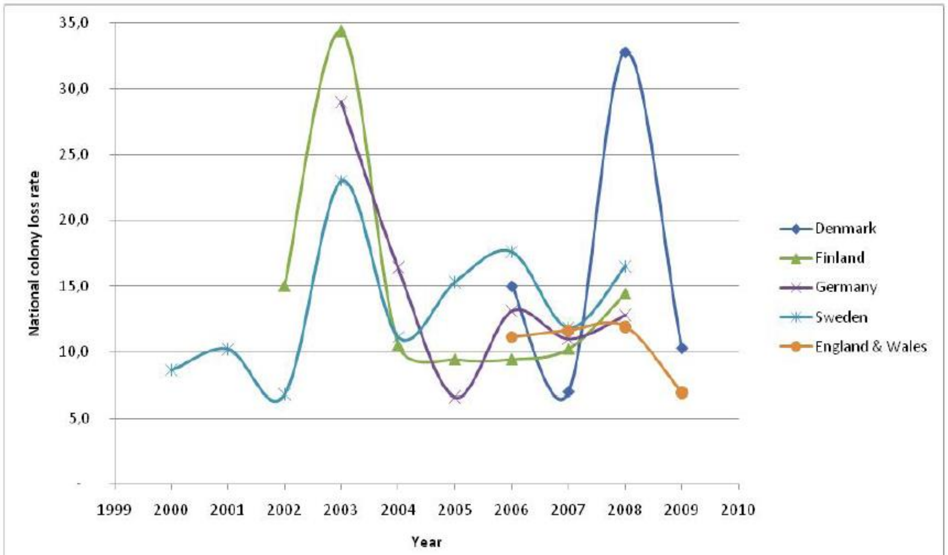

Fig. 8 National percentages of colonies lost during winter from 2000 to 2009 in Denmark, Finland, Germany, Sweden, England and Wales. From Hendrikx, Chauzat, et al. (2009)

"The figure 1 presents the temporal trends for the five surveillance systems that were considered to provide representative data. Results obtained from all other systems are presented from Figure 37 to Figure 49 in the original report. When continuous data were not available, the dataset is represented with dots. All these trends are difficult to interpret, especially when keeping in mind the great variety in quality between those surveillance systems that produced the data. However, the following trend should be noted:
A baseline colony losses rate around 10% is observed at European level (during the period 2000 – 2009 the minimum winter colony loss rate range from 4.8% to 11%, regression line from 9.6% in 2000 to 6.3% in 2009). This baseline loss rate is the normal loss rate admitted for bee production systems;
The project identifies a higher level of colony losses in some countries during the years 2003 and 2008. This apparent finding is, however, based on limited data that varies in representativeness, precision and the indicator calculation methods used. It must therefore be viewed with caution." Hendrikx, Chauzat, et al. (2009).

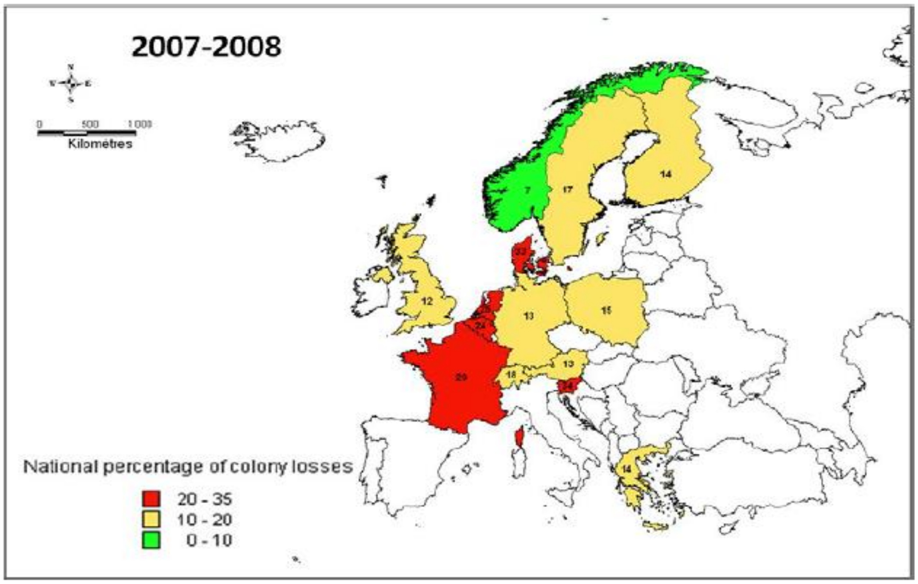

Fig. 9 Percentage of colony losses in some countries of Europe during winter 2007-2008, from Hendrikx, Chauzat, et al. (2009).

Conclusions, recommendations and perspectives related to CCD monitoring

Collection and analysis of data stemming from the colony losses surveillance systems in Europe clearly reveal that there is an absence of shared loss indicators, calculated following the same procedures, and applied to comparable populations. Therefore, the only indicator that could be used in this study was the winter colony loss rate. Even though analyses of temporal trends or geographical incidence seem to suggest some periods of higher winter colony loss rates, these findings should not be over interpreted; this highlights how some existing data collection systems are unsuitable for drawing any comparisons between situations in different European countries, and in the analysis of colony loss trends at the European level.

Therefore, according to the recommendations proposed following the assessment of the surveillance systems, harmonization of surveillance procedures at the European level must include the establishment of a common set of epidemiological indicators, calculated following the same rules in all countries, and produced by comparable active surveillance procedures applied on comparable populations. This recommendation is essential to allow comparison between countries' situations within Europe, and the objective assessment of the trends in colony losses, not only addressing winter colony losses but also summer colony losses giving a more complete view of the phenomenon. An appropriate tool to monitor colony losses at the European level is important as this will provide national or European decision makers and the beekeeping industry with accurate figures about colony mortality, allowing them to focus their collective research and control activities.

The above mentioned scientific monitoring group which should be implemented at the European level for the harmonization of surveillance systems, should be also responsible for the implementation and follow-up of the European data collection, management and interpretation activities. Composed of scientists specialized in bee diseases and bee production and epidemiology, this group would represent the appropriate scientific and technical support to European institutions such as EFSA and European Commission for risk analysis and decision-making."

Hendrikx, Chauzat, et al. (2009)

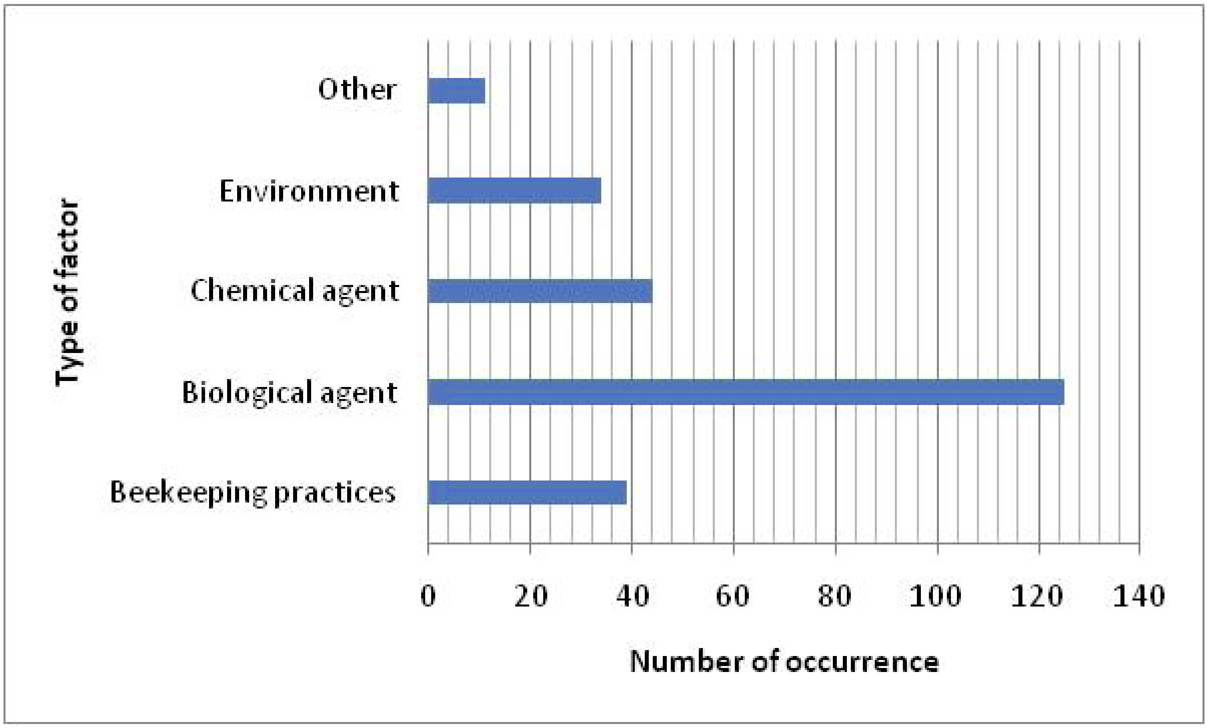

Fig. 10 Types of factors occurring in the studied literature from Hendrikx, Chauzat, et al. (2009).

Comments within the study on Fig.3, based on a very thorough and unusually systematic literature study:

"All risk and causative factors mentioned in the studied references are detailed in this chapter. It has proved difficult to separate risk factors from causative factors. This distinction is rarely drawn in the literature; authors often mix these concepts and, in many cases, misuse them. This is certainly due to the lack of understanding of the origin of the colony losses syndrome and the difficulty scientists are facing, when identifying a link between a factor and the phenomenon, to clearly state if it's a causative or a risk factor. Therefore, this difference has not been highlighted in the following description.
The quantitative data given in this chapter should not be used as evidence to categorize the importance or to qualify the certainty of the involvement of a specific factor. Quantitative data should express more the relative "popularity" of the studied factors. Furthermore, a certain redundancy occurs in the read references, due to the integration of reviews and non peer-reviewed literature mentioning results from the peer-reviewed literature. Therefore, an "amplification effect" may over represent the occurrence of one factor in the literature, without representing any link with its real involvement in the phenomenon.
An attempt to address the link between any factor and the phenomenon has been done by qualifying the probability of this link according to authors' opinion, using a four scale scoring: Unlikely probable, very likely and proven. Once more, due to the lack of evidence of the origin of colony losses and the amount of ongoing research into this, proven effects are very scarce and unsubstantiated authors' opinions should be viewed with caution.
A four-class categorization scale was used to capture the range of factors mentioned in the literature. Figure 4 shows that biological agents are the most represented factors. Each type is detailed and discussed in the following paragraphs." Hendrikx, Chauzat, et al. (2009)

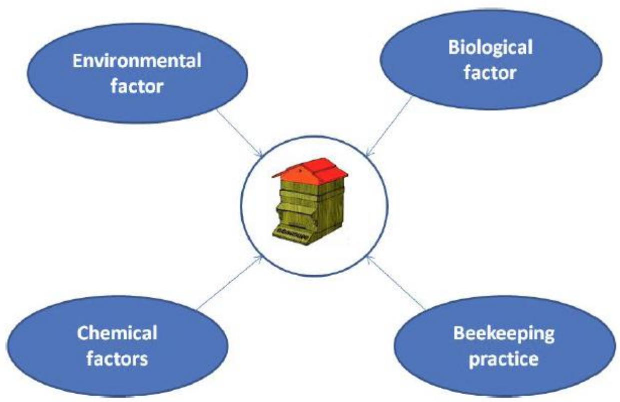

Fig. 11 Factors involved in colony losses from Hendrikx, Chauzat, et al. (2009).

"Conclusion and perspectives related to CCD causal factors
The work package on literature review allowed the development of a specific methodology for literature search and analysis. The "priority 1" references selected and reviewed validate the objectivity of the literature search which is expressed through the variability and the balanced topics included. The results of this work regarding risk and causative factors involved in colony losses have to be taken as a "snap shot" of the scientific community's opinion as they are today; these are also "time sensitive", and evolving due to the amount of ongoing research which will likely lead to new findings and a better understanding of the factors involved in the coming months or years.
To summarize this picture, common consensus amongst the scientific community about the multi-factorial origin of colony losses in Europe and in the United States (in the two aspects of this term: combination of factors at one place and different factors involved according to place and period considered) suggests the following factors are important, namely: beekeeping practices (feeding, migratory beekeeping, colony husbandry, treatments applied and so forth), environmental factors (climate, available forage, biodiversity, etc.), chemical factors (pesticides) or biological agents (Varroa, Nosema spp, etc.) which together create stress, weaken bees' immune systems that then allow pests and pathogens to kill the colony (e.g. one or several parasites, viruses, etc.).
Questions remain about the sequence of events that lead to colony mortality, and future studies should be designed and conducted to address this:
There are many inconsistencies in the ways in which "colony losses" are defined. Up to 17 different definitions for CCD in the literature. This means that involved persons may not always be referring to the same phenomenon, and this creates confusion when trying to explain the origin of what has been identified in the field. The described pathology is varied, with authors/using the same descriptions for different sets of circumstances. A specific study should be undertaken to clearly categorize and quantify the various expressions of colony losses in the field. This study will be closely linked to the strengthening of surveillance systems;
High concentrations of pesticides have rarely been identified in relation to colony losses (CCD in USA and winter colony losses in Europe) although acute events of pesticide toxicity are well described during the production season (and clearly differentiated from CCD and winter colony losses). However, the questions of possible synergistic effects of various pesticides and the effect of chronic exposure to sub-lethal doses of pesticides remains, and requires further investigation;
Biological agents such as parasites, viruses or bacteria, alone or in combination, have clearly been identified as important factors in colony losses. Nevertheless, there is still a lack of knowledge about the exact mechanisms and/or interactions involved, that must also be addressed;
Even though the multi-factorial origin of colony losses is well acknowledged, the respective role of each factor as a risk or causative agent is unknown, and no hierarchy of relative threat posed." Hendrikx, Chauzat, et al. (2009).

Referring to GM crops there is no questionnaire report available, although in the questionnaire the question was included (p. 24)

"GMOs were not mentioned as an origin of colony losses, but neither are they targeted by any of the surveillance systems within this study."
Or p.87 under environmental factors:
"A consensus on these assumptions as authors' opinions is expressed in Table 15. Considering the role of GMOs and electro-magnetic radiations, another consensus arises: the role of either of these two factors on colony losses is absent (Table 15)."

Other comprehensive summaries of the present-day knowledge about the CCD have been published in 2010, a confirmation for a multitude of causes of CCD.

vanEngelsdorp, D. and M. D. Meixner (2010). "A historical review of managed Honeybee populations in Europe and the United States and the factors that may affect them, open source." Journal of Invertebrate Pathology **103**(Supplement 1): S80-S95. http://www.sciencedirect.com/science/article/B6WJV-4XNMC3B-4/2/243a511760d9697483f95c9d96a9794e AND open source http://www.ask-force.org/web/Bees/vanEngelsdorp-Historical-.Review-2010.pdf

Abstract: Honeybees are a highly valued resource around the world. They are prized for their honey and wax production and depended upon for pollination of many important crops. While globally Honeybee populations have been increasing, the rate of increase is not keeping pace with demand. Further, honeybee populations have not been increasing in all parts of the world, and have declined in many nations in Europe and in North America. Managed Honeybee populations are influenced by many factors including diseases, parasites, pesticides, the environment, and socio-economic factors. These factors can act alone or in combination with each other. This review highlights the present day value of Honeybees, followed by a detailed description of some of the historical and present day factors that influence Honeybee populations, with particular emphasis on colony populations in Europe and the United States. vanEngelsdorp and Meixner (2010)

Van Engelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D.R., & Pettis, J.S. (2009)

Colony Collapse Disorder: A Descriptive Study. PLoS ONE, 4, 7, pp Article No.: e6481

openlink: <Go to ISI>://BIOSIS:PREV200900520575 AND http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0006481 AND http://www.ask-force.org/web/Bees/VanEngelsdorp-Descriptive-PLoSone-2009.pdf

"Background: Over the last two winters, there have been large-scale, unexplained losses of managed Honeybee (Apis mellifera L.) colonies in the United States. In the absence of a known cause, this syndrome was named Colony Collapse Disorder (CCD) because the main trait was a rapid loss of adult worker bees. We initiated a descriptive epizootiological study in order to better characterize CCD and compare risk factor exposure between populations afflicted by and not afflicted by CCD.Methods and Principal Findings: Of 61 quantified variables (including adult bee physiology, pathogen loads, and pesticide levels), no single measure emerged as a most-likely cause of CCD. Bees in CCD colonies had higher pathogen loads and were co-infected with a greater number of pathogens than control populations, suggesting either an increased exposure to pathogens or a reduced resistance of bees toward pathogens. Levels of the synthetic acaricide coumaphos (used by beekeepers to control the parasitic mite Varroa destructor) were higher in control colonies than CCD-affected colonies. Conclusions/Significance: This is the first comprehensive survey of CCD-affected bee populations that suggests CCD involves an interaction between pathogens and other stress factors. We present evidence that this condition is contagious or the result of exposure to a common risk factor. Potentially important areas for future hypothesis-driven research, including the possible legacy effect of mite parasitism and the role of Honeybee resistance to pesticides, are highlighted." vanEngelsdorp, Evans, et al. (2009B)

James Tew from the Ohio State University gives an earlier good summary from 2002 on the multiple possible causes of CCD and also sums up the history of this still enigmatic disease:

Tew, J.E. (2002)

Bee Culture's Beeyard. In ECAI 2002. 15th European Conference on Artificial Intelligence Proceedings. Dr. James E. Tew, State Specialist, Beekeeping, The Ohio State University, Wooster, OH 44691

http://www.orsba.org/htdocs/download/Dtew.htm AND http://www.ask-force.org/web/Bees/Tew-Bee-Cultures-Beeyard-2002.PDF

"After several hundred years of observations, there are still plenty of mysteries behind the closed doors of the beehive. As beekeepers, we have always given simple answers to complicated bee questions. In most instances, our only other alternative was to give no answer at all.

During the Spring of 2002, numerous Alabama beekeepers experienced an inexplicable bee colony die-off. There was no obvious cause - even after USDA analysis.

An old diagnosis was called up - The Disappearing Disease of Honeybees.

My first association with this peculiar ailment was about 20 years ago, when I talked to the late Dr. Walter Rothenbuhler about the sickness. He had attempted to work on the problem, but never made much headway. Though he had performed several research projects, no conclusions were ever drawn. Scant research attention has been allocated to this syndrome over the years. Like an urban legend, the disease lives in scattered paragraphs in bee books near the end of the requisite chapter on bee diseases.

Some History

The condition was first described in 1915 and was called Disappearing Disease because the disease was self-limiting and disappeared. Through the years, that name has increasingly been broadened to describe any mysterious instance where adult bees disappear - not the disease. Confusing isn't it? If the bees have disappeared, then the disease is gone, too. Right? From 1915 until this time, no single pathogen has even been isolated. Other possible names for the ailment are: Spring Dwindling, Fall Dwindling, May Disease, and Autumn Collapse. The Isle of Wight Disease, caused by tracheal mites, has many similarities to Disappearing Disease. The reported symptoms are broad and indistinct appearing to be a collage of characteristics.

In 1915, after a particularly wet Spring, significant colony losses were reported. One beekeeper lost 400 hives. The problem was noted in multiple states from Florida to California. Hives came out the Winter in good shape, but adult bees began to vanish at the beginning of the

Spring nectar flow. In afflicted apiaries, at best, honey crops were reduced. At worst, colonies were essentially emptied of adult bees. During subsequent years, now and then, reports were posted presenting Disappearing Disease as the cause of occasional colony losses." Tew (2002)

A very good and rich source on all aspects of CCD is the following Wicki:

http://en.wikipedia.org/wiki/Colony_collapse_disorder

More: An EU project COLOSS, started in December 2008, results are summarized in the next chapter 3.2, but as we will see below, there will be no silver bullet solution with a clear cut explanation, rather we will be confronted with a multifold collection of factors from climate to agricultural management elements which can add to the overall stress of bees:

AFFSA-EFSA (2008), Bee Mortality and Bee Surveillance in Europe (EFSA-Q-2008-428), A Report from the Assessment Methodology Unit in Response to Agence Francaise de Securite Sanitaire des Aliments (AFSSA), The EFSA journal, 154, pp. 1-28, http://www.efsa.europa.eu/cs/BlobServer/Report/AMU_Technical_Report_Bees_EFSA-Q-2008-428_20083007_final,0.pdf?ssbinary=true AND http://www.ask-force.org/web/Bees/AFSSA-EFSA-Bee-Mortality-Project-2008.pdf

3. Standard Methods: Reports on general management of experimentation with bees in laboratories and field.

In the latest final reports from 2013 of the COLOSS project several papers describe best procedures on how to conduct bee experiments in the laboratory and field: Buchler, Andonov, et al. (2013, Crailsheim, Brodschneider, et al. (2013, Delaplane, van der Steen, et al. (2013, Genersch, Gisder, et al. (2013, Hartfelder, Bitondi, et al. (2013, vanEngelsdorp, Lengerich, et al. (2013, Williams, Alaux, et al. (2013)
A recent search in December 2016 in the Web of Science under bees and standard methods revealed a plethora of proposals, mainly coming from COLOSS: 2013 was the major year of publications, here a selection of 30 references.

Akimov, Zaloznaya, et al. (1990, Anderson and Roberts (2013, Becher Matthias A., Volker Grimm, et al. (2014, Borsuk, Olszewski, et al. (2012, Buchler, Andonov, et al. (2013, Chen, Collins, et al. (2013, Crailsheim, Brodschneider, et al. (2013, de Graaf, Alippi, et al. (2013, Delaplane, van der Steen, et al. (2013, Dietemann, Nazzi, et al. (2013, Ellis, Graham, et al. (2013, Evans, Schwarz, et al. (2013, Forsgren, Budge, et al. (2013, Fremuth (1984, Fries, Chauzat, et al. (2013, Garrido, Martin, et al. (2013, Genersch, Gisder, et al. (2013, Hartfelder, Bitondi, et al. (2013, Human, Brodschneider, et al. (2013, Jensen, Aronstein, et al. (2013, Lee, Reuter, et al. (2010, Medrzycki, Giffard, et al. (2013, Meixner, Pinto, et al. (2013, Neumann, Evans, et al. (2013, ORourke and Buchmann (1991, Sammataro, de Guzman, et al. (2013, Scheiner, Abramson, et al. (2013, Tenczar and Krischik (2007, Tofilski (2008, vanEngelsdorp, Lengerich, et al. (2013, Williams, Alaux, et al. (2013, Williams, Dietemann, et al. (2012)

A selection is given below with summaries:

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L. P., Chauzat, M. P., Classen, C., Colin, M. E., Dupont, T., Girolami, V., Johnson, R., Le Conte, Y., Luckmann, J., Marzaro, M., Pistorius, J., Porrini, C., Schur, A., Sgolastra, F., Delso, N. S., van der Steen, J. J. M., Wallner, K., Alaux, C., Biron, D. G., Blot, N., Bogo, G., Brunet, J. L., Delbac, F., Diogon, M., El Alaoui, H., Provost, B., Tosi, S., & Vidau, C. (2013). Standard methods for toxicology research in Apis mellifera. *Journal of Apicultural Research, 52*(4), pp. <Go to ISI>://WOS:000323845800012 AND http://www.ask-force.org/web/Bees/Medrzycki-Standard-Methods-toxicology-research-Apis-mellifera-2013.pdf

Modern agriculture often involves the use of pesticides to protect crops. These substances are harmful to target organisms (pests and pathogens). Nevertheless, they can also damage non-target animals, such as pollinators and entomophagous arthropods. It is obvious that the undesirable side effects of pesticides on the environment should be reduced to a minimum. Western honey bees (Apis mellifera) are very important organisms from an agricultural perspective and are vulnerable to pesticide-induced impacts. They contribute actively to the pollination of cultivated crops and wild vegetation, making food production possible. Of course, since Apis mellifera occupies the same ecological niche as many other species of pollinators, the loss of honey bees caused by environmental pollutants suggests that other insects may experience a similar outcome. ***Because pesticides can harm honey bees and other pollinators, it is important to register pesticides that are as selective as possible. In this manuscript, we describe a selection of methods used for studying pesticide toxicity/selectiveness towards Apis mellifera. These methods may be used in risk assessment schemes and in scientific research aimed to explain acute and chronic effects of any target compound on Apis mellifera.*** *Medrzycki, Giffard, et al. (2013)*

Becher Matthias A., Volker Grimm, Pernille Thorbek, Juliane Horn, Peter J. Kennedy and Osborne, J. L. (2014) BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure 470-482 pp DOI: 10.1111/1365-2664.12222 AND http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12222/epdf AND http://www.ask-force.org/web/Bees/Becher-BEEHAVE-systems-model-honeybee-colony-dynamics-2014.pdf

"Summary
1. A notable increase in failure of managed European honeybee Apis mellifera L. colonies has been reported in various regions in recent years. Although the underlying causes remain unclear, it is likely that a combination of stressors act together, particularly varroa mites and other pathogens, forage availability and potentially pesticides. It is experimentally challenging to address causality at the colony scale when multiple factors interact. In silico experiments offer a fast and cost-effective way to begin to address these challenges and inform experiments. However, none of the published bee models combine colony dynamics with foraging patterns and varroa dynamics.
2. We have developed a honeybee model, BEEHAVE, which integrates colony dynamics, population dynamics of the varroa mite, epidemiology of varroa-transmitted viruses and allows foragers in an agent-based foraging model to collect food from a representation of a spatially explicit landscape.
3. We describe the model, which is freely available online (www.beehave-model.net). Extensive sensitivity analyses and tests illustrate the model's robustness and realism. Simulation experiments with various combinations of stressors demonstrate, in simplified landscape settings, the model's potential: predicting colony dynamics and potential losses with and without varroa mites under different foraging conditions and under pesticide application. We also show how mitigation measures can be tested.
4. Synthesis and applications. BEEHAVE offers a valuable tool for researchers to design and focus field experiments, for regulators to explore the relative importance of stressors to devise management and policy advice and for beekeepers to understand and predict varroa dynamics and effects of management interventions. We expect that scientists and stakeholders will find a variety of applications for BEEHAVE, stimulating further model development and the possible inclusion of other stressors of potential importance to honeybee colony dynamics. From Becher Matthias A., Volker Grimm, et al. (2014)

Buchler, R., S. Andonov, K. Bienefeld, C. Costa, F. Hatjina, N. Kezic, P. Kryger, M. Spivak, A. Uzunov and J. Wilde (2013), Standard methods for rearing and selection of Apis mellifera queens, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300007 AND http://www.ask-force.org/web/Bees/Buechler-Standard-Rearing-Selection-20130901.pdf

"Here we cover a wide range of methods currently in use and recommended in modern queen rearing, selection and breeding. The recommendations are meant to equally serve as standards for both scientific and practical beekeeping purposes. The basic conditions and different management techniques for queen rearing are described, including recommendations for suitable technical equipment. As the success of breeding programs strongly depends on the selective mating of queens, a subchapter is dedicated to the management and quality control of mating stations. Recommendations for the handling and quality control of queens complete the queen rearing section. The improvement of colony traits usually depends on a comparative testing of colonies. ***Standardized recommendations for the organization of performance tests and the measurement of the most common selection characters are presented. Statistical methods and data preconditions for the estimation of breeding values which integrate pedigree and performance data from as many colonies as possible are described as the most efficient selection method for large populations.*** *Alternative breeding programs for small populations or certain scientific questions are briefly mentioned, including also an overview of the young and fast developing field of molecular selection tools. Because the subject of queen rearing and selection is too large to be covered within this paper, plenty of references are given to facilitate comprehensive studies." Buchler, Andonov, et al. (2013)*

Chen, M., Collins, E. M., Tao, L. and Lu, C. S. (2013) Simultaneous determination of residues in pollen and high-fructose corn syrup from eight neonicotinoid insecticides by liquid chromatography-tandem mass spectrometry Analytical and Bioanalytical Chemistry 405 28 9251-9264 pp ISBN/1618-2642 <Go to ISI>://WOS:000326374100027 AND http://www.ask-force.org/web/Bees/Chen-Simultaneous-Determination-2013.pdf

"Theneonicotinoidshaverecentlybeenidentifiedas a potential contributing factor to the sudden decline in adult honeybee population, commonly known as colony collapse disorder (CCD). To protect the health of honeybees and other pollinators,anew,simple,andsensitiveliquidchromatographyelectrospray ionization mass spectrometry method was developed and validated for simultaneous determination of eight neonicotinoids,includingacetamiprid,clothianidin,dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam, in pollen and high-fructose corn syrup (HFCS). In this method, eight neonicotinoids, along with their isotope-labeled internal standards, were extracted from 2 g of pollen or 5 g of HFCS using an optimized quick, easy, cheap, effective, rugged, and safe extraction procedure. The method limits of detection in pollen and HFCS matrices were 0.03 ng/g for acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam and ranged between 0.03 and 0.1 ng/g for nitenpyram and flonicamid. The precision and accuracy were well within the acceptable 20 % range. Selectivity, linearity, lower limit of quantitation, matrix effect, recovery, and stability in autosampler were also evaluated during validation. This validated method has been used successfully in analyzing a set of pollen and HFCS samples collected for evaluating potential honeybee exposure to neonicotinoids." From Chen, Collins, et al. (2013)

Crailsheim, K., R. Brodschneider, P. Aupinel, D. Behrens, E. Genersch, J. Vollmann and U. Riessberger-Galle (2013), Standard methods for artificial rearing of Apis mellifera larvae, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300005 AND http://www.ask-force.org/web/Bees/Crailsheim-Standard-Artificial-Raring-20130901.pdf

"Originally, a method to rear worker Honeybee larvae in vitro was introduced into the field of bee biology to analyse Honeybee physiology and caste development. Recently, it has become an increasingly important method in bee pathology and toxicology. The in vitro method of rearing larvae is complex and can be developed as an art by itself, especially if the aim is to obtain queens or worker bees which, for example, can be re-introduced into the colony as able members. However, a more pragmatic approach to in vitro rearing of larvae is also possible and justified if the aim is to focus on certain pathogens or compounds to be tested. It is up to the researcher(s) to decide on the appropriate experimental establishment and design. ***This paper will help with this decision and provide guidelines on how to adjust the method of in vitro rearing according to the specific needs of the scientific project."*** *Crailsheim, Brodschneider, et al. (2013)*

Delaplane, K. S., J. van der Steen and E. Guzman-Novoa (2013), Standard methods for estimating strength parameters of Apis mellifera colonies, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300003 AND http://www.ask-force.org/web/Bees/Delaplane-Standard-Estimating-Parameters-Apis-Cologies-20130901.pdf

"This paper covers measures of field colony strength, by which we mean population measures of adult bees and brood. There are generally two contexts in which an investigator wishes to measure colony strength: 1. at the beginning of a study as part of manipulations to produce uniform colonies and reduce experimental error and; 2. as response variables during or at the end of an experiment. Moreover, there are two general modes for measuring colony strength: 1. an objective mode which uses empirical measures and; 2. a subjective mode that relies on visual estimates by one or more observers. There is a third emerging mode for measuring colony strength; 3. computer-assisted digital image analysis. A final section deals with parameters that do not directly measure colony strength yet give important indicators of colony state: flight activity at the hive entrance; comb construction; and two proxy measures of colony fitness: production of queen cells and drone brood. " Delaplane, van der Steen, et al. (2013)

Genersch, E., S. Gisder, K. Hedtke, W. B. Hunter, N. Mockel and U. Muller (2013), Standard methods for cell cultures in Apis mellifera research, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300002 AND http://www.ask-force.org/web/Bees/Genersch-Standard-Cell-Cultures-Apis-Mellifera-20130901.pdf

"Cell culture techniques are indispensable in most, if not all life science disciplines to date. Wherever appropriate cell culture models are lacking, scientific development is hampered. Unfortunately this has been and still is the case in Honeybee research, because permanent Honeybee cell lines have not so far been established. To overcome this hurdle, protocols for the cultivation of primary Honeybee cells and of non-permanent Honeybee cell lines have been developed. In addition, heterologous cell culture models for Honeybee pathogens based on non-Apis insect cell lines have recently been developed. ***To further advance this progress and to encourage bee scientists to enter the field of cell biology based research, here we present protocols for the cultivation of Honeybee primary cells and non-permanent cell lines, as well as hints for the cultivation of permanent insect cell lines suitable for Honeybee research****." Genersch, Gisder, et al. (2013)*

Hartfelder, K., M. M. G. Bitondi, C. S. Brent, K. R. Guidugli-Lazzarini, Z. L. P. Simoes, A. Stabentheiner, E. D. Tanaka and Y. Wang (2013), Standard methods for physiology and biochemistry research in Apis mellifera, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300006 AND http://www.ask-force.org/web/Bees/Hartfelder-Standard-Physiology-Chemistry-Apis-Mellifera-20130901.pdf

"Despite their tremendous economic importance, and apart from certain topics in the field of neurophysiology such as vision, olfaction, learning and memory, Honeybees are not a typical model system for studying general questions of insect physiology. The reason is their social lifestyle, which sets them apart from a "typical insect" and, during social evolution, has resulted in the restructuring of certain physiological pathways and biochemical characteristics in this insect. Not surprisingly, the questions that have attracted most attention by researchers working on Honeybee physiology and biochemistry in general are core topics specifically related to social organization, such as caste development, reproductive division of labour and polyethism within the worker caste. With certain proteins playing key roles in these processes, such as the major royal jelly proteins (MRJPs), including royalactin and hexamerins in caste development, and vitellogenin in reproductive division of labour and age polyethism, a major section herein will present and discuss basic laboratory protocols for protein analyses established and standardized to address such questions in bees. A second major topic concerns endocrine mechanisms underlying processes of queen and worker development, as well as reproduction and polyethism, especially the roles of juvenile hormone and ecdysteroids. Sensitive techniques for the quantification of juvenile hormone levels circulating in haemolymph, as well as its synthesis by the corpora allata are described. Although these require certain instrumentation and a considerable degree of sophistication in the analysis procedures, we considered that presenting these techniques would be of interest to laboratories planning to specialize in such analyses. ***Since biogenic amines are both neurotransmitters and regulators of endocrine glands, we also present a standard method for the detection and analysis of certain biogenic amines of interest. Further questions that cross borders between individual and social physiology are related to energy metabolism and thermoregulation. Thus a further three sections are dedicated to protocols on carbohydrate quantification in body fluid, body temperature measurement and respirometry****". Hartfelder, Bitondi, et al. (2013)*

vanEngelsdorp, D., E. Lengerich, A. Spleen, B. Dainat, J. Cresswell, K. Baylis, B. K. Nguyen, V. Soroker, R. Underwood, H. Human, Y. Le Conte and C. Saegerman (2013), Standard epidemiological methods to understand and improve Apis mellifera health, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300008 AND http://www.ask-force.org/web/Bees/vanEngelsdorp-Standard-Epidemiological-Methods-corr-20130901.pdf

"In this paper, we describe the use of epidemiological methods to understand and reduce Honeybee morbidity and mortality. Essential terms are presented and defined and we also give examples for their use. Defining such terms as disease, population, sensitivity, and specificity, provides a framework for epidemiological comparisons. The term population, in particular, is quite complex for an organism like the Honeybee because one can view "epidemiological unit" as individual bees, colonies, apiaries, or operations. The population of interest must, therefore, be clearly defined. Equations and explanations of how to calculate measures of disease rates in a population are provided. There are two types of study design; observational and experimental. The advantages and limitations of both are discussed. Approaches to calculate and interpret results are detailed. Methods for calculating epidemiological measures such as detection of rare events, associating exposure and disease (Odds Ratio and Relative Risk), and comparing prevalence and incidence are discussed. Naturally, for beekeepers, the adoption of any management system must have economic advantage. ***We present a means to determine the cost and benefit of the treatment in order determine its net benefit. Lastly, this paper presents a discussion of the use of Hill's criteria for inferring causal relationships. This framework for judging cause-effect relationships supports a repeatable and quantitative evaluation process at the population or landscape level. Hill's criteria disaggregate the different kinds of evidence, allowing the scientist to consider each type of evidence individually and objectively, using a quantitative scoring method for drawing conclusions. It is hoped that the epidemiological approach will be more broadly used to study and negate Honeybee disease."*** *vanEngelsdorp, Lengerich, et al. (2013)*

Williams, G. R., C. Alaux, C. Costa, T. Csaki, V. Doublet, D. Eisenhardt, I. Fries, R. Kuhn, D. P. McMahon, P. Medrzycki, T. E. Murray, M. E. Natsopoulou, P. Neumann, R. Oliver, R. J. Paxton, S. F. Pernal, D. Shutler, G. Tanner, J. J. M. van der Steen and R. Brodschneider (2013), Standard methods for maintaining adult Apis mellifera in cages under in vitro laboratory conditions, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300004 AND http://www.ask-force.org/web/Bees/Williams-Standard-Maintaining-Adult-Apis-Cages-20130901.pdf

"Adult Honeybees are maintained in vitro in laboratory cages for a variety of purposes. For example, researchers may wish to perform experiments on Honeybees caged individually or in groups to study aspects of parasitology, toxicology, or physiology under highly controlled conditions, or they may cage whole frames to obtain newly emerged workers of known age cohorts. Regardless of purpose, researchers must manage a number of variables, ranging from selection of study subjects (e.g. Honeybee subspecies) to experimental environment (e.g. temperature and relative humidity). Although decisions made by researchers may not necessarily jeopardize the scientific rigour of an experiment, they may profoundly affect results, and may make comparisons with similar, but independent, studies difficult. Focusing primarily on workers, we provide recommendations for maintaining adults under in vitro laboratory conditions, whilst acknowledging gaps in our understanding that require further attention. We specifically describe how to properly obtain Honeybees, and how to choose appropriate cages, incubator conditions, and food to obtain biologically relevant and comparable experimental results. ***Additionally, we provide broad recommendations for experimental design and statistical analyses of data that arises from experiments using caged Honeybees. The ultimate goal of this, and of all COLOSS BEEBOOK papers, is not to stifle science with restrictions, but rather to provide researchers with the appropriate tools to generate comparable data that will build upon our current understanding of Honeybees."*** *Williams, Alaux, et al. (2013)*

Bouga, M., Alaux, C., Bienkowska, M., Buchler, R., Carreck, N. L., Cauia, E., Chlebo, R., Dahle, B., Dall'Olio, R., De la Rua, P., Gregorc, A., Ivanova, E., Kence, A., Kence, M., Kezic, N., Kiprijanovska, H., Kozmus, P., Kryger, P., Le Conte, Y., Lodesani, M., Murilhas, A. M., Siceanu, A., Soland, G., Uzunov, A. and Wilde, J. (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping Journal of Apicultural Research 50 1 51-84 pp ISBN/0021-8839 <Go to ISI>://WOS:000287335500006 AND http://www.ask-force.org/web/Bees/Bouga-review-methods-discrimination-bee-populations-2015.pdf

"Here, scientists from 19 European countries, most of them collaborating in Working Group 4: "Diversity and Vitality" of COST Action FA 0803 "Prevention of honey bee COlony LOSSes" (COLOSS), review the methodology applied in each country for discriminating between honey bee populations. Morphometric analyses (classical and geometric) and different molecular markers have been applied. Even if the approach has been similar, however, different methodologies regarding measurements, landmarks or molecular markers may have been used, as well as different statistical procedures. ***There is therefore the necessity to establish common methods in all countries in order to have results that can be directly compared. This is one of the goals of WG4 of the COLOSS project."*** *From Bouga, Alaux, et al. (2011)*

See also an interesting summary in the times of Brexit of Carreck - on Bees, Britain and the EU: Carreck (2016).

Carreck, N. L. (2016) Bees, Britain and the EU ... Bee World 93 1 1-1 pp ISBN/0005-772X http://dx.doi.org/10.1080/0005772X.2016.1222791 AND http://www.ask-force.org/web/Bees/Carreck-Bees-Britain-and-EU-2016.pdf

4. GM crops ruled out as a possible cause of CCD

An early comprehensive study of Hendrikx, Chauzat, et al. (2009) with an extensive literature analysis and a survey based on questionnaires did a priori rule out any negative impact of transgenic plants on honey bees. More publications supporting those same conclusions:

Behle and Popham (2012, Clairmont, Milne, et al. (1998, Couty, Down, et al. (2001, Kaiser, Pham-Delegue, et al. (2001, Laloi, Sandoz, et al. (1999, Malone and Pham-Delegue (2001, Mehlo, Gahakwa, et al. (2005, Parasharami, Naik, et al. (2006, Pham-Délègue (1992, Ramirez-Romero, Chaufaux, et al. (2005, Ramirez-Romero, Desneux, et al. (2008A, Vazquez-Padron, de la Riva, et al. (2004)

Only two of those publications should be mentioned here with more details:

Ramirez-Romero, R., Chaufaux, J., & Pham-Delegue, M. H. (2005). Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach. *Apidologie, 36*(4), pp. 601-611. <Go to ISI>://000235837400007 AND http://www.ask-force.org/web/Bees/Ramirez-Romero-Effects-2005.pdf

"In a comparative approach, we evaluated the effects of Cry1Ab protoxin, deltamethrin and imidacloprid insecticides on mortality, syrup consumption, foraging activity and olfactory learning capacities of free-flying honeybees. In an indoor flight cage we exposed bee colonies to different syrups containing Cry1Ab protoxin, deltamethrin or imidacloprid at 1000 µg/kg, 500 µg/kg and 48 µg/kg, respectively. Cry1Ab did not affect mortality, syrup consumption or learning capacities. However, foraging activity was reduced during and after the treatment. Deltamethrin and imidacloprid both affected syrup consumption and foraging activity. Deltamethrin also induced a reduction in learning capacities. ***With the tested concentrations, our study suggests that for honeybees, synthetic insecticides such as deltamethrin may induce a greater hazard than Cry1Ab protein, potentially expressed in Bt corn pollen at concentrations lower than 1000 µg/kg."*** *from Ramirez-Romero, Chaufaux, et al. (2005)*

Another feeding study Ramirez-Romero, Desneux, et al. (2008B) comes seemingly to negative results, but considering the artificially high concentration of 5000 ppb of Cry1Ab, which did disturb the learning performances or food consumption, these results do not fit to reality.
In addition, when you read about the details of the paper, you discover that in the feeding experiments **with realistic amounts of Bt-toxin (3ppb- parts per billion)**, the Honeybees do not show any reaction in behavior and feeding time. People just reading the abstract will not be aware of those facts crucial for real agriculture. In a way, the abstract is misleading, since it avoids communicating some crucial experimental data, see statements within the main text:

"The consumption of uncontaminated syrup per day was significantly lower in Honeybees subjected to sub-chronic exposure of 5000 ppb Cry1Ab compared to those from the control group (Z = -2.776; P = 0.006) (Fig. 2).
In contrast, Cry1Ab at 3 ppb did not significantly affect the consumption rate of uncontaminated syrup (Z = -1.686; P = 0.092), nor did imidacloprid when compared to the control group (Z = -1.120; P = 0.263)."Ramirez-Romero, Desneux, et al. (2008B)

Ramirez-Romero, R., Desneux, N., A., D., Chaffiol, A., & Pham-Delegue, M. H. (2008). Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? *Ecotoxicology and Environmental Safety, In Press, Corrected Proof*, pp. http://www.sciencedirect.com/science/article/pii/S0147651307003065 AND http://www.ask-force.org/web/Bt/Ramirez-Romero-Affect-Bees-2008.pdf

"Genetically modified Bt-crops are increasingly used worldwide but side effects and especially sublethal effects on beneficial insects remain poorly studied. Honeybees are beneficial insects for natural and cultivated ecosystems through pollination. The goal of the present study was to

assess potential effects of two concentrations of Cry1Ab protein (3 and 5000 ppb) on young adult Honeybees. Following a complementary bioassay, our experiments evaluated effects of the Cry1Ab on three major life traits of young adult Honeybees: (a) survival of Honeybees during sub-chronic exposure to Cry1Ab, (b) feeding behavior, and (c) learning performance at the time that Honeybees become foragers. The latter effect was tested using the proboscis extension reflex (PER) procedure. The same effects were also tested using a chemical pesticide, imidacloprid, as positive reference. The tested concentrations of Cry1Ab protein did not cause lethal effects on Honeybees. However, Honeybee feeding behavior was affected when exposed to the highest concentration of Cry1Ab protein, with Honeybees taking longer to imbibe the contaminated syrup. ***Moreover, Honeybees exposed to 5000 ppb of Cry1Ab had disturbed learning performances. Honeybees continued to respond to a conditioned odor even in the absence of a food reward. Our results show that transgenic crops expressing Cry1Ab protein at 5000 ppb may affect food consumption or learning processes and thereby may impact Honeybee foraging efficiency. The implications of these results are discussed in terms of risks of transgenic Bt-crops for Honeybees.*** *" Ramirez-Romero, Desneux, et al. (2008B).*

A different matter was the report of Hans Hinrich Kaatz which was long withheld and is still not published in a peer reviewed journal, despite good results in the second round of the Monitor project, clearly stating that there is no hint of negative impact of Bt maize (at this time the old, more toxic Bt 176 event), despite of false press statements: Kaatz (2004):

Kaatz, H. H. (2004), Effects of Bt maize pollen on the honeybee, (2001 - 2004) Jena University, Institute of Nutrition and Environment, No. pp. 2 and 48, http://www.ask-force.org/web/Bt/Kaatz-Effects-Ask-force.org/webMaize-Pollen-2004.pdf AND German http://www.ask-force.org/web/Bt/Kaatz-Bienen-Biosicherheit-2004.pdf AND German original report: http://www.ask-force.org/web/Bees/Kaatz-Abschlussbericht-Verbundprojekt-54169383-2004.pdf

Overall it was not possible to prove the existence of any chronic toxic effects of Bt176 and Mon810 Bt maize varieties on healthy honeybee colonies. In view of the extreme conditions under which the trial was carried out (six-week duration, high Bt toxin content), **the wide-ranging investigations carried out show that toxic effects on healthy bees under natural conditions can be excluded with a high degree of certainty**. This result is further supported by the fact that honeybees only collect small quantities of maize pollen, even in areas cultivated with large maize plots, when other plants are available as sources of pollen (less than three percent). Kaatz (2004)

More citations and graphs from the report:

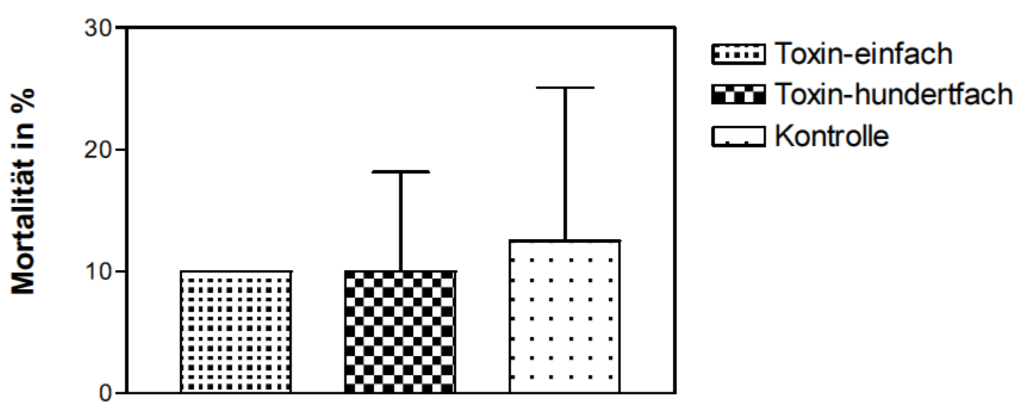

Abb.1 Wirkung von *Bacillus thuringiensis* Toxin CryIAb auf die Mortalität von Stockbienen
Durchschnittliche Mortalität ± SD in Prozent im Käfigversuch bei Fütterung mit dem Futterteig-Pollen-Gemisch 2:1 mit bzw. ohne Toxin; n = 4 Käfige mit je 10 Bienen; kein signifikanter Unterschied im U-Test, p>0,05.

Fig. 12 Impact of Bacillus thuringiensis toxin Cry1Ab on the mortality of honey bees: Average mortality +- SD in Percent in Cage experiment with feeding of a mixture of feed-paste 2:1 with and without toxin; n=4 cages, each with 10 bees, no significant difference in U-Test, p>0.05.

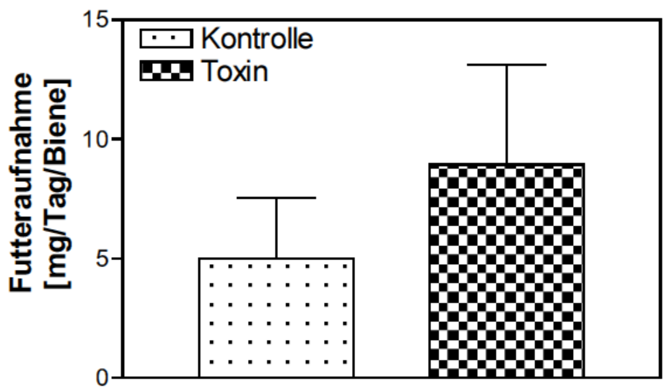

Abb.3 Wirkung von *Bacillus thuringiensis* Toxin CryIAb (100-fach)auf die Futteraufnahme von Jungbienen
Durchschnittlicher Futterverbrauch vom Futterteig-Pollen-Gemisch 2:1 mit bzw. ohne Toxin in mg ± SD/Tag/Biene im Käfigversuch; n = 12 Käfige mit je 10 Bienen; U-Test nicht signifikant, α=0,05

Fig. 13 Impact of Bacillus thuringiensis toxin Cry1Ab on the mortality of *young* honey bees: Average mortality +- SD/day/bee in Percent in Cage experiment with feeding of a mixture of feed-paste 2:1 with and without toxin (x100); n=12 cages, each with 10 bees, no significant difference in U-Test, p>0.05.

Conclusion: *The toxin altered through genetic engineering does not have any negative impact during the four days of exposure on juvenile and adult bees compared to the control. The acute amount of Cry1Ab in the Bt 176 maize plant can be seen for adult bees as harmless. Kaatz (2004) p. 6, translated by K.Ammann.*

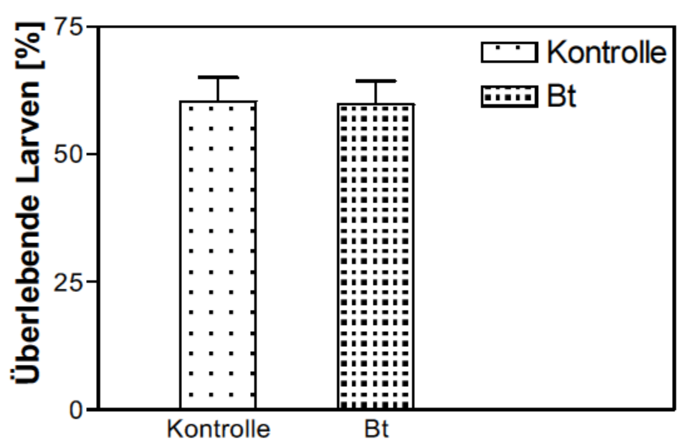

Abb. 9 Überlebende Larven nach Exposition zu Bt-Toxin *in vitro*
Je 9 Ansätze a 60 Larven wurden verglichen

Fig. 14 Surviving larvae after Bt toxin exposure in vitro, 9 assays with 60 larvae each one compared

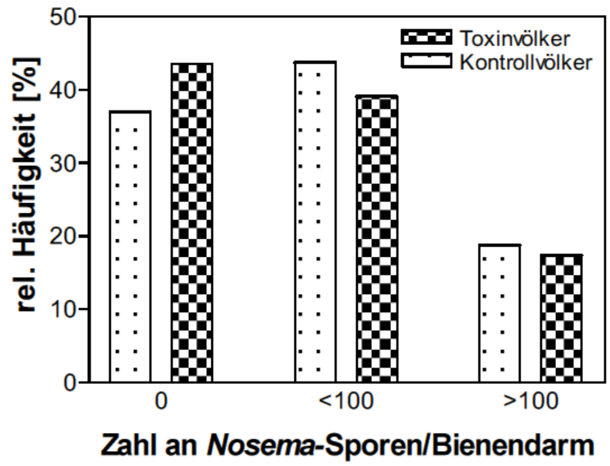

Abb. 11 Vorkommen von Sporen des Mikrosporidiums *Nosema apis* in Därmen von sterbenden und toten Bienen.
4 - 10Bienen/Volk aus mit Bt gefütterten Bienenvölkern und Kontrollbienenvölkern.

Fig. 15 Occurrence of Nosema apis microsporidium spores in the intestines of dying or dead bees: 4-10 bees per populations from Bt fed bee populations and control populations

Comment: The weakening of the bee populations is caused by the Nosema infection, not by the Bt toxin exposure, however Bt impact seems to be higher due to Nosema infection, as shown for the case of interaction between Nosema apis impact and the mortality of Ostrinia nubilalis by Pierce, Solter, et al. (2001). This effect would not have been manifest with short term cage experiments, because the time between infection and manifestation of symptoms is too long.Kaatz (2004) p. 18, translation by K.Ammann.

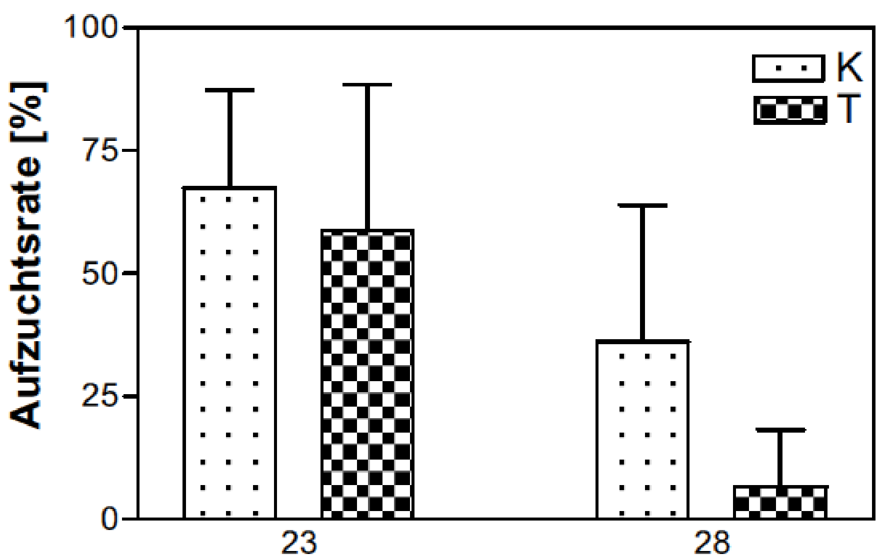

Abb.18 Aufzuchtsrate der Larven
Versuch I; n = 8 Völker, nicht signifikant unterschiedlich nach U-Test, $p<0,05$

Fig. 16 Breeding rate of larvae: Experiment I; n= 8 populations, not significantly different according to U-test, $p<0,05$ Kaatz (2004) p. 22.

"Conclusion: The survival rate of the capped brood with more than 80% in both groups is clearly higher than compared to the values in eggs and larvae. Also the differences between the groups are non-significant, this means that the populations attend the brood (as visible in Fig. 16) in the same way until capping." Kaatz (2004) p. 22, translated by K. Ammann

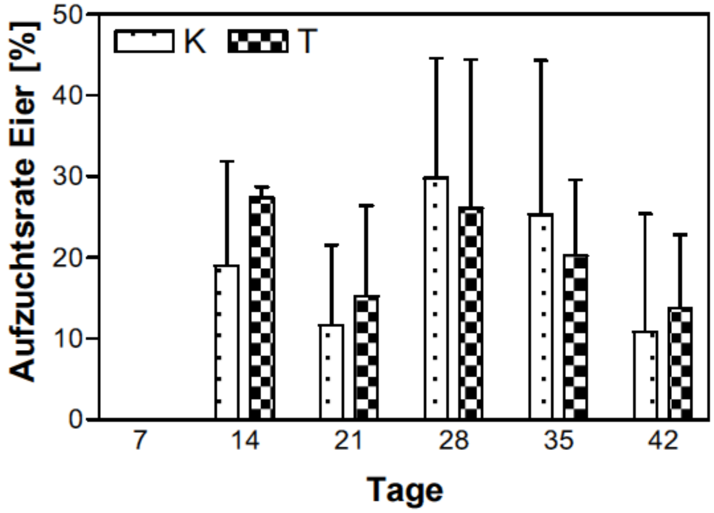

Abb.23 Aufzuchtsrate der Eier ± SD
Versuch II; n = 8 Völker; nicht signifikant verschieden nach U-Test; $p<0{,}05$

Fig. 17 Experiment II; n=8 populations; not significantly different after U-test; p<0,05

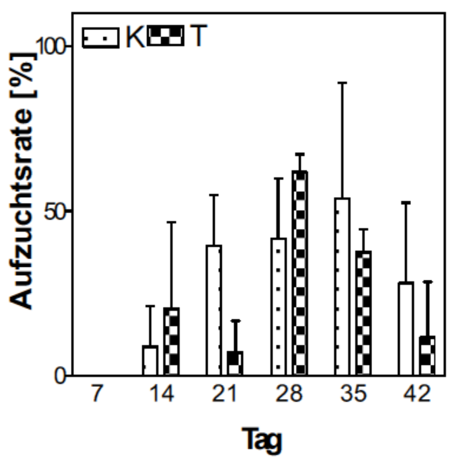

Abb.24 Aufzuchtsrate Larven L1-L3 ± SD

Fig. 18 Abb. 24: breeding rate of larvae L1-l3 +- SD

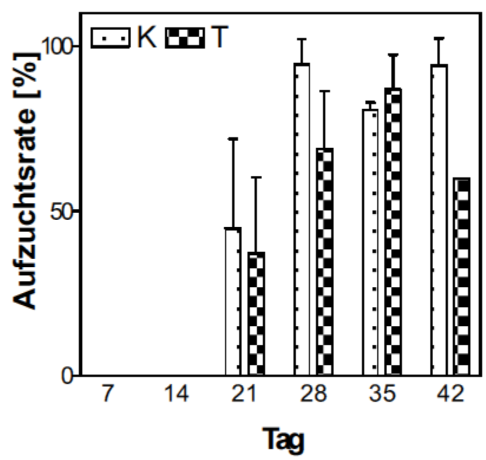

Abb.25 Aufzuchtsrate Larven L4-L5 ± SD

Fig. 19: breeding rate of Larvae L4-L5 +1 SD Kaatz (2004) p.26

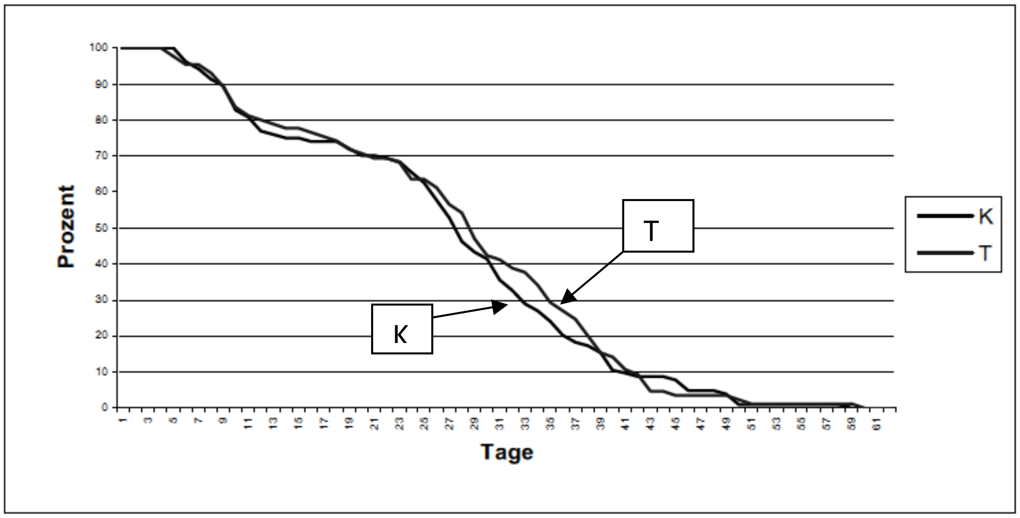

Abb.26 Lebensdauer der adulten Bienen im Vergleich
Versuch II; n_K = 104 Bienen, n_T = 85 Bienen

Fig. 20 Survival time of adult bees compared: K: control group n=104 bees, T: group n= 85 bees with Bt maize Kaatz (2004) p.28

General conclusions including the results of a total of 5 Experiments:

Zusammenfassung

Generell kann aufgrund der Ergebnisse der Halbfreiland und Freilandversuche eine chronisch toxische Wirkung von Bt-Mais der Sorten NovBt176 und Mon810 auf gesunde Honigbienenvölker nicht nachgewiesen werden. Berücksichtigt man dazu noch die gegenüber den herkömmlichen, gesetzlich vorgeschriebenen Pflanzenschutzmittelprüfungen wesentlich erhöhten Stichprobenzahlen und die extremen 6-wöchigen Expositionen mit den Bt-Toxinen, dann kann eine toxische Wirkung auf gesunde Bienen unter natürlichen Bedingungen nach den erfolgten umfangreichen Untersuchungen mit großer Sicherheit ausgeschlossen werden. Dieses zentrale Ergebnis wird noch dadurch untermauert, dass Honigbienen selbst in den ostdeutschen Agrarräumen mit großen Maisschlägen nur wenig Maispollen sammeln, wenn andere Pflanzen als Pollenquellen zur Verfügung stehen. In unseren Untersuchungen betrug der Anteil an Maispollen unter 5% des gesammelten Pollens. Selbst dann, wenn Bienenvölker ausschließlich Pollen der o.a. GV-Maispflanzen sammeln würden, wie es in den Zeltversuchen simuliert wurde, kann mit der durch die Stichprobenzahl bedingten Power ausgeschlossen werden, dass sich Bt-Maispollen der Sorten Mon810 und Novartis Bt176 negativ auf gesunde Bienenvölker auswirken.
Die eingangs aufgestellte Hypothese, dass der großflächige Anbau von Bt-Mais Auswirkungen auf wichtige phytophage Nichtziel-Organismen wie die Honigbiene habe, kann durch durch das vorliegende Datenmaterial nicht gestützt werden. Auch die Hypothese, dass die Wirkung der Bt-Endotoxine bei der Aufnahme mit dem Pollen stärker ist als die Wirkung von schon seit Jahren im Pflanzenschutz eingesetzten Bt-Präparate, trifft angesichts der fehlenden Wirkung von Bt-Toxinen auf gesunde Bienenvölker nicht zu.

Original text of the general conclusions of the full report p.42 Kaatz (2004)

In the wake of the first draft report, Dr. Hans Hinrich Kaatz leaked to some journalists from the Spiegel press his doubts about the safety of GM crops for bees derived from preliminary results of the first round of experiments (notably under strict embargo agreements). The Spiegel then clearly broke the embargo commitment and released preliminary, and in a boulevard style clearly misleading alarmist statements, and the author Prof. Kaatz was left helpless in the background, not able to really place scientifically correct rebuttals. Details see chapter 1.1.

Modern experimental feeding experiments and field data are clearly ruling out Bt crops as a possible cause of CCD.

The EFSA has made it clear in many statements its position of possible negative influences of Bt maize EFSA-Opinion (2005A, EFSA-Opinion (2005B, EFSA-Opinion (2005C, EFSA-Opinion (2005D, EFSA-Opinion (2005E, EFSA-Opinion (2005F, EFSA-Opinion (2005G, EFSA-Opinion (2005H, EFSA-Opinion (2005I, EFSA-Opinion (2006A, EFSA-Opinion (2006B, EFSA-Opinion (2008A, EFSA-Opinion (2008B, EFSA-Opinion (2008C, EFSA-Opinion (2008D, EFSA-Opinion (2009A, EFSA-Opinion (2009B) that Bt maize products have no detrimental impact on the environment, and they state also in the last opinion (as also in earlier ones) without any doubts that bees are not harmed by Bt maize:

In the last published summary EFSA-Opinion (2009A) it is concluded:

"While the EFSA GMO Panel agrees that in field settings honeybees might face additional stresses that could theoretically affect their susceptibility to Cry proteins or generate indirect effects, ***it concludes that the likelihood of adverse effects on honeybees is expected to be very low. The EFSA GMO Panel has no reason to consider that maize MON810 will cause reductions to pollinating insects that are significantly greater from those caused by conventional farming."***

There is an extensive literature published on bees related to Bt crops. One of the reliable meta-analysis summarized important papers and does not reveal any negative effects of Bt crops on bees has been published by Duan, Marvier, et al. (2008):

Duan, J. J., M. Marvier, J. Huesing, G. Dively and Z. Y. Huang (2008). "A Meta-Analysis of Effects of Bt Crops on Honeybees (Hymenoptera: Apidae)." PLoS ONE **3**(1): e1415. http://dx.doi.org/10.1371%2Fjournal.pone.0001415 AND http://www.ask-force.org/web/Bt/Duan-Meta-Analysis-Effects-Bees-2008.pdf

"Background: Honeybees (Apis mellifera L.) are the most important pollinators of many agricultural crops worldwide and are a key test species used in the tiered safety assessment of genetically engineered insect-resistant crops. There is concern that widespread planting of these transgenic crops could harm Honeybee populations. Methodology/Principal Findings: We conducted a meta-analysis of 25 studies that independently assessed potential effects of Bt Cry proteins on Honeybee survival (or mortality). Our results show that Bt Cry proteins used in genetically modified crops commercialized for control of lepidopteran and coleopteran pests do not negatively affect the survival of either Honeybee larvae or adults in laboratory settings. ***Conclusions/Significance: Although the additional stresses that Honeybees face in the field could, in principle, modify their susceptibility to Cry proteins or lead to indirect effects, our findings support safety assessments that have not detected any direct negative effects of Bt crops for this vital insect pollinator."*** *Duan, Marvier, et al. (2008)*

Another bee feeding study comes to the same conclusion: There were no significant differences in any of the parameters measured between larvae that were fed transgenic canola pollen and those fed non-transgenic corn pollen. Results from this study suggest that transgenic canola pollen does not have adverse effects on Honeybee development and that the use of transgenic canola does not pose any threat to Honeybees.

Huang, Z. Y., A. V. Hanley, et al. (2004). "Field and semi-field evaluation of impacts of transgenic canola pollen on survival and development of worker Honeybees." Journal of Economic Entomology **97**(5): 1517-1523.
<Go to ISI>://000224653200005 AND http://www.ask-force.org/web/Bees/Huang-Field-Bees-2004.pdf

"A 2-yr field trial (2001 and 2002) and 1-yr semifield trial (2002) were conducted to evaluate the effect of transgenic herbicide (glyphosate)-tolerant canola Brassica napus L. pollen oil larval and adult Honeybee, Apis mellifera L., workers. In the field trial, colonies of Honeybees were moved to transgenic or nontransgenic canola fields (each at least 40 hectares) during bloom and then sampled for larval survival and adult recovery, pupal weight, and hemolymph protein concentrations. No differences in larval survival, adult recovery, and pupal weight were detected between colonies placed in nontransgenic canola fields and those in transgenic canola fields. Colonies placed in the transgenic canola fields in the 2002 field experiment showed significantly higher hemolymph protein in newly emerged bees compared with those placed in nontransgenic canola field; however, this difference was not detected in the 2001 field experiment. In the semifield trial, bee larvae were artificially fed with bee-collected transgenic and nontransgenic canola pollen and returned to their original colonies. Larval survival, pupal survival, pupal weight, and hemolymph protein concentration of newly emerged adults were measured. ***There were no significant differences in any of the parameters measured between larvae that were fed transgenic canola pollen and those fed nontransgenic corn pollen. Results from this study suggest that transgenic canola pollen does not have adverse effects on Honeybee development and that the use of transgenic canola dose not pose any threat to Honeybees."*** *Huang, Hanley, et al. (2004)*

The same results of Bt toxins being tested negatively for bees come from the research group of Dai et al.

Dai, P. L., Zhou, W., Zhang, J., Cui, H. J., Wang, Q., Jiang, W. Y., Sun, J. H., Wu, Y. Y., & Zhou, T. (2012). Field assessment of Bt cry1Ah corn pollen on the survival, development and behavior of Apis mellifera ligustica. *Ecotoxicology and Environmental Safety, 79*, pp. 232-237. <Go to ISI>://WOS:000302107000032 AND http://www.ask-force.org/web/Bees/Dai-Field-assessment-Ask-force.org/webcry1Ah-corn-Apis-2012.pdf

Dai, P. L., Zhou, W., Zhang, J., Jiang, W. Y., Wang, Q., Cui, H. J., Sun, J. H., Wu, Y. Y., & Zhou, T. (2012). The effects of Bt Cry1Ah toxin on worker honeybees (Apis mellifera ligustica and Apis cerana cerana). *Apidologie, 43*(4), pp. 384-391. <Go to ISI>://WOS:000306214600002 AND http://www.ask-force.org/web/Bees/Dai-Effects-Ask-force.org/webCry1Ah-toxin-honeybees-2012.pdf

Dai, P.-L., Zhou, W., Zhang, J., Lang, Z.-H., Zhou, T., Wang, Q., Cui, H.-J., Jiang, W.-Y., & Wu, Y.-Y. (2015). Effects of Bt cry1Ah corn pollen on immature worker survival and development of Apis cerana cerana. *Journal of Apicultural Research, 54*(1), pp. 72-76. http://dx.doi.org/10.1080/00218839.2015.1035075 AND http://www.ask-force.org/web/Bees/Dai-Effects-Ask-force.org/webCry1Ah-corn-pollen-immature-worker-survival-2015.pdf

"The honey bee may be exposed to insecticidal proteins from transgenic plants via pollen. An assessment of the impact of such exposures on the honey bee is an essential part of the risk assessment process for transgenic Bacillus thuringiensis (Bt) corn. The effects of dietary transgenic Bt corn pollen on honey bee immature workers of Apis cerana cerana were examined by feeding trials in colonies. Four- to six-day-old honey bee worker larvae were fed various pollens (cry1Ah corn pollen, regular corn pollen, mixed bee pollen and control) and then sampled to record their survival and development. There were no significant differences in capping rate, emergence rate and immature stage among treatments. ***Our studies suggest that cry1Ah corn pollen carries no risk for the survival and development of A. c. cerana immature workers."*** *From Dai, Zhou, et al. (2015)*

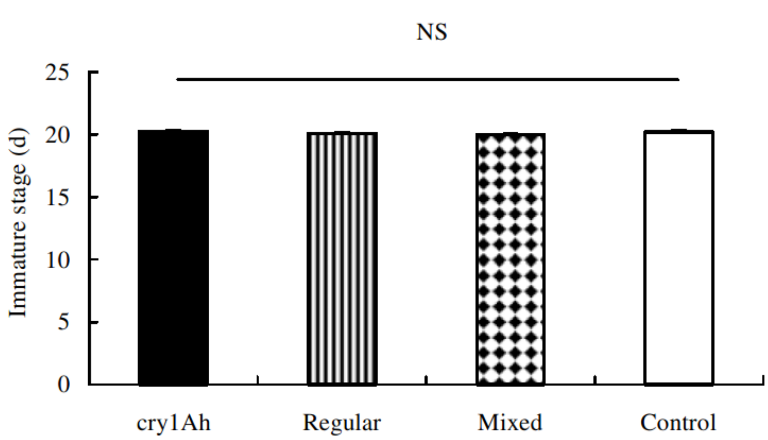

Fig. 21 Duration of immature stage (mean ± standard error) of the honey bee A. c. cerana larvae fed cry1Ah gene corn pollen, regular (non-transgenic corn pollen), mixed (bee collected pollen) and control (larvae not fed) (40 individual per treatment). P < .05 indicated a significant difference; NS: not significant. From Dai, Zhou, et al. (2015)

An interesting long term study needs to be discussed critically here: Inhibition of brain protein-synthesis by cycloheximide does not affect formation of long-term-memory in honeybees after olfactory conditioning: Wittstock, Kaatz, et al. (1993), the result: according to the results no negative effects.

Wittstock, S., Kaatz, H. H., & Menzel, R. (1993). Inhibition of Brain Protein-Synthesis by Cycloheximide Does Not Affect Formation of Long-Term-Memory in Honeybees after Olfactory Conditioning. *Journal of Neuroscience, 13*(4), pp. 1379-1386. <Go to ISI>://A1993KV71100006 AND http://www.ask-force.org/web/Bees/Wittstock-Inhibition-Brain-Protein-Synthesis-Cycloheximide-Not-Affect-1993.pdf

"The honeybee forms a long-term memory in different training situations that lasts for a lifetime, but the cellular mechanisms of long-term memory formation are not known. We analyzed the dependency of long-term memory on the de nova brain protein synthesis. The protein synthesis inhibitor cycloheximide was injected via the median ocellus directly into the brain. 3H-leucine incorporation into brain proteins was inhibited by >95% for >3 hr. The time of protein synthesis inhibition was prolonged by a second injection of the same dose. Worker honeybees were conditioned to an olfactory stimulus at different times before and after injection. The proboscis extension response (PER) of bees restrained in tubes was classically conditioned with sugar water applied first to the antennae followed by feeding (unconditioned stimulus) paired with odor presentation (conditioned stimulus). The bees were tested by presenting the odor alone at different times up to 24 hr after injection. ***No significant reduction in the probability of the conditioned response in cycloheximide-treated bees was found when compared to the Ringer-injected controls in 4 series of experiments. Since protein synthesis was inhibited between 7 hr pre- and 7 hr postconditioning without affecting the formation of long-term memory, a possible role of de nova protein synthesis in the formation of long-term memory after olfactory conditioning of the PER is not supported by these experiments****. From Wittstock, Kaatz, et al. (1993)*

4.1. Hidden GMOs not substantiated in relation to bee mortality

Recently, totally unsubstantiated scares have been produced by beekeepers, not giving the slightest hint about real data, that transgenic sunflowers could kill bees: Anonymous and Mieli d-Italia (20130806, Life (20130820, Rivolet Patrick, Belval Olivier, et al. (20130729, Wyle Claude (20130517). Most refer to a French report on herbicide tolerance: Beckert M. (2011), but there is not a single scientific reference there on bee mortality in connection with glyphosate or other herbicides, hence it is just cheap GMO opposition hearsay with false citations in the hope that readers trust the text and do not go to the original report.

5. Possible protein deficiency as a follow up of a Picornia–like viral infection

Johnson, Evans, et al. (2009) report that Microarray analysis revealed unusual ribosomal RNA fragments that were conspicuously more abundant in the guts of CCD bees. The presence of these fragments may be a possible consequence of picornia-like viral infection, including deformed wing virus and Israeli acute paralysis virus, and may be related to arrested translation. Ribosomal fragment abundance and presence of multiple viruses may prove to be useful diagnostic markers for colonies afflicted with CCD.

Johnson, R.M., Evans, J.D., Robinson, G.E., & Berenbaum, M.R. (2009a)
Changes in transcript abundance relating to colony collapse disorder in Honeybees (Apis mellifera). Proceedings of the National Academy of Sciences, pp. 14790–14795 http://www.pnas.org/content/early/2009/08/21/0906970106.abstract AND http://www.ask-force.org/web/Bees/Johnson-Changes-Transscript-CCD-2009.pdf

"Colony collapse disorder (CCD) is a mysterious disappearance of Honeybees that has beset beekeepers in the United States since late 2006. Pathogens and other environmental stresses, including pesticides, have been linked to CCD, but a causal relationship has not yet been demonstrated. Because the gut acts as a primary interface between the Honeybee and its environment as a site of entry for pathogens and toxins, we used whole-genome microarrays to compare gene expression between guts of bees from CCD colonies originating on both the east and west coasts of the United States and guts of bees from healthy colonies sampled before the emergence of CCD. Considerable variation in gene expression was associated with the geographical origin of bees, but a consensus list of 65 transcripts was identified as potential markers for CCD status. Overall, elevated expression of pesticide response genes was not observed. Genes involved in immune response showed no clear trend in expression pattern despite the increased prevalence of viruses and other pathogens in CCD colonies. Microarray analysis revealed unusual ribosomal RNA fragments that were conspicuously more abundant in the guts of CCD bees. The presence of these fragments may be a possible consequence of picorna-like viral infection, including deformed wing virus and Israeli acute paralysis virus, and may be related to arrested translation. Ribosomal fragment abundance and presence of multiple viruses may prove to be useful diagnostic markers for colonies afflicted with CCD." Johnson, Evans, et al. (2009)

In the conclusions of the article:

Although gene transcript analysis did not clearly identify a specific cause for CCD, our study documents several patterns suggestive of a causal mechanism. The reduced protein synthetic capabilities that would accompany ribosomal hijacking by multiple picorna-like viruses would leave bees unable to respond to additional stresses from pesticides, nutrition, or pathogens. Although any interpretation of the presence of these rRNA fragments is speculative, the reported interaction between bee picorna-like viruses and rRNA is suggestive of a possible root cause of CCD. ***To establish a causal relationship, the quantitative association between multiple picorna-like virus infections and polyadenyl-ated rRNA fragment abundance merits further exploration. In addition, the consequences of viral infection and CCD on the function of ribosomes should be explored through assays of translational efficiency. Because of the potential for translational interference, studies on immune suppression should focus on bioassays or protein abundance rather than on immune gene transcripts.*** *Johnson, Evans, et al. (2009)*

This hypothesis with some evidence might well be the solution of the CCD problem, although the same authors ask for more confirmation, and recently they published another hypothesis about a combined case of toxicity related to varroa fumigation:

Johnson, R.M., Pollock, H.S., & Berenbaum, M.R. (2009b)
Synergistic Interactions Between In-Hive Miticides in Apis mellifera. Journal of Economic Entomology, 102, 2, pp 474-479
<Go to ISI>://WOS:000264899500003 AND http://www.ask-force.org/web/Bees/Johnson-Synergistic-Actions-2009.pdf

"The varroa mite, Varroa destructor Anderson & Trueman, is a devastating pest of Honeybees, Apis mellifera L. that has been primarily controlled over the last 15 yr with two in-hive miticides; the organophosphate coumaphos (Checkmite(-)), and the pyrethroid tan-fluvalinate (Apistan). Both the hive where they are stable and have the potential to build up over repeated treatments such that bees could be exposed to both compounds simultaneously. Although these compounds were chosen as in-hive miticides due to their low toxicity to Honeybees, that low toxicity depends, at least in part, on rapid detoxification mediated by cytochrome P450 monooxygenase enzymes (P450s). In this laboratory study, we observed a large increase in the toxicity of tan-fluvalinate to 3-d-old bees that had been treated previously with coumaphos, and a

moderate increase in the toxicity of coumpahos in bees treated previously with tan-fluvalinate. The observed synergism may result from competition between miticides for access to detoxicative P450. ***These results suggest that Honeybee mortality may occur with the application of other wise sublethal doses of miticide when tan-fluvalinate and coumaphos are simultaneously present in the hive****" Johnson, Pollock, et al. (2009)*

More on the deformed wing virus from Lamp Lamp, Url, et al. (2016)

Lamp, B., Url, A., Seitz, K., Eichhorn, J., Riedel, C., Sinn, L. J., Indik, S., Köglberger, H. and Rümenapf, T. (2016) Construction and Rescue of a Molecular Clone of Deformed Wing Virus (DWV) PLOS ONE 11 11 e0164639 pp http://dx.doi.org/10.1371%2Fjournal.pone.0164639 AND http://www.ask-force.org/web/Bees/Lamp-Construction-Rescue-Molecular-Clone-Deformed-Wing-Virus.pdf

"European honey bees are highly important in crop pollination, increasing the value of global agricultural production by billions of dollars. Current knowledge about virulence and pathogenicity of Deformed wing virus (DWV), a major factor in honey bee colony mortality, is limited. With this study, we close the gap between field research and laboratory investigations by establishing a complete in vitro model for DWV pathogenesis. Infectious DWV was rescued from a molecular clone of a DWV-A genome that induces DWV symptoms such as crippled wings and discoloration. ***The expression of DWV proteins, production of infectious virus progeny, and DWV host cell tropism could be confirmed using newly generated antiDWV monoclonal antibodies. The recombinant RNA fulfills Koch's postulates circumventing the need of virus isolation and propagation of pure virus cultures. In conclusion, we describe the development and application of a reverse genetics system for the study of DWV pathogenesis****." From Lamp, Url, et al. (2016)*

6. Imidacloprid (and neonicotinoids) or other pesticides and fungicides as possible cause of CCD?

6.1. Papers of general interest, emphasizing the precautionary approach

First some basic literature on pesticides and Toxicology: The classic book with an US perspective of Wagner is still worth-while to read: Wagner Sheldon L. (1983).

Some basic views on the complex system of social insects comes from Straub, Williams, et al. (2015)

Straub, L., Williams, G. R., Pettis, J., Fries, I., & Neumann, P. (2015). Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. *Current Opinion in Insect Science, 12*, pp. 109-112. <Go to ISI>://WOS:000369017900016 AND http://www.ask-force.org/web/Bees/Straub-Superorganism-resilience-2015.pdf

"Insects provide crucial ecosystem services for human food security and maintenance of biodiversity. It is therefore not surprising that major declines in wild insects, combined with losses of managed bees, have raised great concern. Recent data suggest that honey bees appear to be less susceptible to stressors compared to other species. Here we argue that eusociality plays a key role for the susceptibility of insects to environmental stressors due to what we call superorganism resilience, which can be defined as the ability to tolerate the loss of somatic cells (=workers) as long as the germ line (=reproduction) is maintained. Life history and colony size appear critical for such resilience. Future conservation efforts should take superorganism resilience into account to safeguard ecosystem services by insects." From Straub, Williams, et al. (2015)

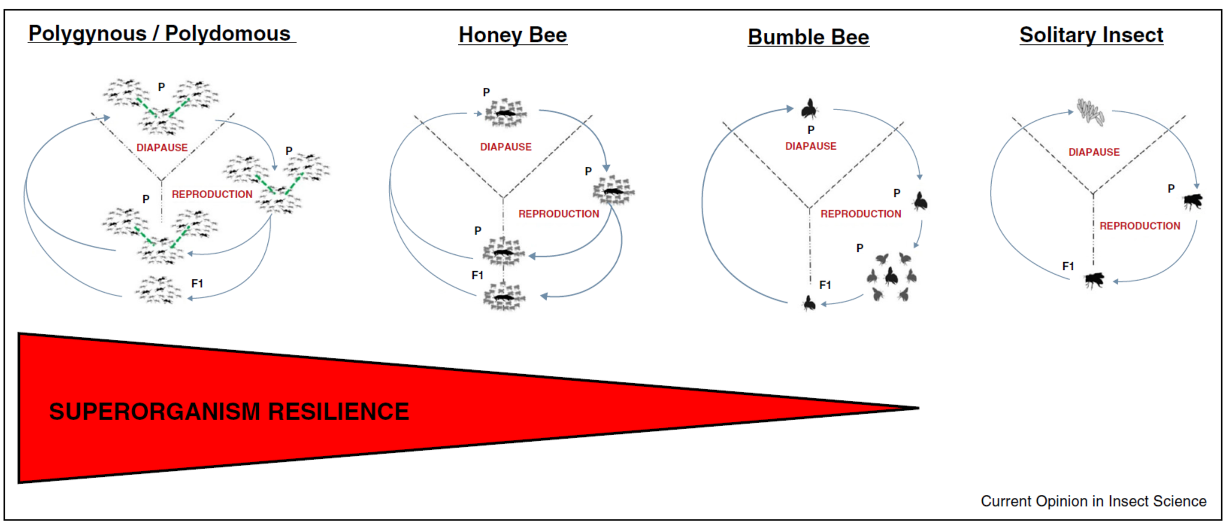

Fig. 22 Eusociality, life history and relative superorganism resilience in insects. The four major groups, their life history and relative superorganism resilience are shown. Polygynous and polydomous species with large perennial nests and dependent nest founding possess the largest superorganism resilience (some ants). Monogynous, monodomous species with large nests and dependent nest founding are less resilient (e.g. honey bees). Species with a solitary phase (e.g. during diapause) and independent nest founding as well as small colonies are even more vulnerable (e.g. bumblebees). Lastly, solitary insects have no superorganism resilience, because each female is reproductive. Black irregulars, sexual reproductive; grey irregulars, workers; blue lines indicate life history development, green lines indicate connection between nests, P, parental; F1, first filial). From Straub, Williams, et al. (2015)

The most recent account on the CCD:

A recent review comes to positive conclusions, but is asking for more research: Sheets, Li, et al. (2016)

"A comprehensive review of published and previously unpublished studies was performed to evaluate the neonicotinoid insecticides for evidence of developmental neurotoxicity (DNT). These insecticides have favorable safety profiles, due to their preferential affinity for nicotinic receptor (nAChR) subtypes in insects, poor penetration of the mammalian blood–brain barrier, and low application rates.

Sheets, L. P., Li, A. A., Minnema, D. J., Collier, R. H., Creek, M. R. and Peffer, R. C. (2016) A critical review of neonicotinoid insecticides for developmental neurotoxicity Critical Reviews in Toxicology 46 2 153-190 pp ISBN/1040-8444 <Go to ISI>://WOS:000368449400003 AND http://www.ask-force.org/web/Bees/Sheens-Critical-review-neonicotinoid-developmental-neurotoxicity-2016.pdf

"A comprehensive review of published and previously unpublished studies was performed to evaluate the neonicotinoid insecticides for evidence of developmental neurotoxicity (DNT). These insecticides have favorable safety profiles, due to their preferential affinity for nicotinic receptor (nAChR) subtypes in insects, poor penetration of the mammalian blood–brain barrier, and low application rates. *Nevertheless, examination of this issue is warranted, due to their insecticidal mode of action and potential exposure with agricultural and residential uses. This review identified in vitro, in vivo, and epidemiology studies in the literature and studies performed in rats in accordance with GLP standards and EPA guidelines with imidacloprid, acetamiprid, thiacloprid, clothianidin, thiamethoxam, and dinotefuran, which are all the neonicotinoids currently registered in major markets. For the guideline-based studies, treatment was administered via the diet or gavage to primiparous female rats at three dose levels, plus a vehicle control (20/dose level), from gestation day 0 or 6 to lactation day 21. F1 males and females were evaluated using measures of motor activity, acoustic startle response, cognition, brain morphometry, and neuropathology. The principal effects in F1 animals were associated with decreased body weight (delayed sexual maturation, decreased brain weight, and morphometric measurements) and acute toxicity (decreased activity during exposure) at high doses, without neuropathology or impaired cognition. No common effects were identified among the neonicotinoids that were consistent with DNT or the neurodevelopmental effects associated with nicotine. Findings at high doses were associated with evidence of systemic toxicity, which indicates that these insecticides do not selectively affect the developing nervous system." Sheets, Li, et al. (2016)*

And the positive conclusions:

"The collective information that is available shows no consistent pattern of effects among the six neonicotinoids or for any of these insecticides compared with nicotine. Dinotefuran produced no adverse effects in the guideline-compliant study at any dose level (EPA 2013b). The principal effects with the other neonicotinoids are associated with decreased body weight of F1 animals during development (e.g. delayed sexual maturation, decreased brain weight, and morphometric measurements) and acute toxicity (decreased motor activity associated with peak exposure via the diet and milk). Other findings among the individual studies, including differences in various brain measurements or performance in a neurobehavioral test, are inconsistent with a selective effect on the developing nervous system. ***These results may be useful for risk assessment purposes but the collective evidence indicates the neonicotinoid insecticides are not developmental neurotoxicants. This outcome is consistent with the relatively low activity of neonicotinoid insecticides toward nAChR subtypes that are expressed in mammals and other vertebrates and the absence of any other effect to suggest these compounds would be developmental neurotoxicants."*** *From Sheets, Li, et al. (2016)*

A recent account should also be mentioned here, the study had a major political impact in France, despite its self-declared critical points related to the negative conclusions:

In France, the use of Imidacloprid (a neonicotinoid) was blamed for the cause of CCD and was therefore promptly banned by the French Government *as early as 2003* Doucet-Personeni C., MP. Halm, et al. (20030918).

« Devant le volume de travail, et afin d'éviter la dispersion à laquelle peut conduire une analyse centrifuge de nombreux paramètres, le groupe de travail s'est principalement attaché à étudier ce qui au départ a conduit à la décision ministérielle, c'est à dire l'éventuel rôle du Gaucho et de l'imidaclopride dans les troubles observés antérieurement. Ce rapport fait un bilan de l'état actuel des connaissances sur les risques liés à l'utilisation de l'imidaclopride comme traitement de semences sur tournesol et maïs pour les abeilles. Il présente les conclusions du sous-groupe métrologie qui ont été validées par l'ensemble des membres du CST. La rédaction suit le plan classique d'une évaluation de risques pour l'environnement, en distinguant l'analyse de l'exposition de celle des effets.

Enfin, devant les problèmes rencontrés lors de la validation des différentes données, un chapitre « recommandations » à été intégré a ce rapport en vue d'une amélioration de la pertinence des études futures.
Il va de soi que cette approche centrée sur le phénomène de départ sera élargie à d'autres facteurs, c'est à dire à d'autres produits phytosanitaires, à la combinaison des effets de ceux-ci avec des pathologies, aux pratiques apicoles particulières, aux mauvais usages agricoles, etc. » From Doucet-Personeni C., MP. Halm, et al. (20030918)

And with more details the recommendations for an improvement of the study:

8 RECOMMANDATIONS CONCERNANT LES DONNEES DʻEXPOSITION DES ABEILLES
8.1 Données à acquérir concernant l'imidaclopride et ses métabolites
Les experts du CST ont conclu à la validation de la méthode analytique de dosages d'imidaclopride pour les sols, végétaux et pollen. En ce qui concerne les dosages d'imidaclopride dans le nectar et le miel, les limites de quantification et de détection sont actuellement trop élevées (>1 ppb et 0,3 ppb, respectivement). Une amélioration de la technique de dosage dans ces produits permettant une diminution de ces limites est donc demandée.
Devant la difficulté de prélever des quantités suffisantes de nectar à partir des fleurs, les experts du CST proposent de prélever le nectar dans le jabot des butineuses sur le champ ou à l'entrée de la ruche.
Parallèlement d'autres prélèvements et dosages doivent être envisagés afin de conclure quant à :

- *la présence de métabolites de l'imidaclopride dans les parties végétales visitées par les abeilles (pollen, nectar, miel)*
- *la possibilité d'accumulation d'imidaclopride et de ses métabolites dans le sol après plusieurs traitements successifs. A ce titre les experts du CST préconisent des études conduites en milieu*
- *non ouvert.*
- *la possibilité d'absorption d'imidaclopride résiduel par des plantes non traitées Gaucho mais cultivées sur sol ayant reçu un traitement Gaucho les années précédentes.*
- *la présence d'imidaclopride dans les différents produits de la ruche, à savoir : la bouillie nutritive des larves d'ouvrières, la gelée royale, le miel, le pollen stocké en rayon (pain d'abeille), la cire…*
- *la stabilité chimique de l'imidaclopride au cours du stockage et de la transformation du pollen et du nectar dans la ruche.*

8.2 Recommandations générales

- *Les échantillons doivent être prélevés selon les Bonnes Pratiques de Laboratoire. Un historique détaillé doit accompagner chaque échantillon afin d'assurer une bonne traçabilité. La nature et l'historique des échantillons lors de l'analyse des données doivent être en accord avec les fiches de prélèvement.*
- *Le nombre d'échantillons doit être suffisant pour chaque condition. Un minimum de 20 échantillons nous semble nécessaire.*
- *Les échantillons doivent être ensuite rapidement congelés et stockés sans interruption de la chaîne du froid au minimum à -20°C afin d'éviter toute dégradation de la substance active. La possible dégradation de la substance active lors du stockage des échantillons doit être préalablement étudiée en fonction du délai et des conditions de stockage.*
- *Enfin les résultats complets (données brutes et analysées) doivent être présentés clairement.*

9 RECOMMANDATIONS CONCERNANT LES DONNEES DE TOXICITE PAR ADMINISTRATION REITEREE DE SUBSTANCE ACTIVE
9.1 Données à acquérir concernant l'imidaclopride et ses métabolites
Il est indispensable d'acquérir des données sur la toxicité de l'imidaclopride et de ces métabolites pour les larves. Les études devront être conduites en laboratoire et en conditions de semi-champ grâce à des méthodes adéquates qui seront à mettre au point et à valider.
De la même façon il est nécessaire d'évaluer la sensibilité des nourrices au produit en conditions de laboratoire et de semi champ.
9.2 Recommandations générales relatives aux études des effets létaux ou sublétaux.
Devant la relative hétérogénéité des résultats et le nombre d'études non validées il sera nécessaire de développer des protocoles standardisés pour les études de toxicité par traitement réitéré. Ces protocoles devront être établis par des experts en Apidologie.
Parmi les points importants que devront respecter ces protocoles, nous suggérons :

  - *Le respect des critères de validation cités dans le rapport.*
  - *Lors des études d'intoxication suite à des traitements réitérés administrés par voie orale, la quantité de substance active (imidaclopride ou dérivé) testée doit être mesurée en quantité absorbée (ng / abeille)..*
  - *Les concentrations d'imidaclopride et de ses métabolites doivent être vérifiées en fin d'expérience à cause de sa dégradation à la lumière au cours du temps*
  - *Les nourrisseurs contenant de l'imidaclopride doivent être bien protégés de la lumière (mise au point d'un nourrisseur type)*
  - *Chaque étude doit comporter un traitement témoin, un traitement avec un produit chimique de haute toxicité (dimethoate) comme référence (0,11µg/abeille<DL50<0,26 µg/abeille et 0,11µg/abeille<DL50>0,33 µg/abeille pour une intoxication topique et une intoxication orale*

- *respectivement), un traitement avec chaque dose de substance active à tester (3 concentrations différentes minimum). Le traitement de référence doit permettre de contrôler qu'une absence éventuelle de toxicité n'est pas due à un caractère particulier des abeilles utilisées dans le test.*

- *Lors d'une dissolution du pesticide dans un solvant (ou d'une anesthésie des abeilles), 2 groupes témoins doivent être utilisés : un groupe témoin « non –traité », nourri avec un sirop constitué uniquement de saccharose (ou non anesthésié), un groupe témoin « solvant », nourri*
- *avec du sirop contenant le solvant utilisé à la même concentration que dans les groupes traités (ou anesthésié)*
  - *La répartition aléatoire des animaux afin de limiter la variabilité des études.*
  - *L'utilisation d'une mortalité corrigée dans le traitement des résultats ainsi que la prise en compte des abeilles mortes lors de l'évaluation de la quantité de sirop ingéré. Les résultats bruts devront apparaître dans le rapport final.*
  - *L'utilisation de tests statistiques permettant de montrer des effets significatifs ou non entre les groupes témoins et les animaux traités devra être systématique. Ces tests devront être appropriés aux conditions expérimentales et prendre en compte la répétition des intoxications (suggestions : ANOVA à mesures répétées).*
- *9.3 Recommandations générales relatives aux études en enceinte et en champ*
- *En plus des précédentes recommandations, les critères suivants devront être respectés :*
  - *Les études sur colonies (développement, production, consommation, etc.) doivent comprendre un effectif minimum de quelques milliers d'abeilles (10 % de l'effectif d'une colonie normale, dans les conditions naturelles)*

Les observations comportementales doivent:
être ciblées (grille de comportement à préciser),
être étalées dans le temps (plusieurs jours ou plusieurs semaines),
être régulières (fréquence et date),
comprendre la période post-test (effets retardés)
utiliser des abeilles marquées,
According to the authors own comments, the study lacks a proper comparison of possible other effects on the studied organisms. The French government decision is therefore based on dubious grounds and again, as in many other cases, based on politics, not on science.

- *Les études qui testent l'effet de pollens, nectars ou miels contaminés devraient, au préalable,*

vérifier la teneur en imidaclopride dans ces matrices (limite de quantification inférieure ou égale à 1 ppb) et la biodégradation du contaminant au cours du temps.

- *L'historique des cultures traitées doit être connu (traitements, cultures précédentes etc…)*
- *Lors de tests en plein champs avec intoxication « sur fleurs », l'aire des cultures doit être suffisamment importante pour minimiser la probabilité que les abeilles exploitent d'autres cultures. De même, lorsque la substance active est disposée sur nourrisseur, la distance entre la*

ruche et le nourrisseur doit être suffisamment grande.

- *Une analyse pollinique doit être exigée sur un échantillon de butineuses (pollen dans le contenu stomacal et dans les pelotes) pour déterminer l'exposition des abeilles aux plantes contaminées*

10. TRAVAUX A REALISER POUR COMPLETER L'EDUDE MULTIFACTORIELLE
Le rapport devra être progressivement enrichi des travaux futurs des membres du sous-groupe métrologie du CST. Il s'agira de :

- *Réaliser pour le fipronil une évaluation des risques du même type que celle effectuée pour l'imidaclopride,.*
- *Analyser les autres facteurs impliqués dans les pertes d'abeilles (maladies, pratiques apicoles et agricoles, variétés génétiques pour les plantes cultivées et traitées, influence des terpènes, …) en étroite collaboration avec le sous-groupe réseau.*
- *Faire l'inventaire des troubles des abeilles constatés dans les autres pays.*

L'évaluation des risques pour les abeilles engendrés par l'enrobage Gaucho® de semences a été menée en prenant en compte l'ensemble des données d'exposition, de toxicité et en élaborant des scénarios reflétant au mieux, l'intoxication des abeilles dans leurs environnement naturel. La démarche utilisée pour l'évaluation de risques, les différents scénarios mis au point ainsi que les recommandations établies suite aux problèmes rencontrés lors de l'évaluation des données devront être utilisés en phase d'homologation lors des études de risques d'autres produits insecticides à propriétés systémiques utilisés en traitement de sol ou de semences. L'extension aux autres types d'insecticides est également envisageable.

But a clearly more reliable report comes from the EASAC: The European Academies published in April 2015 EASAC (201504):

EASAC. (201504). *Ecosystem services, agriculture and neonicotinoids* Halle Germany: European Academies. ISBN 978-3-8047-3437-1 http://www.easac.eu/home/reports-and-statements/detail-view/article/ecosystem-se.html AND http://www.ask-force.org/web/Bees/EASAC-Policy-Report-26-Neonicotinoid-201504.pdf

"A focus on honey bees has distorted the debate around neonicotinoids. But there is more and more evidence that widespread use of neonicotinoids has severe effects on a range of organisms that provide ecosystem services like pollination and natural pest control, as well as on biodiversity.
Public and political attention has focused on whether honey bee colonies are being affected by neonicotinoids. But studying honey bee colony numbers does not show what is happening to the many other species providing the ecosystem services of pollination, natural pest control, soil productivity or the underpinning of biodiversity. Honey bees are just one pollinator - others include bumble bees, solitary bees, hoverflies, butterflies and moths. Other pollinators have generally declined across Europe as honey bee colony numbers have fluctuated.
In addition, honey bee colony structure provides a buffer against losses of foragers and workers, which is overlooked by many studies looking at the impact of neonicotinoid use on honey bee colony survival. In contrast, bumble bees have much less buffering capacity - and solitary bees none at all. Protecting honey bees is not enough to ensure sustainable agriculture.

Some intensive agriculture has become reliant on neonicotinoids, with proponents arguing that their withdrawal would have serious economic and food security implications. However, EASAC notes that some recent research has questioned the benefits of routine use as seed dressing against occasional or secondary pests. In some cases, neonicotinoid use has even made pest problems worse by eliminating insects which provided natural pest control.
As the EASAC report acknowledges, all pesticides involve a balancing act between the desired effect on food production and the inevitable risk of collateral damage to non-target species and the environment. In the case of the neonicotinoids, the increase in scientific knowledge over the last two years suggests that the current balance requires reassessment. From EASAC (201504).

In the European Community, the neonicotinoids (Similar products: thiamethoxam and imidacloprid, from Takeda and Bayer, other trade names include Gaucho, Admire, Merit, Advantage, Confidor, Provado, and Winne) were banned due to a peer reviewed conclusion of EFSA related to Clothianidin, emphasizing the precautionary approach: EFSA Conclusion (20130314) (which replaces the conclusion from January 2013):

EFSA Conclusion (20130314), Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin, EFSA Journal, 11, 1, pp. http://www.efsa.europa.eu/en/efsajournal/doc/3066.pdf AND http://www.ask-force.org/web/Bees/EFSA-Conclusion-Clothianidin-Bees-20130314.pdf

"Abstract:
The European Food Safety Authority (EFSA) was asked by the European Commission to perform a risk assessment of neonicotinoids, including clothianidin, as regards the risk to bees. In this context the conclusions of EFSA concerning the risk assessment for bees for the active substance clothianidin are reported. The context of the evaluation was that required by the European Commission in accordance with Article 21 of Regulation (EC) No 1107/2009 to review the approval of active substances in light of new scientific and technical knowledge and monitoring data. The conclusions were reached on the basis of the evaluation of the uses of clothianidin applied as a seed treatment or granules on a variety of crops currently authorized in Europe. ***The reliable endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the submitted studies and literature data as well as the available EU evaluations and monitoring data, are presented. Missing information identified as being required to allow for a complete risk assessment is listed. Concerns are identified.***

Summary:
Clothianidin was included in Annex I to Directive 91/414/EEC on 1 August 2006 by Commission Directive 2006/41/EC, and has been deemed to be approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/2011, as amended by Commission Implementing Regulation (EU) No 541/2011.
The specific provisions of the approval were amended by Commission Directive 2010/21/EU, to permit use as a seed treatment only where the seed coating is performed in professional seed treatment facilities, which must apply the best available techniques to ensure that the release of dust during application to the seed, storage and transport can be minimized, and where adequate drilling equipment is used to ensure a high degree of incorporation in soil, minimization of spillage and minimization of dust emission.
In accordance with Article 21 of Regulation (EC) No 1107/2009 to review the approval of active substances in light of new scientific and technical knowledge and monitoring data, in April 2012 the European Commission requested the EFSA to provide conclusions as regards the risk of neonicotinoid active substances for bees, in particular with regard to the acute and chronic effects on colony survival and development, taking into account effects on bee larvae and bee behavior, and the effects of sub-lethal doses on bee survival and behavior. Following discussions at the Standing Committee on the Food Chain and Animal Health (SCFCAH) in June / July 2012, and taking into account the outcome of the EFSA statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorized in Europe (EFSA Journal 2012;10(6):2752), the EFSA received an updated request from the European Commission to prioritize the review of 3 neonicotinoid substances, including clothianidin, and to perform an evaluation of the currently authorized uses of these substances as seed treatments and granules. The conclusions laid down in this report were reached on the basis of the evaluation of the studies submitted for the approval of the active substance at EU level and for the authorization of plant protection products containing clothianidin at Member State level, for the uses as seed treatments or granules applied on a variety of crops in Europe. In addition, the EFSA Scientific Opinion on the science behind the development of a risk assessment of plant protection products on bees (EFSA Journal 2012;10 (5):2668), some relevant literature data, as well as monitoring data available at national level were also considered in the current evaluation.
Several data gaps were identified with regard to the risk to Honeybees from exposure via dust, from consumption of contaminated nectar and pollen, and from exposure via guttation fluid for the authorized uses as seed treatment and granules. Furthermore, the risk assessment for pollinators other than Honeybees, the risk assessment following exposure to insect honey dew and the risk assessment from exposure to succeeding crops could not be finalized on the basis of the available information. A high-risk was indicated or could not be excluded in relation to certain aspects of the risk assessment for Honeybees for some of the authorized uses. For some exposure routes it was possible to identify a low-risk for some of the authorized use." EFSA Conclusion (20130314)

According to the EFSA, *the doubts prevail*, whether Clothianidin can still be used, as a *precautionary measure*, the substance has been banned.

Two wikis offer, as usual, a mix of lots of contradicting, peer-reviewed and non-peer-reviewed information: http://www.en.wikipedia.org/wiki/Imidacloprid_effects_on_bee_population and http://en.wikipedia.org/wiki/Clothianidin

It's of course difficult to be sure if this particular insecticide group is the cause of the problem, it was banned in France 2003, finally also by the EU (EFSA) in 2013, but many different *other* pesticides could cause this problem (or at least be part of it). The FAO website gives a list of relative toxicity of pesticides.
http://www.fao.org/docrep/X0083E/X0083E09.htm

6.2. Denial of negative effects of neonicotinoids:

Two papers of science journalist Jon Entine from 2013 and 2014 Entine Jon (20130430, Entine Jon (20141125) claim, that negative effects of neonicotinoids cannot be taken as proven:

Entine Jon (20130430), The Politics of Bees Turns Science on its Head -- Europe Bans Neonics While Local Beekeepers, Scientists Say Action is Precipitous, in: FORBES, pp. 9, FORBES, New York, http://www.forbes.com/sites/jonentine/2013/04/30/the-politics-of-bees-turns-science-on-its-head-europe-bans-neonics-while-local-beekeepers-scientists-say-action-is-precipitous/ AND http://www.ask-force.org/web/Bees/Entine-Politics-Bees-Turns-Science-20130430.pdf

"Although a vote by the 27 member states of the European Union to suspend use of the insecticides failed to reach a qualified majority—voting in the EU is weighted, and Britain, Italy and many other nations remain steadfastly opposed—EU rules now give final discretion to the commissioners. They have announced that the ban will likely become effective at the end of the year even though the scientific questions as to what has caused the bee deaths remain largely unanswered.
Farmers in Europe and elsewhere are almost universally opposed to even a temporary ban absent definitive real world research, calling it reckless. As they note, because bans exist on more toxic organophosphates—the chemicals that neonics replaced because of their more benign safety profile—there are no real alternatives." From Entine Jon (20130430, Entine Jon (20141125)

A confirmation of the results above that neonicotinoids do not harm bee colonies comes from Craptree and Wager: Crabtree Bill and Wager Robert (20160810), the text and in particular also the 197 comments are worth reading:

Crabtree Bill and Wager Robert (20160810) Are bees in peril from neonicotinoids? Farmer evidence challenges doomsayers and 197 comments glp Washington DC 4 text and 37 commentss pp https://www.geneticliteracyproject.org/2016/08/10/bees-peril-neonicotinoids-farmer-evidence-challenges-doomsayers/ AND http://www.ask-force.org/web/Bees/Crabtree-Are-bees-in-peril-neonicotinoids-20160810.pdf

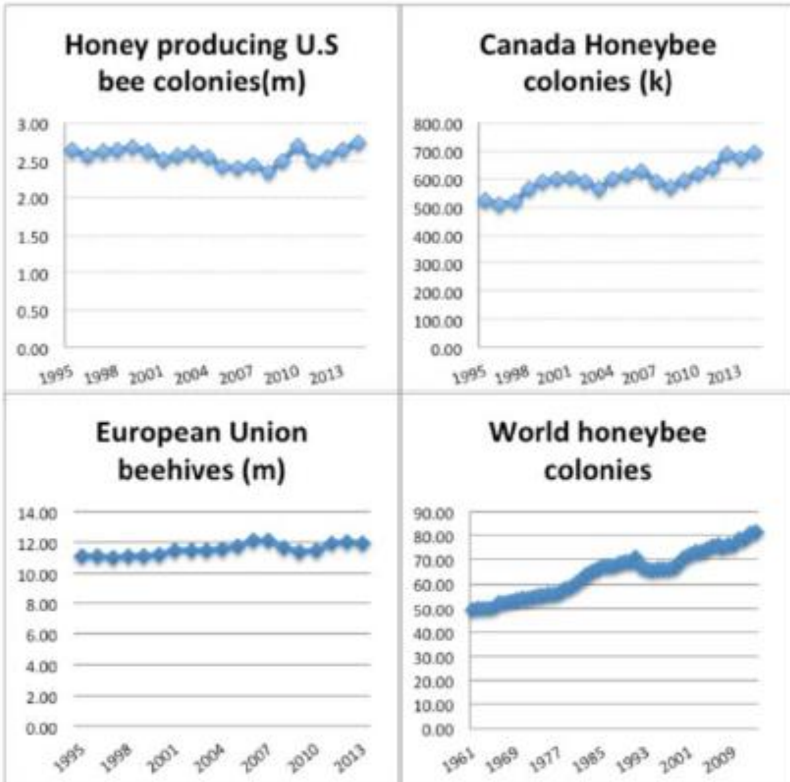

Fig. 23 The "Bee-pocalypse" has been cancelled. Global bee populations are rising and are now near historic highs. In Canada, the bee populations are up 13 percent since 2011, from 637,920 colonies to 721,106 in 2015. While there are serious threats to bees, we assert that the use of neonicotinoid pesticides (or neonics) in agriculture is not one of them. Continued focus on neonics by environmental groups detracts from and potentially worsens real threats to bees. From the text in Crabtree Bill and Wager Robert (20160810)

Finally, it should be mentioned that Syngenta recently challenged the negative impact of their pesticides on bee colonies: Anonymous and PhysOrg (20130827, Upton John (20130827), the outcome is open.

Anonymous and PhysOrg (20130827), Syngenta challenges EU's bee-saving pesticide ban, publ: PhysOrg, http://phys.org/print296805291.html AND http://www.ask-force.org/web/Bees/PhysOrg-Syngenta-challenges-EU-ban-20130827.pdf

"Swiss agrichemical giant Syngenta said on Tuesday it was taking the European Commission to court over its suspension of the use of an insecticide it blames for killing bees.
"We would prefer not to take legal action but have no other choice given our firm belief that the Commission wrongly linked thiamethoxam to the decline in bee health," Syngenta chief operating officer John Atkin said in a statement: The company is bringing the case before the European Court of Justice in Luxembourg, a spokeswoman told AFP." From Anonymous and PhysOrg (20130827)

And

Upton John (20130827), Syngenta to take a continent to court to upend pesticide ban, Grist, a beacon in the smog, pp. 9, http://grist.org/news/syngenta-to-take-a-continent-to-court-to-upend-pesticide-ban/ AND http://www.ask-force.org/web/Bees/Upton-Syngenta-Takes-Continent-Court-20130827.pdf

The European Farmers and European Agri-Cooperatives (Copa-Cogeca), the European Seed Association (ESA) and the European Crop Protection Association (ECPA) support a study by HFFA (Humboldt Forum for Food and Agriculture), which warns of premature regulatory decisions against Imidacloprid seed treatment with negative followup: Noleppa Steffen and Hahn Thomas (2013) with lots of details and a thorough regional analysis:

Noleppa Steffen and Hahn Thomas (2013), The Value of Neonicotinoid seed treatment in the European Union. A socioeconomic and environmental review., HFFA Working Paper, 01, pp. 96, http://www.hffa.info/files/wp_1_13_1.pdf AND http://www.ask-force.org/web/Bees/Noleppa-Value-Neonicotinoid-Seed-Treatment-2013.pdf

"**Neonicotinoid seed treatment is a key and currently often irreplacable technology available to farmers today that helps to secure the competitiveness of European Agriculture - with all the discussed socio-economic and global environmental benefits - as well as achieve a level of productivity that supports the stability of agricultural markets, while also supporting the food security for a growing world population.** The authors strongly recommend that these factors are considered in any regulatory decision making process that addresses this technology." Noleppa Steffen and Hahn Thomas (2013)

In a recent paper, Feltham, Park, et al. (2014) come to negative conclusions related to imidacloprid impact on bumblebees under field realistic dosages.

Feltham, H., K. Park and D. Goulson (2014), Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency, Ecotoxicology, pp. 1-7, http://dx.doi.org/10.1007/s10646-014-1189-7 AND http://www.ask-force.org/web/Pesticides/Feltham-Field-realistic-doses-Imidacloprid-2014.pdf AND http://www.ask-force.org/web/Pesticides/Feltham-Field-Release-Imidacloprid-Press-2014.pdf

*"Bumblebees and other pollinators provide a vital ecosystem service for the agricultural sector. Recent studies however have suggested that exposure to systemic neonicotinoid insecticides in flowering crops has sub-lethal effects on the bumblebee workforce, and hence in reducing queen production. The mechanism behind reduced nest performance, however, remains unclear. Here we use Radio Frequency Identification (RFID) technology to test whether exposure to a low, field realistic dose (0.7 ppb in sugar water and 6 ppb in pollen) of the neonicotinoid imidacloprid, reduces worker foraging efficiency. Whilst the nectar foraging efficiency of bees treated with imidacloprid was not significantly different than that of control bees, treated bees brought back pollen less often than control bees (40 % of trips vs 63 % trips, respectively) and, where pollen was collected, treated bees brought back 31 % less pollen per hour than controls. **This study demonstrates that field-realistic doses of these pesticides substantially impacts on foraging ability of bumblebee workers when collecting pollen, and we suggest that this provides a causal mechanism behind reduced queen production in imidacloprid exposed colonies.**" Feltham, Park, et al. (2014)*

A journalistic comment in the Science 2.0 demonstrates the controversy, if you take also into account the comments to the editor: Science20 (20151023): Bees Addicted To Neonics: A Failure of Science Journalism:

Science20. (20151023). Bees Addicted To Neonics: A Failure of Science Journalism. Science20 Science Blogging, pp. http://www.science20.com/news_articles/bees_addicted_to_neonics_a_failure_of_science_journalism-158154 AND http://www.ask-force.org/web/Bees/Science-2-0-Bees-Addicted-to-Neonics-Failure-Science-Journalism-20151023.pdf

At the EU country-level we've witnessed severe crop damage:
 Germany has experienced a six per cent decline of OSR growing area. Ninety per cent of OSR damaged and 30 per cent of the 1.309 million hectares was seen to suffer from severe flea beetle attack. A lot more insecticide sprays now. There's also confirmed increase in resistance issues towards pyrethroid insecticides.
 In the UK, 38,000 ha were not planted due to lack of crop protection products. In total, yield of OSR in the UK decreased by 60,000 ha or 10 per cent primarily due to flea beetle. Foliar insecticide spraying increased four-fold, reaching 100 per cent treated area in parts of the east and southeast.
 And in Sweden, the area of spring oilseed rape significantly decreased: 54,000 ha (2013) > 14,700 ha (2014) -> 6,000 ha (2015) = 90 per cent reduction. Increase in number of sprays from 2 per ha to 5.5 sprays per ha.

Beyond the EU, in May 2015, U.S. President Barack Obama's administration published its National Pollinator Strategy, which clearly argues that there are multiple factors impacting bee health, rather than just one. Thus far, the U.S. government along with virtually all other major governments around the world have continued to support the use of neonicotinoid pesticide seed treatment technology, which remains one of the most innovative and environmentally friendly forms of crop protection.

An excellent Nature article brings good evidence of the bees having this addiction to neonicotinoids: Kessler, Tiedeken, et al. (2015)

Kessler, S. C., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C., & Wright, G. A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature, 521*(7550), pp. 74-U145. <Go to ISI>://WOS:000354040900035 AND http://www.ask-force.org/web/Bees/Kessler-Bees-prefer-foods-containing-neonicotinoid-pesticides-2015.pdf

"The impact of neonicotinoid insecticides on insect pollinators is highly controversial. Sublethal concentrations alter the behaviour of social bees and reduce survival of entire colonies1–3. However, critics argue that the reported negative effects only arise from neonicotinoid concentrations that are greater than those found in the nectar and pollen of pesticide-treated plants DEFRA (20130327). Furthermore, it has been suggested that bees could choose to forage on other available flowers and hence avoid or dilute exposure DEFRA (20130327), Godfray, Blacquière, et al. (2014) . Here, using a two-choice feeding assay, we show that the honeybee, Apis mellifera, and the buff-tailed bumblebee, Bombus terrestris, do not avoid nectar-relevant concentrations of three of the most commonly used neonicotinoids, imidacloprid (IMD), thiamethoxam (TMX), and clothianidin (CLO), in food. ***Moreover, bees of both species prefer to eat more of sucrose solutions laced with IMD or TMX than sucrose alone. Stimulation with IMD, TMX and CLO neither elicited spiking responses from gustatory neurons in the bees' mouthparts, nor inhibited the responses of sucrose-sensitive neurons. Our data indicate that bees cannot taste neonicotinoids and are not repelled by them. Instead, bees preferred solutions containing IMD or TMX, even though the consumption of these pesticides caused them to eat less food overall. This work shows that bees cannot control their exposure to neonicotinoids in food and implies that treating flowering crops with IMD and TMX presents a sizeable hazard to foraging bees."*** *From Kessler, Tiedeken, et al. (2015)*

See above also Stoner and Eitzer (2012) with sub-lethal effects on honeybees and bumblebees.

Stoner, K. A. and Eitzer, B. D. (2012) Movement of Soil-Applied Imidacloprid and Thiamethoxam into Nectar and Pollen of Squash (Cucurbita pepo) Plos One 7 6 ISBN/1932-6203 <Go to ISI>://WOS:000305825800019 AND http://www.ask-force.org/web/Bees/Stoner-Movement-Soil-Applied-Imidacloprid-Squash-2012.pdf

Two more papers denying negative effects of neonicotinoids on bees coming from industry sources: Georg (20151005, Schindle Shannon and Zienkiewicz (20140930)

Schindle Shannon and Zienkiewicz, M. (20140930) EU Feeling Impact of Neonics Ban. European growers and industry are feeling the sting of the two-year restriction on the use of neonicotinoids eruopeanseed 1 1 5 pp http://european-seed.com/eu-feeling-impact-neonics-ban/ AND http://www.ask-force.org/web/Bees/Shannon-Zinkiewicz-EU-feeling-impact-on-Neonics-ban-20140930.pdf

"The ban has had some negative economic effects on the seed sector, and we expect this to get worse as we move into 2015." Essentially, EFSA was to assess each seed treatment on its acute and chronic effects on bee colony survival and development; its effects on bee larvae and bee behaviour; and the risks posed by sublethal doses of the three substances. Despite that in some cases EFSA was unable to finalize its assessments due to a lack of available data, the European Commission enacted restrictions on the use of neonicotinoids beginning Dec. 1, 2013 for several bee-attractive crops and included a twoyear period to further review conditions for registration of neonicotinoids in these crops. The European Seed Association (ESA) also says the ban has had a number of negative effects for industry. "This ban means a big gap in the area of insecticide seed treatment in many key crops, such as oilseed rape and maize," says Ana Silva, ESA communications manager. "The ban has had some negative economic effects on the seed sector, and we expect this to get worse as we move into 2015." From Schindle Shannon and Zienkiewicz (20140930)

Georg, G. (20151005) Neonicotinoids and Bees: Separating Fact From Fiction. THE SEED INDUSTRY SPEAKS OUT ON THE BAN OF NEONICOTINOIDS SEED TREATMENTS AS THE FULL IMPACT OF THE BAN IS FELT ON EU CROP PRODUCTION europeanseed 2 3 5 pp http://european-seed.com/neonicotinoids-and-bees-separating-fact-from-fiction/ AND http://www.ask-force.org/web/Bees/Goeres-Neonicotinoids-and-Bees-Separating-Fact-From-Fiction-20151005.docx AND http://www.ask-force.org/web/Bees/Goeres-Neonicotinoids-and-Bees-Separating-Fact-From-Fiction-20151005.pdf

At the EU country-level we've witnessed severe crop damage:
Germany has experienced a six per cent decline of OSR growing area. Ninety per cent of OSR damaged and 30 per cent of the 1.309 million hectares was seen to suffer from severe flea beetle attack. A lot more insecticide sprays now. There's also confirmed increase in resistance issues towards pyrethroid insecticides.
In the UK, 38,000 ha were not planted due to lack of crop protection products. In total, yield of OSR in the UK decreased by 60,000 ha or 10 per cent primarily due to flea beetle. Foliar insecticide spraying increased four-fold, reaching 100 per cent treated area in parts of the east and southeast.
And in Sweden, the area of spring oilseed rape significantly decreased: 54,000 ha (2013) > 14,700 ha (2014) -> 6,000 ha (2015) = 90 per cent reduction. Increase in number of sprays from 2 per ha to 5.5 sprays per ha.
Beyond the EU, in May 2015, U.S. President Barack Obama's administration published its National Pollinator Strategy, which clearly argues that there are multiple factors impacting bee health, rather than just one. Thus far, the U.S. government along with virtually all other major governments around the world have continued to support the use of neonicotinoid pesticide seed treatment technology, which remains one of the most innovative and environmentally friendly forms of crop protection. From Georg (20151005)

6.3. More detailed information about the Imidacloprid controversy

It's not sure if imidacloprid is still used in the US - some insects might have developed a resistance to this pesticide, but Nguyen, Saegerman, et al. (2009) **demonstrate that imidacloprid seed-treated maize has *no negative impact on Honeybees*:**

Nguyen, B.K., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Tuirionet, B., Verheggen, F.J., Berkvens, D., De Pauw, E., & Haubruge, E. (2009) Does Imidacloprid Seed-Treated Maize Have an Impact on Honeybee Mortality? Journal of Economic Entomology, 102, 2, pp 616-623 <Go to ISI>://WOS:000264899500021 AND http://www.ask-force.org/web/Bees/Nguyen-Imidacloprid-Seed-2009.pdf

*"Beekeepers suspected maize. Zea mays L., treated with imidacloprid to result in substantial loss of Honeybee (Hymenoptera: Apidae) colonies in Belgium. The objective of this study was to investigate the potential impact of maize grown from imidacloprid-treated seeds on Honeybee mortality. A survey of 16 apiaries was carried out, and all maize fields treated or not with imidacloprid were located within a radius of 3,000 m around the observed apiaries. Samples of honey, beeswax, and bees were collected in three colonies per apiary and analyzed for pesticide contain by liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry. We first found significant correlation between the number of colonies per apiary and the mortality rates in an apiary**. In addition, this mortality rate was inversely correlated with the surface of maize fields treated and not with imidacloprid, suggesting that this pesticide do not interact with bees fitness.** Moreover, a very large number of our samples contained acarcides either prohibited or ineffective against varroa destructor (Anderson & Trueman) (Acari: Varroidae), suggesting that the treatment method used by the beekeepers to be inadequate or mite control. Our results support the hypothesis that imidacloprid seed-treated maize has no negative impact on Honeybees.*

Nguyen, Saegerman, et al. (2009)

A more alarming review of pesticide impact on CCD syndromes is published by Pettis et al: Pettis, vanEngelsdorp, et al. (2012),

Pettis, J. S., D. vanEngelsdorp, J. Johnson and G. Dively (2012), Pesticide exposure in Honeybees results in increased levels of the gut pathogen Nosema, Naturwissenschaften, 99, 2, pp. 153-158, <Go to ISI>://WOS:000300682000008 AND http://www.ask-force.org/web/Bees/Pettis-Pesticide-Exposure-Nosema-2012.pdf

"Global pollinator declines have been attributed to habitat destruction, pesticide use, and climate change or some combination of these factors, and managed Honeybees, Apis mellifera, are part of worldwide pollinator declines. Here we exposed Honeybee colonies during three brood generations to sub-lethal doses of a widely used pesticide, imidacloprid, and then subsequently challenged newly emerged bees with the gut parasite, Nosema spp. The pesticide dosages used were below levels demonstrated to cause effects on longevity or foraging in adult Honeybees. Nosema infections increased significantly in the bees from pesticide-treated hives when compared to bees from control hives demonstrating an indirect effect of pesticides on pathogen growth in Honeybees. We clearly demonstrate an increase in pathogen growth within individual bees reared in colonies exposed to one of the most widely used pesticides worldwide, imidacloprid, at below levels considered harmful to bees. The finding that individual bees with undetectable levels of the target pesticide, after being reared in a sub-lethal pesticide environment within the colony, had higher Nosema is significant. ***Interactions between pesticides and pathogens could be a major contributor to increased mortality of Honeybee colonies, including colony collapse disorder, and other pollinator declines worldwide****."Pettis, vanEngelsdorp, et al. (2012)*

And one year later by the same research lead author comes with more details: Pettis, Lichtenberg, et al. (2013B) hints to the relationship between pesticide exposure and higher susceptibility to the pathogen Nosema:

Pettis, J. S., E. M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose and D. Vanengelsdorp (2013), Crop Pollination Exposes Honeybees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae, Plos One, 8, 7, pp. <Go to ISI>://WOS:000322167900111 AND http://www.ask-force.org/web/Bees/Pettis-Crop-Pollination-Exposes-Bees-Pesticides-2013.pdf

"Recent declines in Honeybee populations and increasing demand for insect-pollinated crops raise concerns about pollinator shortages. Pesticide exposure and pathogens may interact to have strong negative effects on managed Honeybee colonies. Such findings are of great concern given the large numbers and high levels of pesticides found in Honeybee colonies. Thus it is crucial to determine how field-relevant combinations and loads of pesticides affect bee health. We collected pollen from bee hives in seven major crops to determine 1) what types of pesticides bees are exposed to when rented for pollination of various crops and 2) how field-relevant pesticide blends affect bees' susceptibility to the gut parasite Nosema ceranae. Our samples represent pollen collected by foragers for use by the colony, and do not necessarily indicate foragers' roles as pollinators. In blueberry, cranberry, cucumber, pumpkin and watermelon bees collected pollen almost exclusively from weeds and wildflowers during our sampling. Thus more attention must be paid to how Honeybees are exposed to pesticides outside of the field in which they are placed. We detected 35 different pesticides in the sampled pollen, and found high fungicide loads. The insecticides esfenvalerate and phosmet were at a concentration higher than their median lethal dose in at least one pollen sample. ***While fungicides are typically seen as fairly safe for Honeybees, we found an increased probability of Nosema infection in bees that consumed pollen with a higher fungicide load. Our results highlight a need for research on sub-lethal effects of fungicides and other chemicals that bees placed in an agricultural setting are exposed to.*** *" Pettis, Lichtenberg, et al. (2013B)*

About the interaction between Nosema and neonicotinoids see also Alaux et al., suggesting in the long term a higher susceptibility of the colony to pathogens Alaux, Brunet, et al. (2010):

Alaux, C., J. L. Brunet, C. Dussaubat, F. Mondet, S. Tchamitchan, M. Cousin, J. Brillard, A. Baldy, L. P. Belzunces and Y. Le Conte (2010), Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera), Environmental Microbiology, 12, 3, pp. 774-782, <Go to ISI>://WOS:000274942300019 AND http://www.ask-force.org/web/Bees/Alaux-Interaction-Neonics-Nosema-2010.pdf

"Global pollinators, like honeybees, are declining in abundance and diversity, which can adversely affect natural ecosystems and agriculture. Therefore, we tested the current hypotheses describing honeybee losses as a multifactorial syndrome, by investigating integrative effects of an infectious organism and an insecticide on honeybee health. We demonstrated that the interaction between the microsporidia Nosema and a neonicotinoid (imidacloprid) significantly weakened honeybees. In the short term, the combination of both agents caused the highest individual mortality rates and energetic stress. By quantifying the strength of immunity at both the individual and social levels, we showed that neither the haemocyte number nor the phenoloxidase activity of individuals was affected by the different treatments. ***However, the activity of glucose oxidase, enabling bees to sterilize colony and brood food, was significantly decreased only by the combination of both factors compared with control, Nosema or imidacloprid groups, suggesting a synergistic interaction and in the long term a higher susceptibility of the colony to pathogens. This provides the first evidences that interaction between an infectious organism and a chemical can also threaten pollinators, interactions that are widely used to eliminate insect pests in integrative pest management.****" Alaux, Brunet, et al. (2010)*

Another major review has been published by Blacquiere, Smagghe, et al. (2012)*, stating that the data existing do not allow for a total ban and negative conclusions related to Honeybees*:

Blacquiere, T., G. Smagghe, C. A. M. van Gestel and V. Mommaerts (2012), Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment, Ecotoxicology, 21, 4, pp. 973-992, <Go to ISI>://WOS:000302800900002 AND http://www.ask-force.org/web/Bees/Blacquiere-Neonicotinoids-Bees-Concentrations-2012.pdf

"Via the plant sap transport neonicotinoids are translocated to different plant parts. In general, the few reported residue levels of neonicotinoids in nectar (average of 2 lg kg-1) and pollen (average of 3 lg kg-1) were below the acute and chronic toxicity levels; however, there is a lack of reliable data as analyses are performed near the detection limit. Similarly, also the levels in bee-collected pollen, in bees and bee products were low. ***But before drawing a conclusion, it is strongly encouraged to conduct more studies as so far only a few large studies have been undertaken in apiaries in France, Germany and North America. Moreover, the wide and increasing application of neonicotinoids in pest control will likely cause an accumulation of neonicotinoids in the environment in the future.***
Many lethal and sublethal effects of neonicotinoid insecticides on bees have been described in laboratory studies, however, no effects were observed in field studies with field-realistic dosages". *From Blacquiere, Smagghe, et al. (2012)*

In his master thesis Snart conducted a transcriptomic analysis of neonicotinoid exposure on honey bee larvae Snart (2011). Whilst these investigations have drawn differing conclusions, it is possible that imidacloprid contamination may result in some differential gene expression. Although rt-qPCR has shown that this level of contamination does not result in differential expression of the Dnmts, the differential candidates identified during the transcriptome analysis may be equally important for maintaining normal colony function. As a result, any change in their expression may be damaging to overall colony fitness. However, of these nine microRNA's, *only one* has been significantly documented. This is miR-9a which has been previously investigated within genetic model organisms.

Snart, C. J. (2011), Transcriptome analysis of honey bee larvae following neonicotinoid exposure, Masters Degree, University of Nottingham, P. R. Stoger, pp. http://etheses.nottingham.ac.uk/2333/2/Transcriptome__Analysis_of_Honey_Bee_Larvae_following_Neonicotinoid_Exposure.pdf AND http://www.ask-force.org/web/Bees/Snart-Transcriptom-Analysis-Bee-Neonicotinoid-2011.pdf

The current decline of the European Honey Bee (Apis Mellifera) has been linked to the increasing use of neonicotinoid pesticides within agriculture. Whilst the toxicity of these pesticides to Apis has long been established, the possibility of low dosages inducing molecular stress has not yet been fully explored. Of particular interest is the action of these nicotine derivatives on the nicotinic acetylcholine receptor, and its association with the DNA methyltransferase family (Dnmts).
An experimental group of three hives were exposed to sugar water contaminated with a low concentration of imidacloprid (2µg/l). From these hives, 12 third instar larvae were selected. A corresponding number of larvae were also selected from three control hives, for a total of 12 samples. Using quantitative reverse transcription-PCR, known Dnmt transcripts were detected and amplified from these larval samples. Specially designed oligonucleotide primers were used containing gene specific sequences that linked to universal DNA sequences, ensuring that PCR amplification products were of predetermined sizes. Products of this amplification were resolved by capillary electrophoresis and detected by fluorescence spectrophotometry. Simultaneously, the transcriptomes of 3 larval samples each from the control and experimental groups were generated using SOLiD platform sequencing. The statistical package EdgeR was then utilised to identify differential candidates of known honey bee microRNA's.
Statistical analysis utilising a one-way Analysis of Variance (ANOVA) found no significant differences in the expression levels of known Dnmt transcripts between control and experimental groups. However, comparisons of sequenced control and experimental transcriptomes identified a number of differential microRNA candidates, most notably miR-9/14. Snart (2011)

Rat experiments of Bal et al. Bal, Erdogan, et al. (2010, Bal, Naziroglu, et al. (2012, Bal, Turk, et al. (2013, Bal, Turk, et al. (2012A, Bal, Turk, et al. (2012B) show partial impact of neonics:

Bal, R., G. Turk, M. Tuzcu, O. Yilmaz, T. Kuloglu, G. Baydas, M. Naziroglu, Z. Yener, E. Etem and Z. Tuzcu (2013), Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats, Drug and Chemical Toxicology, 36, 4, pp. 421-429, <Go to ISI>://WOS:000323931300005 AND http://www.ask-force.org/web/Pesticides/Bal-Effects-Neonicoinoid-Insects-2013.pdf

Clothianidin (CTD) is a novel, broad-spectrum insecticide. In the current study, it was aimed to study the effect of subchronic exposure to low doses of CTD (2, 8 and 24 mg/kg body weight/day) on the reproductive system in adult rats. CTD treatment did not significantly change serum testosterone level or sperm parameters (e. g. concentration, motility and morphology), but caused significant decreases in weights of epididymis, right cauda epididymis and seminal vesicles. ***CTD treatment did not cause sperm DNA fragmentation and did not change the apoptotic index in the seminiferous tubules and levels of alpha-tocopherol and glutathione, but increased the level of thiobarbituric acid-reactive substances and cholesterol levels significantly at all doses. CTD exposure caused significant elevations in palmitic, linoleic and arachidonic acids in testis in all CTD-exposed groups. There was a drop in 20:4/18:2 (arachidonic acid/linoleic acid) ratio and an increase in 18:1n-9/18:0 (oleic acid/stearic acid) ratios in all CTD groups, in comparison to the control group. In conclusion, CTD had little detectable detrimental effects on the reproductive system of male rats over the measured parameters. Bal, Turk, et al. (2013)***

The latest warning, that neonicotinoid insecticides can serve as inadvertent insect contraceptives comes from the team of Straub in Bern:

Straub, L., Villamar-Bouza, L., Bruckner, S., Chantawannakul, P., Gauthier, L., Khongphinitbunjong, K., Retschnig, G., Troxler, A., Vidondo, B., Neumann, P., & Williams, G. R. (2016). Neonicotinoid insecticides can serve as inadvertent insect contraceptives. *Proceedings of the Royal Society of London B: Biological Sciences, 283*(1835), pp. http://rspb.royalsocietypublishing.org/content/royprsb/283/1835/20160506.full.pdf AND http://www.ask-force.org/web/Bees/Straub-neonicotinoid-inadvertent-insect-contraceptives-2016.pdf

"There is clear evidence for sublethal effects of neonicotinoid insecticides on non-target ecosystem service-providing insects. However, their possible impact on male insect reproduction is currently unknown, despite the key role of sex. Here, we show that two neonicotinoids (4.5 ppb thiamethoxam and 1.5 ppb clothianidin) significantly reduce the reproductive capacity of male honeybees (drones), Apis mellifera. Drones were obtained from colonies exposed to the neonicotinoid insecticides or controls, and subsequently maintained in laboratory cages until they reached sexual maturity. While no significant effects were observed for male teneral (newly emerged adult) body mass and sperm quantity, the data clearly showed reduced drone lifespan, as well as reduced sperm viability (percentage living versus dead) and living sperm quantity by 39%. ***Our results demonstrate for the first time that neonicotinoid insecticides can negatively affect male insect reproductive capacity, and provide a possible mechanistic explanation for managed honeybee queen failure and wild insect pollinator decline. The widespread prophylactic use of neonicotinoids may have previously overlooked inadvertent contraceptive effects on non-target insects, thereby limiting conservation efforts."*** *From Straub et al. 2016.*

6.4. Sub-lethal doses of imidacloprid and other pesticides

Sub-lethal neonicotinoid-impact is postulated with data from an impressive list of publications: El Hassani, Dacher, et al. (2008, Matsumoto (2013, Sandrock, Tanadini, et al. (2014, Schneider, Tautz, et al. (2012, Shi, Jiang, et al. (2011, Williamson, Baker, et al. (2013, Wu-Smart and Spivak (2016, Yang, Chuang, et al. (2008) LU Chensheng (2014), if you widen the search to imidacloprid you get a list of 34 publications on sub-lethal effects, and the list of sub-lethal effects related to pesticides as a whole yields 62 references, they are given here all in one list, all including full text links:

Lu, C. S., Warchol, K. M. and Callahan, R. A. (2014) Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder Bulletin of Insectology 67 1 125-130 pp ISBN/1721-8861 <Go to ISI>://WOS:000336561400016 AND http://www.ask-force.org/web/Bees/Lu-Sub-Lethal-Exposure-Neonicotinoids-Bees-2014.pdf

Honey bee (Apis mellifera L.) colony collapse disorder (CCD) that appeared in 2005/2006 still lingers in many parts of the world. Here we show that sub-lethal exposure of neonicotinoids, imidacloprid or clothianidin, affected the winterization of healthy colonies that subsequently leads to CCD. We found honey bees in both control and neonicotinoid-treated groups progressed almost identically through the summer and fall seasons and observed no acute morbidity or mortality in either group until the end of winter.

Bees from six of the twelve neonicotinoid-treated colonies had abandoned their hives, and were eventually dead with symptoms resembling CCD. However, we observed a complete opposite phenomenon in the control colonies in which instead of abandonment, they were re-populated quickly with new emerging bees. ***Only one of the six control colonies was lost due to Nosemalike infection. The observations from this study may help to elucidate the mechanisms by which sub-lethal neonicotinoids exposure caused honey bees to vanish from their hives****. LU Chensheng (2014)*

F. Hatjina, C. Papaefthimiou, L. Charistos, T. Dogaroglu, M. Bouga, C. Emmanouil and G. Arnold (2013)

Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo, Apidologie, 44, 4, 467-480<Go to ISI>://WOS:000319878900011 AND http://www.ask-force.org/web/Bees/Hatjina-Sublethal-Doses-Imidacloprid-2013.pdf

"Most studies that have shown negative sublethal effects of the pesticide imidacloprid on honeybees concern behavioral effects; only a few concern physiological effects. Therefore, we investigated sublethal effects of imidacloprid on the development of the hypopharyngeal glands (HPGs) and respiratory rhythm in honeybees fed under laboratory conditions. We introduced newly emerged honeybees into wooden mesh-sided cages and provided sugar solution and pollen pastry ad libitum. Imidacloprid was administered in the food: 2 mu g/kg in the sugar solution and 3 mu g/kg in the pollen pastry. The acini, the lobes of the HPGs of imidacloprid-treated honeybees, were 14.5 % smaller in diameter in 9-day-old honeybees and 16.3 % smaller in 14-day-old honeybees than in the same-aged untreated honeybees; the difference was significant for both age groups. ***Imidacloprid also significantly affected the bursting pattern of abdominal ventilation movements (AVM) by causing a 59.4 % increase in the inter-burst interval and a 56.99 % decrease in the mean duration of AVM bursts. At the same time, the quantity of food consumed (sugar solution and pollen pastry) per honeybee per day was the same for both treated and untreated honeybees.****" Hatjina, Papaefthimiou, et al. (2013)*

K. P. Yanez, J. L. Bernal, M. J. Nozal, M. T. Martin and J. Bernal (2013)

"Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column, Journal of Chromatography A, 1285, 110-117
<Go to ISI>://WOS:000317167400013 AND http://www.ask-force.org/web/Bees/Yanez-Determination-Seven-Neonicotinoid-Bee-Wax-2013.pdf

A new method has been developed to measure seven neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam) in beeswax using liquid chromatography (LC) coupled to electrospray ionization mass spectrometry (ESI-MS) detection. Beeswax was melted and diluted in an n-hexane/isopropanol (8:2, v/v) mixture. After this, liquid extraction with water was performed followed by a clean-up on diatomaceous material based cartridges. The compounds were eluted with acetone, and the resulting solution was evaporated until dry and reconstituted with a mixture of water and acetonitrile 50:50 (v/v). The separation of all compounds was achieved in less than 15 min using a C-18 reverse-phase fused-core column (Kinetex (R) C-18, 150 mm x 4.6 mm i.d.) and a mobile phase composed of a mixture of 0.1% formic acid in water and acetonitrile in gradient elution mode at 0.5 mL/min. ***This method was fully validated in terms of selectivity, linearity, precision and recovery. Low limits of detection and quantification could be achieved for all analytes ranging from 0.4 to 2.3 mu g/kg, and from 1.5 to 7.0 mu g/kg, respectively. Finally, the proposed method was applied to an analysis of neonicotinoid residues in beeswax samples from apiaries located close to fruit orchards****." Yanez, Bernal, et al. (2013)*

S. M. Williamson and G. A. Wright (2013)

Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees, Journal of Experimental Biology, 216, 10, 1799-1807

"Pesticides are important agricultural tools often used in combination to avoid resistance in target pest species, but there is growing concern that their widespread use contributes to the decline of pollinator populations. Pollinators perform sophisticated behaviors while foraging that require them to learn and remember floral traits associated with food, but we know relatively little about the way that combined exposure to multiple pesticides affects neural function and behavior. The experiments reported here show that prolonged exposure to field-realistic concentrations of the neonicotinoid imidacloprid and the organophosphate acetylcholinesterase inhibitor coumaphos and their combination impairs olfactory learning and memory formation in the honeybee. Using a method for classical conditioning of proboscis extension, honeybees were trained in either a massed or spaced conditioning protocol to examine how these pesticides affected performance during learning and short- and long-term memory tasks. We found that bees exposed to imidacloprid, coumaphos, or a combination of these compounds, were less likely to express conditioned proboscis extension towards an odor associated with reward. Bees exposed to imidacloprid were less likely to form a long-term memory, whereas bees exposed to coumaphos were only less likely to respond during the short-term memory test after massed conditioning. Imidacloprid, coumaphos and a combination of the two compounds impaired the bees' ability to differentiate the conditioned odor from a novel odor during the memory test. ***Our results demonstrate that exposure to sublethal doses of combined cholinergic pesticides significantly impairs important behaviours involved in foraging, implying that pollinator population decline could be the result of a failure of neural function of bees exposed to pesticides in agricultural landscapes."*** *Williamson and Wright (2013)*
<Go to ISI>://WOS:000318483600013 AND http://www.ask-force.org/web/Bees/Williamson-Exposure-Multiple-Cholinergic-Pesticides-2013.pdf

S. M. Williamson, D. D. Baker and G. A. Wright (2013)

Acute exposure to a sublethal dose of imidacloprid and coumaphos enhances olfactory learning and memory in the honeybee Apis mellifera, Invertebrate Neuroscience, 13, 1, 63-70

The decline of honeybees and other pollinating insects is a current cause for concern. A major factor implicated in their decline is exposure to agricultural chemicals, in particular the neonicotinoid insecticides such as imidacloprid. Honeybees are also subjected to additional chemical exposure when beekeepers treat hives with acaricides to combat the mite Varroa destructor. Here, we assess the effects of acute sublethal doses of the neonicotinoid imidacloprid, and the organophosphate acaricide coumaphos, on Honeybee learning and memory. ***Imidacloprid had little effect on performance in a six-trial olfactory conditioning assay, while coumaphos caused a modest impairment. We report a surprising lack of additive adverse effects when both compounds were administered simultaneously, which instead produced a modest improvement in learning and memory.*** Williamson, Baker, et al. (2013)
<Go to ISI>://WOS:000320000900008 AND http://www.ask-force.org/web/Bees/Williamson-Acute-Exposure-Sublethal-Dose-Imidacloprid-2013.pdf

Wu-Smart, J. and Spivak, M. (2016) Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development Scientific Reports 6 32108 pp http://dx.doi.org/10.1038/srep32108 AND http://www.ask-force.org/web/Bees/Wu-Smart-Sublethal-effects-dietary-neonic-exposure-bee-fecundity-2016.pdf

"Many factors can negatively affect honey bee (Apis mellifera L.) health including the pervasive use of systemic neonicotinoid insecticides. Through direct consumption of contaminated nectar and pollen from treated plants, neonicotinoids can affect foraging, learning, and memory in worker bees. Less well studied are the potential effects of neonicotinoids on queen bees, which may be exposed indirectly through trophallaxis, or food-sharing. To assess effects on queen productivity, small colonies of different sizes (1500, 3000, and 7000 bees) were fed imidacloprid (0, 10, 20, 50, and 100 ppb) in syrup for three weeks. We found adverse effects of imidacloprid on queens (egg-laying and locomotor activity), worker bees (foraging and hygienic activities), and colony development (brood production and pollen stores) in all treated colonies. Some effects were less evident as colony size increased, suggesting that larger colony populations may act as a buffer to pesticide exposure. ***This study is the first to show adverse effects of imidacloprid on queen bee fecundity and behavior and improves our understanding of how neonicotinoids may impair short-term colony functioning. These data indicate that risk-mitigation efforts should focus on reducing neonicotinoid exposure in the early spring when colonies are smallest and queens are most vulnerable to exposure."*** *From Wu-Smart and Spivak (2016)*

T. C. Van Dijk, M. A. Van Staalduinen and J. P. Van der Sluijs (2013)
Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid, Plos One, 8, 5,

"Imidacloprid is one of the most widely used insecticides in the world. Its concentration in surface water exceeds the water quality norms in many parts of the Netherlands. Several studies have demonstrated harmful effects of this neonicotinoid to a wide range of non-target species. Therefore we expected that surface water pollution with imidacloprid would negatively impact aquatic ecosystems. Availability of extensive monitoring data on the abundance of aquatic macro-invertebrate species, and on imidacloprid concentrations in surface water in the Netherlands enabled us to test this hypothesis. Our regression analysis showed a significant negative relationship (P<0.001) between macro-invertebrate abundance and imidacloprid concentration for all species pooled. A significant negative relationship was also found for the orders Amphipoda, Basommatophora, Diptera, Ephemeroptera and Isopoda, and for several species separately. The order Odonata had a negative relationship very close to the significance threshold of 0.05 (P <0.051). However, in accordance with previous research, a positive relationship was found for the order Actinedida. We used the monitoring field data to test whether the existing three water quality norms for imidacloprid in the Netherlands are protective in real conditions. Our data show that macro-fauna abundance drops sharply between 13 and 67 ng l(-1). For aquatic ecosystem protection, two of the norms are not protective at all while the strictest norm of 13 ng l(-1) (MTR) seems somewhat protective. ***In addition to the existing experimental evidence on the negative effects of imidacloprid on invertebrate life, our study, based on data from large-scale field monitoring during multiple years, shows that serious concern about the far-reaching consequences of the abundant use of imidacloprid for aquatic ecosystems is justified*.**" Van Dijk, Van Staalduinen, et al. (2013).
<Go to ISI>://WOS:000319167000043 AND http://www.ask-force.org/web/Bees/Van-Dijk-Macro-Invertebrate-Decline-Water-Imidacloprid-2013.pdf

A. Tapparo, C. Giorio, L. Solda, S. Bogialli, D. Marton, M. Marzaro and V. Girolami (2013)
UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations, Analytical and Bioanalytical Chemistry, 405, 2-3, 1007-1014

In the understanding of colony loss phenomena, a worldwide crisis of honeybee colonies which has serious consequences for both apiculture and bee-pollination-dependent farm production, analytical chemistry can play an important role. For instance, rapid and accurate analytical procedures are currently required to better assess the effects of neonicotinoid insecticides on honeybee health. Since their introduction in agriculture, neonicotinoid insecticides have been blamed for being highly toxic to honeybees, possibly at the nanogram per bee level or lower. As a consequence, most of the analytical methods recently optimized have focused on the analysis of ultratraces of neonicotinoids using liquid chromatography-mass spectrometry techniques to study the effects of sublethal doses. ***However, recent evidences on two novel routes-seedling guttations and seed coating particulate, both associated with corn crops-that may expose honeybees to huge amounts of neonicotinoids in the field, with instantly lethal effects, suggest that selected procedures need optimizing.*** *In the present work, a simplified ultra-high-performance liquid chromatography-diode-array detection method for the determination of neonicotinoids in single bees has been*

optimized and validated. The method ensures good selectivity, good accuracy, and adequate detection limits, which make it suitable for the purpose, while maintaining its ability to evaluate exposure variability of individual bees. It has been successfully applied to the analysis of bees in free flight over an experimental sowing field, with the bees therefore being exposed to seed coating particulate released by the pneumatic drilling machine. Tapparo, Giorio, et al. (2013).
<Go to ISI>://WOS:000313650800048 AND http://www.ask-force.org/web/Bees/Tapparo-UHPLC-DAD-Method-Determination-Neonicotinoid-2013.pdf

C. D. Rossi, T. C. Roat, D. A. Tavares, P. Cintra-Socolowski and O. Malaspina (2013)
Effects of sublethal doses of imidacloprid in malpighian tubules of africanized Apis mellifera (Hymenoptera, Apidae), Microscopy Research and Technique, 76, 5, 552-558
In Brazil, imidacloprid is a widely used insecticide on agriculture and can harm bees, which are important pollinators. The active ingredient imidacloprid has action on the nervous system of the insects. However, little has been studied about the actions of the insecticide on nontarget organs of insects, such as the Malpighian tubules that make up the excretory and osmo-regulatory system. Hence, in this study, we evaluated the effects of chronic exposure to sublethal doses of imidacloprid in Malpighian tubules of Africanized Apis mellifera. In the tubules of treated bees, we found an increase in the number of cells with picnotic nuclei, the lost of part of the cell into the lumen, and a homogenization of coloring cytoplasm. Furthermore, we observed the presence of cytoplasmic vacuolization. We confirmed the increased occurrence of picnotic nuclei by using the Feulgan reaction, which showed the chromatin compaction was more intense in the tubules of bees exposed to the insecticide. We observed an intensification of the staining of the nucleus with Xylidine Ponceau, further verifying the cytoplasmic negative regions that may indicate autophagic activity. Additionally, immunocytochemistry experiments showed TUNEL positive nuclei in exposed bees, implicating increased cell apoptosis after chronic imidacloprid exposure. **In conclusion, our results indicate that very low concentrations of imidacloprid lead to cytotoxic activity in the Malpighian tubules of exposed bees at all tested times for exposure and imply that this insecticide can alter Honeybee physiology.** *Rossi, Roat, et al. (2013).*
<Go to ISI>://WOS:000317299500015 AND http://www.ask-force.org/web/Bees/Rossi-Effect-Sublethal-Doses-Imidacloprid-2013.pdf

M. J. Palmer, C. Moffat, N. Saranzewa, J. Harvey, G. A. Wright and C. N. Connolly (2013)
Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees, Nature Communications, 4,
"*Pesticides that target cholinergic neurotransmission are highly effective, but their use has been implicated in insect pollinator population decline. Honeybees are exposed to two widely used classes of cholinergic pesticide: neonicotinoids (nicotinic receptor agonists) and organophosphate miticides (acetylcholinesterase inhibitors). Although sublethal levels of neonicotinoids are known to disrupt honeybee learning and behaviour, the neurophysiological basis of these effects has not been shown. Here, using recordings from mushroom body Kenyon cells in acutely isolated honeybee brain, we show that the neonicotinoids imidacloprid and clothianidin, and the organophosphate miticide coumaphos oxon, cause a depolarization-block of neuronal firing and inhibit nicotinic responses. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with combined application.* ***Our findings demonstrate a neuronal mechanism that may account for the cognitive impairments caused by neonicotinoids, and predict that exposure to multiple pesticides that target cholinergic signalling will cause enhanced toxicity to pollinators."*** *Palmer, Moffat, et al. (2013)*
<Go to ISI>://WOS:000318873900087 AND http://www.ask-force.org/web/Bees/Palmer-Cholinergic-Pesticides-Cause-Mushroom-Body-2013.pdf

T. Matsumoto (2013)
Reduction in homing flights in the Honeybee Apis mellifera after a sublethal dose of neonicotinoid insecticides, Bulletin of Insectology, 66, 1, 1-9
The negative effects of a commonly applied systemic insecticide, neonicotinoid, on the Honeybee Apis mellifera L. are of great concern worldwide, as the use of the chemical is expanding. Recently, special attention has been paid to the sublethal effects of insecticides. An increasing number of studies has identified sublethal effects on the Honeybee in the laboratory or in experimental cages, but so far, few studies have examined sublethal effects in the field. To reveal sublethal effects under field conditions, I examined whether the proportion of successful homing flights by foraging Honeybees during 30 min after release decreased after bees were topically exposed to insecticides. Honeybees were treated with two types of neonicotinoid insecticide (clothianidin, dinotefuran) and two types of previously common insecticide (etofenprox [pyrethroid] and fenitrothion [organophosphate]) at five different doses (one-halt one-fourth, one-tenth, one-twentieth, and one-fortieth of their median lethal dose - LD50). Then the bees were released 500 m from their hives in the field. The proportions of successful homing flights by bees exposed to neonicotinoids and pyrethroid decreased with doses of one-tenth LD50 (2.18 ng/head for clothianidin, 7.5 ng/head for dinotefuran) or more and one-fourth LD50 (32.5 ng/head for pyrethroid) or more, respectively, whereas bees exposed to organophosphate did not significantly show a response at any sublethal dose though the trend in decline appeared to. Flight times were not significantly different among treatments at any dose. ***These results indicate that neonicotinoid and pyrethroid exposure reduced successful homing flights at doses far below the LD50 in the field. Moreover, neonicotinoid caused reductions at relatively lower exposure than pyrethroid****. Matsumoto (2013)*
<Go to ISI>://WOS:000319310300001 AND http://www.ask-force.org/web/Bees/Matsumoto-Reduction-Homing-Flights-Neonicotinoid-2013.pdf

K. A. Stoner and B. D. Eitzer (2012)
Movement of Soil-Applied Imidacloprid and Thiamethoxam into Nectar and Pollen of Squash (Cucurbita pepo), Plos One, 7, 6, *"There has been recent interest in the threat to bees posed by the use of systemic insecticides. One concern is that systemic insecticides may translocate from the soil into pollen and nectar of plants, where they would be ingested by pollinators. This paper reports on the movement of two such systemic*

neonicotinoid insecticides, imidacloprid and thiamethoxam, into the pollen and nectar of flowers of squash (Cucurbita pepo cultivars "Multipik," "Sunray" and "Bush Delicata") when applied to soil by two methods: (1) sprayed into soil before seeding, or (2) applied through drip irrigation in a single treatment after transplant. All insecticide treatments were within labeled rates for these compounds. Pollen and nectar samples were analyzed using a standard extraction method widely used for pesticides (QuEChERS) and liquid chromatography mass spectrometric analysis. The concentrations found in nectar, 10 +/- 3 ppb (mean +/- s.d) for imidacloprid and 11 +/- 6 ppb for thiamethoxam, are higher than concentrations of neonicotinoid insecticides in nectar of canola and sunflower grown from treated seed, and similar to those found in a recent study of neonicotinoids applied to pumpkins at transplant and through drip irrigation. ***The concentrations in pollen, 14 +/- 8 ppb for imidacloprid and 12 +/- 9 ppb for thiamethoxam, are higher than those found for seed treatments in most studies, but at the low end of the range found in the pumpkin study. Our concentrations fall into the range being investigated for sub-lethal effects on Honeybees and bumble bees."*** *Stoner and Eitzer (2012)*
<Go to ISI>://WOS:000305825800019 AND http://www.ask-force.org/web/Bees/Stoner-Movement-Soil-Applied-Imidacloprid-Squash-2012.pdf

Recent literature on neonicotinoid pesticides and their impact on Honeybees, needs still some interpretation: It is difficult from the contradicting - over 500 publications on Neonicotinoids to make up a final verdict.
With a view on the precautionary approach it is in the eyes of the author of this review justified to maintain a ban on neonicotinoids until further thorough systems studies and new recipes for applications could give green light again. See more conclusive remarks below, see chapter 7.9. p. 65.

Studies from the University of Bern, Bee research Department, come to at least partially negative conclusions: Retschnig, Williams, et al. (2014)

Retschnig, G., Williams, G. R., Mehmann, M. M., Yanez, O., de Miranda, J. R., & Neumann, P. (2014). Sex-Specific Differences in Pathogen Susceptibility in Honey Bees (Apis mellifera). *PLoS ONE, 9*(1), pp. <Go to ISI>://WOS:000330237000028 AND http://www.ask-force.org/web/Bees/Retschnig-Sex-Specific-Differences-Pathogen-Susceptibility-Bees-2014.pdf

"Sex-related differences in susceptibility to pathogens are a common phenomenon in animals. In the eusocial Hymenoptera the two female castes, workers and queens, are diploid and males are haploid. The haploid susceptibility hypothesis predicts that haploid males are more susceptible to pathogen infections compared to females. Here we test this hypothesis using adult male (drone) and female (worker) honey bees (Apis mellifera), inoculated with the gut endo-parasite Nosema ceranae and/or black queen cell virus (BQCV). These pathogens were chosen due to previously reported synergistic interactions between Nosema Apis and BQCV. Our data do not support synergistic interactions between N. ceranae and BQCV and also suggest that BQCV has limited effect on both drone and worker health, regardless of the infection level. However, the data clearly show that, despite lower levels of N. ceranae spores in drones than in workers, Nosema-infected drones had both a higher mortality and a lower body mass than non-infected drones, across all treatment groups, while the mortality and body mass of worker bees were largely unaffected by N. ceranae infection, suggesting that drones are more susceptible to this pathogen than workers. ***In conclusion, the data reveal considerable sex-specific differences in pathogen susceptibility in honey bees and highlight the importance of ultimate measures for determining susceptibility, such as mortality and body quality, rather than mere infection levels.*** *" From Retschnig, Williams, et al. (2014)*

And about Thiacloprid-Nosema ceranae interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose: Retschnig, Neumann, et al. (2014):

Retschnig, G., Neumann, P., & Williams, G. R. (2014). Thiacloprid-Nosema ceranae interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose. *Journal of Invertebrate Pathology, 118*, pp. 18-19. <Go to ISI>://WOS:000335541000003 AND http://www.ask-force.org/web/Bees/Retschnig-Thiacloprid-Nosema-ceranae-interactions-bees-2014.pdf

"Interactions between stressors contribute to the recently reported increase in losses of honey bee colonies. Here we demonstrated that a synergistic effect on mortality by the low toxic, commonly used neonicotinoid thiacloprid and the nearly ubiquitous gut parasite Nosema ceranae is dependent on the pesticide dose. Furthermore, thiacloprid had a negative influence on N. ceranae reproduction. ***Our results highlight that interactions among honey bee health stressors can be dynamic and should be studied across a broader range of combinations****." From Retschnig, Neumann, et al. (2014)*

6.5. Bees might be addicted to Neonics, a parallel to the impact of Nicotin

A journalistic comment in the Science 2.0 demonstrates the controversy, if you take also into account the comments to the editor: Science20 (20151023): Bees Addicted To Neonics: A Failure of Science Journalism:

Science20. (20151023). Bees Addicted To Neonics: A Failure of Science Journalism. *Science20 Science Blogging*, pp. http://www.science20.com/news_articles/bees_addicted_to_neonics_a_failure_of_science_journalism-158154 AND http://www.ask-force.org/web/Bees/Science-2-0-Bees-Addicted-to-Neonics-Failure-Science-Journalism-20151023.pdf

Researchers don't always get it right. Scientists used to toiling in obscurity on arcane subjects can be lured into presenting hyperbolic conclusions from a media that demands sensational headlines, and confirmation bias remains a powerful psychological force within the scientific community. So what does the media do when honest researchers realize their attention-getting findings were simply wrong? If this case of "bee addiction" is any indicator, the answer is nothing. Back in April, a provocative press release about a paper [1] by researchers at Newcastle University and Trinity College Dublin that suggested bees are 'hooked' on nectar containing pesticides, in the same way that a meth addict is hooked on stimulants. They concluded that bees simply couldn't resist neonicotinoids. In the words of lead scientist, Geraldine Wright: "We now have evidence that bees prefer to eat pesticide-contaminated food. ***Neonicotinoids target the same mechanisms in the bee brain that are affected by nicotine in the human brain. The fact that bees show a preference for food containing neonicotinoids is concerning as it suggests that like nicotine, neonicotinoids may act like a drug to make foods containing these substances more rewarding."***
From Science20 (20151023)

An excellent Nature article brings good evidence of the bees having this addiction to neonicotinoids: Kessler, Tiedeken, et al. (2015)

Kessler, S. C., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C., & Wright, G. A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature, 521*(7550), pp. 74-U145. <Go to ISI>://WOS:000354040900035 AND http://www.ask-force.org/web/Bees/Kessler-Bees-prefer-foods-containing-neonicotinoid-pesticides-2015.pdf

"The impact of neonicotinoid insecticides on insect pollinators is highly controversial. Sublethal concentrations alter the behaviour of social bees and reduce survival of entire colonies1–3. However, critics argue that the reported negative effects only arise from neonicotinoid concentrations that are greater than those found in the nectar and pollen of pesticide-treated plants DEFRA (20130327). Furthermore, it has been suggested that bees could choose to forage on other available flowers and hence avoid or dilute exposure DEFRA (20130327), Godfray, Blacquière, et al. (2014) . Here, using a two-choice feeding assay, we show that the honeybee, Apis mellifera, and the buff-tailed bumblebee, Bombus terrestris, do not avoid nectar-relevant concentrations of three of the most commonly used neonicotinoids, imidacloprid (IMD), thiamethoxam (TMX), and

clothianidin (CLO), in food. ***Moreover, bees of both species prefer to eat more of sucrose solutions laced with IMD or TMX than sucrose alone. Stimulation with IMD, TMX and CLO neither elicited spiking responses from gustatory neurons in the bees' mouthparts, nor inhibited the responses of sucrose-sensitive neurons. Our data indicate that bees cannot taste neonicotinoids and are not repelled by them. Instead, bees preferred solutions containing IMD or TMX, even though the consumption of these pesticides caused them to eat less food overall. This work shows that bees cannot control their exposure to neonicotinoids in food and implies that treating flowering crops with IMD and TMX presents a sizeable hazard to foraging bees."*** *From Kessler, Tiedeken, et al. (2015)*

6.6. Other pesticides and application modes and their impact on bee colonies

Pesticide dust from treated seeds could also count for negative effects due to significant aerial powdering:

D. Nuyttens, W. Devarrewaere, P. Verboven and D. Foque (2013)
Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review, Pest Management Science, 69, 5, 564-575
<Go to ISI>://WOS:000317621500002 AND http://www.ask-force.org/web/Bees/Nuyttens-Pesticide-Laden-Dust-Emission-Seeds-3013.pdf

Dressing seeds with pesticides to control pests is a widespread practice with important advantages. Recent incidents of bee losses, however, have directed attention to the emission of abraded pesticide-coated seed particles to the environment during sowing. This phenomenon of drift of pesticide dust can lead to pesticide contamination of air, water and other natural resources in crop-growing areas. This review article presents the state of the art of the phenomenon of dust emission and drift from pesticide seed dressing during sowing and its consequences. Firstly, pesticide seed treatment is defined and its pros and cons are set out, with the focus on dust, dust emission and dust drift from pesticide-coated seed. ***The factors affecting emission of pesticide dust (e.g. seed treatment quality, seed drilling technology and environmental conditions) are considered, along with its possible effects****. The measuring techniques and protocols and models currently in use for calculating the behavior of dust are reviewed, together with their features and limitations. Finally, possible mitigation measures are discussed, such as improving the seed quality and the use of modified seed drilling technology, and an overview of regulations and stewardship activities is given. Nuyttens, Devarrewaere, et al. (2013)*

V. Girolami, M. Marzaro, L. Vivan, L. Mazzon, C. Giorio, D. Marton and A. Tapparo (2013)
Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers, Journal of Applied Entomology, 137, 1-2, 35-44
<Go to ISI>://WOS:000313517400004 AND http://www.ask-force.org/web/Bees/Girolami-Aerial-Powdering-Bees-Neonicotinoid-2013.pdf

"Sudden losses of bees have been observed in spring during maize sowing. The death of bees has been correlated with the use of neonicotinoid-coated seed and the toxic particulates emitted by pneumatic drilling machines. The contamination of foragers in flight over the ploughed fields has been hypothesized. The airborne contamination has been proven, both with bees inside fixed cages around the field and in free flight near the driller. A new trial involving mobile cages has been established and consists of making rapid passes with single bees inside cages fixed to an aluminium bar. The bar was moved by two operators at different distances from the working drilling machine. A single pass was shown as sufficient to kill all the bees exposed to exhaust air on the emission side of the drill, when bees were subsequently held in high relative humidity. The extent of toxic cloud around driller was evaluated at the height of 0.5, 1.8 and 3.5 m and proved to be about 20 m in diameter, with an ellipsoidal shape. The shape may be influenced by working speed of the drill and environmental parameters, and is easily shown by adding talc powder to the seed in the machine hopper. A new driller equipment was evaluated consisting of two tubes inclined towards the soil that direct the exhaust air towards the ground. The survival rate of the bees was not substantially increased using the modified drill and was lower than 50%. ***Chemical analyses show up to 4000 ng of insecticide in single bees with an average content around 300 ng. Similar quantities were observed at increased distances from the modified or unmodified drillers. This new evaluation of bee mortality in the field is an innovative biological test to verify the hypothetical efficiency (or not) of driller modifications."*** *Girolami, Marzaro, et al. (2013)*

Traynor, K. S., Pettis, J. S., Tarpy, D. R., Mullin, C. A., Frazier, J. L., Frazier, M. and vanEngelsdorp, D. (2016) In-hive Pesticide Exposome: Assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States Scientific Reports 6 33207 pp
http://dx.doi.org/10.1038/srep33207 AND 10.1038/srep33207 AND http://www.nature.com/articles/srep33207#supplementary-information AND http://www.ask-force.org/web/Bees/Traynor-In-Hive-Pesticide-Eyposome-US-2016.pdf

"This study measured part of the in-hive pesticide exposome by analyzing residues from live in-hive bees, stored pollen, and wax in migratory colonies over time and compared exposure to colony health. We summarized the pesticide burden using three different additive methods: (1) the hazard quotient (HQ), an estimate of pesticide exposure risk, (2) the total number of pesticide residues, and (3) the number of relevant residues. Despite being simplistic, these models attempt to summarize potential risk from multiple contaminations in real-world contexts. Colonies performing pollination services were subject to increased pesticide exposure compared to honey-production and holding yards. We found clear

links between an increase in the total number of products in wax and colony mortality. In particular, we found that fungicides with particular modes of action increased disproportionally in wax within colonies that died. The occurrence of queen events, a significant risk factor for colony health and productivity, was positively associated with all three proxies of pesticide exposure. ***While our exposome summation models do not fully capture the complexities of pesticide exposure, they nonetheless help elucidate their risks to colony health. Implementing and improving such models can help identify potential pesticide risks, permitting preventative actions to improve pollinator health.*** *From Traynor, Pettis, et al. (2016)*

6.7. Genetic differences of bee colonies related to impact of pesticides

The question of genetic differences has been discovered years ago:

Statistically significant differences were observed for clothianidin and thiamethoxam, but not for imidacloprid, but reasons are complex and can only partially be explained by genomic differences of the bee colonies:

D. Laurino, A. Manino, A. Patetta and M. Porporato (2013)
Toxicity of neonicotinoid insecticides on different Honeybee genotypes, Bulletin of Insectology, 66, 1, 119-126
<Go to ISI>://WOS:000319310300017 AND http://www.ask-force.org/web/Bees/Laurino-Toxicity-Neonicotinoid-Insecticides-2013.pdf

"Toxicity effects of the neonicotinoid insecticides clothianidin, imidacloprid, and thiamethoxam were tested in the laboratory on different Honeybee (Apis mellifera L.) genotypes belonging to the following subspecies: Apis mellifera mellifera L., Apis mellifera ligustica Spinola, and Apis mellifera carnica Pollmann Oral and indirect contact trials were carried out on adult worker Honeybees for each pesticide, using commercial formulations. The acute oral toxicity (AOT) LD50 and the acute indirect contact toxicity (ICT) LC50 were calculated. Mean AOT LD50 values at 24 hours (clothianidin 3.53 ng/Honeybee; imidacloprid 118.74 ng/Honeybee; thiamethoxam 4.40 ng/Honeybee), 48 hours (clothianidin 3.35 ng/Honeybee; imidacloprid 90.09 ng/Honeybee; thiamethoxam 4.27 ng/Honeybee), and 72 hours (clothianidin 3.28 ng/Honeybee; imidacloprid 69.68 ng/Honeybee; thiamethoxam 4.16 ng/Honeybee) from test start were of the same order of magnitude of those reported in the literature for all three neonicotinoids. Statistically significant differences emerged in a few instances between groups of Honeybees coming from the different hives tested for clothianidin, between the groups of Honeybees coming from the single A. m. mellifera hive and the four A. m. ligustica hives tested for imidacloprid, and more extensively between the two A. m. carnica, the single A. m. mellifera, and the six A. m. ligustica groups of Honeybees tested for thiamethoxam. ICT LC50 values were obtained for a reduced number of hives: the single A. m. mellifera and two A. m. ligustica hives for clothianidin, the single A. m. mellifera and one A. in. ligustica hive for imidacloprid, the single A. m. mellifera, three A. m. ligustica hives, and one A. in. carnica hive for thiamethoxam. ***Nevertheless statistically significant differences were observed for clothianidin and thiamethoxam, but not for imidacloprid. The results confirm that genetic differences in the response to pesticide toxic action exist in the Honeybee, but they do not constitute the key factor involved in the uneven results observed in toxicity tests. In any case, the LD50 or other similar toxicity indexes should not be determined on a single colony.*** *" Laurino, Manino, et al. (2013).*

Easton, A. H. and D. Goulson (2013), The Neonicotinoid Insecticide Imidacloprid Repels Pollinating Flies and Beetles at Field-Realistic Concentrations, Plos One, 8, 1, pp. <Go to ISI>://WOS:000315211500032 AND http://www.ask-force.org/web/Bees/Easton-Neonicotinoid-Insecticide-Repels-Pollinating-2013.pdf

"The Neonicotinoid Insecticide Imidacloprid Repels Pollinating Flies and Beetles at Field-Realistic Concentrations, Plos One, 8, 1, Neonicotinoids are widely used systemic insecticides which, when applied to flowering crops, are translocated to the nectar and pollen where they may impact upon pollinators. Given global concerns over pollinator declines, this potential impact has recently received much attention. Field exposure of pollinators to neonicotinoids depends on the concentrations present in flowering crops and the degree to which pollinators choose to feed upon them. Here we describe a simple experiment using paired yellow pan traps with or without insecticide to assess whether the commonly used neonicotinoid imidacloprid repels or attracts flying insects. Both Diptera and Coleoptera exhibited marked avoidance of traps containing imidacloprid at a field-realistic dose of 1 mu g L-1, with Diptera avoiding concentrations as low as 0.01 mu g L-1. This is to our knowledge the first evidence for any biological activity at such low concentrations, which are below the limits of laboratory detection using most commonly available techniques. Catch of spiders in pan traps was also slightly reduced by the highest concentrations of imidacloprid used (1 mu g L-1), but catch was increased by lower concentrations. ***It remains to be seen if the repellent effect on insects occurs when neonicotinoids are present in real flowers, but if so then this could have implications for exposure of pollinators to neonicotinoids and for crop pollination****." Easton and Goulson (2013)*

R. M. Wang, Z. H. Wang, H. Yang, Y. Z. Wang and A. P. Deng (2012)

Highly sensitive and specific detection of neonicotinoid insecticide imidacloprid in environmental and food samples by a polyclonal antibody-based enzyme-linked immunosorbent assay, Journal of the Science of Food and Agriculture, 92, 6, 1253-1260

*"BACKGROUND: Imidacloprid is one of the main neonicotinoid insecticides widely used in agriculture owing its broad spectrum of activity and low bioaccumulation. However, imidacloprid is toxic to Honeybees and other beneficial organisms, and its residues may occur in environmental and food samples, posing a potential hazard to consumers. In this study the imidacloprid derivative bearing a three-atom length spacer was synthesized and coupled to carrier proteins. Highly sensitive and specific polyclonal antibodies against imidacloprid were successfully produced and the polyclonal antibody-based enzyme-linked immunosorbent assay (pAb-ELISA) was developed. RESULTS: The ELISA standard curve was constructed within the concentration range 0.1-100 ng mL(-1). The IC50 value for nine standard curves was in the range 1.2-3.0 ng mL(-1) and the limit of detection was 0.03-0.16 ng mL(-1). The sensitivity of the assay was one order of magnitude higher than that in most published papers. There was almost no cross-reactivity of the antibody with four structurally related compounds (acetamiprid, nicotine, clothianidin and nitenpyram) and six other compounds, indicating that the assay displays not only high sensitivity but also high specificity. No detectable imidacloprid was found in 11 collected environmental and food samples by the assay. **For imidacloprid-spiked samples, acceptable recoveries of 73.4-94.4% and intra-assay coefficients of variation of 2.2-12.8% were obtained. The assay was also validated with high-performance liquid chromatography (HPLC) and a good correlation of ELISA with HPLC was achieved. CONCLUSION: The proposed ELISA provides a sensitive, specific, simple and cost-effective quantitative/screening method for detecting imidacloprid in environmental and food samples. (C) 2011 Society of Chemical Industry.**" Wang, Wang, et al. (2012)*

<Go to ISI>://WOS:000301645900013 AND http://www.ask-force.org/web/Bees/Wang-Highly-Sensitive-Detection-Neonicotinoid-2012.pdf

A. Tapparo, D. Marton, C. Giorio, A. Zanella, L. Solda, M. Marzaro, L. Vivan and V. Girolami (2012)

Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds, Environmental Science & Technology, 46, 5, 2592-2599

*"Since seed coating with neonicotinoid insecticides was introduced in the late 1990s, European beekeepers have reported severe colony losses in the period of corn sowing (spring). As a consequence, seed-coating neonicotinoid insecticides that are used worldwide on corn crops have been blamed for honeybee decline. In view of the currently increasing crop production, and also of corn as a renewable energy source, the correct use of these insecticides within sustainable agriculture is a cause of concern. In this paper, a probable but so far underestimated route of environmental exposure of honeybees to and intoxication with neonicotinoid insecticides, namely, the atmospheric emission of particulate matter containing the insecticide by drilling machines, has been quantitatively studied. Using optimized analytical procedures, quantitative measurements of both the emitted particulate and the consequent direct contamination of single bees approaching the drilling machine during the foraging activity have been determined. **Experimental results show that the environmental release of particles containing neonicotinoids can produce high exposure levels for bees, with lethal effects compatible with colony losses phenomena observed by beekeepers**." Tapparo, Marton, et al. (2012)*

<Go to ISI>://WOS:000301023700018 AND
http://www.ask-force.org/web/Bees/Tapparo-Assessment-Environmental-Exposure-Honeybees-2012.pdf

A recent review Gibbons, Morrissey, et al. (2014) with a focus on *vertebrates* concludes: "Despite the lack of research and the difficulty in assigning causation, indirect effects may be as—or even more—important than direct toxic effects on vertebrates, as modern systemic insecticides are more effective at killing the invertebrate prey of vertebrates than the vertebrates themselves. Given the data here, current risk assessment procedures for neonicotinoids and other systemic pesticides need to consider the associated risks from both direct and indirect effects to vertebrate wildlife."

Neonicotinoid and fipronil insecticides can exert their impact on vertebrates either directly, through their overt toxicity, or indirectly, for example, by reducing their food supply. Marked variation exists among taxa and different systemic insecticides in acute toxicity (as measured by LD50 and LC50), while a range of sub-lethal effects can occur at concentrations orders of magnitude below those causing lethality. Overall, at concentrations relevant to field exposure scenarios from seed treatments (birds) or water concentrations (fish), imidacloprid and clothianidin can be considered a risk to granivorous bird species, while fipronil may pose a similar risk to sensitive fish species. Except in the most extreme cases, however, concentrations of imidacloprid and clothianidin that fish and amphibians are exposed to appear to be substantially below thresholds to cause mortality, although sub-lethal effects have not been widely studied.
Despite the lack of research and the difficulty in assigning causation, indirect effects may be as—or even more—important than direct toxic effects on vertebrates, as modern systemic insecticides are more effective at killing the invertebrate prey of vertebrates than the vertebrates themselves. Given the data here, current risk assessment procedures for neonicotinoids and other systemic pesticides need to consider the associated risks from both direct and indirect effects to vertebrate wildlife.

Another pesticide, *Fipronil*, got under scrutiny, but is according to a thorough peer study of EFSA dismissed.

EFSA conclusion, (20130322), Conclusion on the peer review of the pesticide risk assessment for bees for the active substance fipronil, EFSA Journal 2013, 11, 5, pp. 3158, 10.2903/j.efsa.2013.3158 AND www.efsa.europa.eu/efsajournal AND http://www.ask-force.org/web/EFSA/EFSA-Conclusion-Pesticide-Fipronil-20130323.pdf

"The European Food Safety Authority (EFSA) was asked by the European Commission to perform a risk assessment for the active substance fipronil and provide conclusions as regards the risk to bees. In this context the conclusions of EFSA following the peer review of the risk assessment for bees for the active substance fipronil are reported. The context of the evaluation was that required by the European Commission in accordance with Article 21 of Regulation (EC) No 1107/2009 to review the approval of active substances in light of new scientific and technical knowledge and monitoring data. The conclusions were reached on the basis of the evaluation of the currently authorised uses of fipronil applied on a variety of crops in Europe. The reliable
endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the submitted studies and scientific publications including data available at EU and national level, are presented. Missing information identified as being required to allow for a complete risk assessment is listed. Concerns are identified." EFSA conclusion (20130322)

6.8. Fungicides might be the real culprit, not pesticides

Pettis, J. S., E. M. Lichtenberg, M. Andree, J. Stitzinger, R. Rose and D. vanEngelsdorp (2013), Crop Pollination Exposes Honeybees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen *Nosema ceranae*, PLoS ONE, 8, 7, pp. e70182, http://dx.doi.org/10.1371%2Fjournal.pone.0070182 AND http://www.ask-force.org/web/Bees/Pettis-Crop-Pollination-Exposes-Pesticides-2013.pdf

"Recent declines in Honeybee populations and increasing demand for insect-pollinated crops raise concerns about pollinator shortages. Pesticide exposure and pathogens may interact to have strong negative effects on managed Honeybee colonies. Such findings are of great concern given the large numbers and high levels of pesticides found in Honeybee colonies. Thus it is crucial to determine how field-relevant combinations and loads of pesticides affect bee health. We collected pollen from bee hives in seven major crops to determine 1) what types of pesticides bees are exposed to when rented for pollination of various crops and 2) how field-relevant pesticide blends affect bees' susceptibility to the gut parasite Nosema ceranae. Our samples represent pollen collected by foragers for use by the colony, and do not necessarily indicate foragers' roles as pollinators. In blueberry, cranberry, cucumber, pumpkin and watermelon bees collected pollen almost exclusively from weeds and wildflowers during our sampling. Thus more attention must be paid to how Honeybees are exposed to pesticides outside of the field in which they are placed. We detected 35 different pesticides in the sampled pollen, and found high fungicide loads. The insecticides esfenvalerate and phosmet were at a concentration higher than their median lethal dose in at least one pollen sample. While fungicides are typically seen as fairly safe for Honeybees, we found an increased probability of Nosema infection in bees that consumed pollen with a higher fungicide load. Our results highlight a need for research on sub-lethal effects of fungicides and other chemicals that bees placed in an agricultural setting are exposed to." Pettis, Lichtenberg, et al. (2013A)

Also Mullin, Frazier, et al. (2010), co-authored also by vanEngelsdorp, give fungicides a high level importance (mentioned in the text 61 times):

Mullin, C. A., M. Frazier, J. L. Frazier, S. Ashcraft, R. Simonds, D. vanEngelsdorp and J. S. Pettis (2010), High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honeybee Health, PLoS ONE, 5, 3, pp. Go to ISI>://WOS:000275809700007 AND http://www.ask-force.org/web/Bees/Mullin-High-Levels-Miticides-Agrochemicals-2010.pdf

"Background: Recent declines in Honeybees for crop pollination threaten fruit, nut, vegetable and seed production in the United States. A broad survey of pesticide residues was conducted on samples from migratory and other beekeepers across 23 states, one Canadian province and several agricultural cropping systems during the 2007–08 growing seasons.
Methodology/Principal Findings: We have used LC/MS-MS and GC/MS to analyze bees and hive matrices for pesticide residues utilizing a modified QuEChERS method. We have found 121 different pesticides and metabolites within 887 wax, pollen, bee and associated hive samples. Almost 60% of the 259 wax and 350 pollen samples contained at least one systemic pesticide, and over 47% had both in-hive acaricides fluvalinate and coumaphos, and chlorothalonil, a widely-used fungicide. In bee pollen were found chlorothalonil at levels up to 99 ppm and the insecticides aldicarb, carbaryl, chlorpyrifos and imidacloprid, fungicides boscalid, captan and myclobutanil, and herbicide pendimethalin at 1 ppm levels. Almost all comb and foundation wax samples (98%) were contaminated with up to 204 and 94 ppm, respectively, of fluvalinate and coumaphos, and lower amounts of amitraz degradates and chlorothalonil, with an average of 6 pesticide detections per sample and a high of 39. There were fewer pesticides found in adults and brood except for those linked with bee kills by permethrin (20 ppm) and fipronil (3.1 ppm).

Conclusions/Significance: The 98 pesticides and metabolites detected in mixtures up to 214 ppm in bee pollen alone represents a remarkably high level for toxicants in the brood and adult food of this primary pollinator. This represents over half of the maximum individual pesticide incidences ever reported for apiaries. While exposure to many of these neurotoxicants elicits acute and sublethal reductions in Honeybee fitness, the effects of these materials in combinations and their direct association with CCD or declining bee health remains to be determined. Mullin, Frazier, et al. (2010)

Mullins et al. cite older literature mentioning fungicides as important factors:
It seems that also fungicides, besides pesticides in general, where already early in the focus of Bee colony decline: Finley, Camazine, et al. (1996) and Biesmeijer, Roberts, et al. (2006)

Finley, J., S. Camazine and M. Frazier (1996), The epidemic of Honeybee colony losses during the 1995-1996 season, American Bee Journal, 136, 11, pp. 805-808, <Go to ISI>://WOS:A1996VR28100022 AND Year 1996 not in NEBIS, not in Google Scholar

"Thousands of Honeybee colonies died in a region-wide epidemic in the northeastern United States during the winter and spring of 1995-96. In an effort to assess the tremendous colony losses, Pennsylvania beekeepers were asked to provide information on their colony losses and treatments they applied, In all, 252 Pennsylvania beekeepers provided information on 6,054 colonies, or about 22% of the colonies in our state, The average colony mortality was 53%, Colony losses were even higher (71.6%) among beekeepers who did not treat colonies for mites or disease. This is similar to the 85% mortality that we saw in feral colonies in central Pennsylvania, ***Apistan, Terramycin extender patties, and Fumidil-B all significantly decreased colony mortality****, Tracheal mite treatments, including menthol and grease (vegetable shortening) patties, did not reduce colony losses. Many beekeepers applied tracheal mite treatments at the wrong time of year, which most likely lead to ineffective tracheal mite control. overall, we conclude that aggressive treatment for Honeybee mites and other diseases significantly increases colony survival." Finley, Camazine, et al. (1996)*

And

Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. P. Schaffers, S. G. Potts, R. Kleukers, C. D. Thomas, J. Settele and W. E. Kunin (2006), Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands, Science, 313, 5785, pp. 351-354, <Go to ISI>://WOS:000239154300044 AND
http://www.ask-force.org/web/Bees/Biesmeijer-Parallel-Declines-Pollinators-2006.pdf

"Despite widespread concern about declines in pollination services, little is known about the patterns of change in most pollinator assemblages. By studying bee and hoverfly assemblages in Britain and the Netherlands, we found evidence of declines (pre- versus post-1980) in local bee diversity in both countries; however, divergent trends were observed in hoverflies. Depending on the assemblage and location, pollinator declines were most frequent in habitat and flower specialists, in univoltine species, and/or in nonmigrants. ***In conjunction with this evidence, outcrossing plant species that are reliant on the declining pollinators have themselves declined relative to other plant species. Taken together, these findings strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species"****. Biesmeijer, Roberts, et al. (2006)*

More on fungicides:

Lefebvre, B. and D. Bassand (2001), Bee selectivity of MAVRIK((R)) (tau-fluvalinate) in tank mix with ERIA((R)) (Difenoconazole, Ergosterol Biosynthesis Inhibitor-EBI). Short, medium and long term effects under semi-field conditions, Hazards of Pesticides to Bees, 98, pp. 71-77, <Go to ISI>://WOS:000170386300003 NO full text available

"Several publications reported that under laboratory conditions, EBI fungicides have been found to synergize the toxicity of pyrethroids to the honeybee. Two semi-field studies (tent and tunnel) were conducted in order to study this phenomenon between tau-fluvalinate (MAVRIK (R)) and difenoconazole (ERIA (R)). In the tent study, no effects were observed on mortality or foraging activity with the tank mix or tau-fluvalinate alone. In the tunnel study, mortality was negligibly higher in the tank mix (+l% compared to the control); foraging activity was markedly disturbed just after treatment but was completely restored 3 hours later. ***No medium (before Winter) or long term effects (after Winter) were observed (mortality, development of the hive: brood, youngs, food reserves). Bees, at no time, demonstrated any signs of disorientation, enhanced aggressiveness or excitability. As a conclusion, no synergistic effects were observed.****Lefebvre and Bassand (2001)*

Standardized methods are now ready to be installed in fungicides.

A clear opinion to blame fungicides comes from US entomologist Richard Levine Levine Richard (20130725), who comments on the latest Pettis paper Pettis, Lichtenberg, et al. (2013A), commenting specifically the senior author of the Pettis paper:

Levine Richard (20130725), Research Shows Fungicide Affects Honeybee Health, Entomology Today, publ: Entomological Society of America, http://entomologytoday.org/2013/07/25/research-shows-fungicide-affects-Honeybee-health/ AND http://www.ask-force.org/web/Bees/Levine-Richard-Bees-Fungicides-20130725.pdf

"While many studies have been conducted on how insecticides used on crops may affect Honeybee health, a new study by researchers at the University of Maryland and the U.S. Department of Agriculture suggests that Honeybees may be negatively affected by something completely different — fungicides.
The researchers gathered pollen from Honeybee hives and analyzed it to see what agricultural chemicals it contained, including fungicides, insecticides, herbicides and miticides. They then fed the pollen to Honeybees and tested their ability to resist infection from Nosema ceranae, a pathogen of adult Honeybees that has been linked to a lethal phenomenon known as Colony Collapse Disorder (CCD).
In the study's most surprising result, bees that were fed pollen containing a fungicide called chlorothonatil were nearly three times more likely to be infected by Nosema than bees that were not exposed to it.
"We don't think of fungicides as having a negative effect on bees, because they're not designed to kill insects," said Dennis vanEngelsdorp, one of the authors. "But the study's finding that common fungicides can be harmful at real world dosages is new, and points to a gap in existing regulations."
In another unexpected finding, most of the crops that the bees were pollinating appeared to provide their hives with little nourishment. When the researchers collected pollen from bees foraging on native North American crops such as blueberries and watermelon, they found the pollen came from other flowering plants in the area, not from the crops. This is probably because Honeybees, which evolved in the Old World, are not efficient at collecting pollen from New World crops, even though they can pollinate these crops."Levine Richard (20130725)

4.6. A novel way of intoxication of Honeybees:
Translocation through guttation drops
Experiments with imidacloprid from coated corn seedlings.

A recent paper claims detrimental impact of pesticides through guttation: Girolami, Mazzon, et al. (2009): Translocation of Neonicotinoid Insecticides From Coated Seeds to Seedling Guttation Drops. The concentration of neonicotinoids in guttation drops can be near those of active ingredients commonly applied in Þeld sprays for pest control, or even higher. When bees
consume guttation drops, collected from plants grown from neonicotinoid-coated seeds, they encounter death within few minutes. According to the research teams own conclusion, there needs still some additional research to be done on the dose-response dependency.

Girolami, V., L. Mazzon, et al. (2009). "Translocation of Neonicotinoid Insecticides From Coated Seeds to Seedling Guttation Drops: A Novel Way of Intoxication for Bees." Journal of Economic Entomology **102**(5): 1808-1815.
<Go to ISI>://WOS:000270605700011 AND http://www.ask-force.org/web/Bees/Girolami-Translocation-Neonicotinoid-Insecticides-2009.pdf

"The death of honeybees, Apis mellifiera L., and the consequent colony collapse disorder causes major losses in agriculture and plant pollination worldwide. The phenomenon showed increasing rates in the past years, although its causes are still awaiting a clear answer. Although neonicotinoid systemic insecticides used for seed coating of agricultural crops were suspected as possible reason, studies so far have not shown the existence of unquestionable sources capable of delivering directly intoxicating doses in the fields. Guttation is a natural plant phenomenon causing the excretion of xylem fluid at leaf margins. Here, we show that leaf guttation drops of all the corn plants germinated from neonicotinoid-coated seeds contained amounts of insecticide constantly higher than 10 mg/l, with maxima up to 100 mg/l for thiamethoxam and clothianidin, and up to 200 mg/l for imidacloprid. ***The concentration of neonicotinoids in guttation drops can be near those of active ingredients commonly applied in field sprays for pest control, or even higher. When bees consume guttation drops, collected from plants grown from neonicotinoid-coated seeds, they encounter death within few minutes****."* *Gonzalez (2010).*

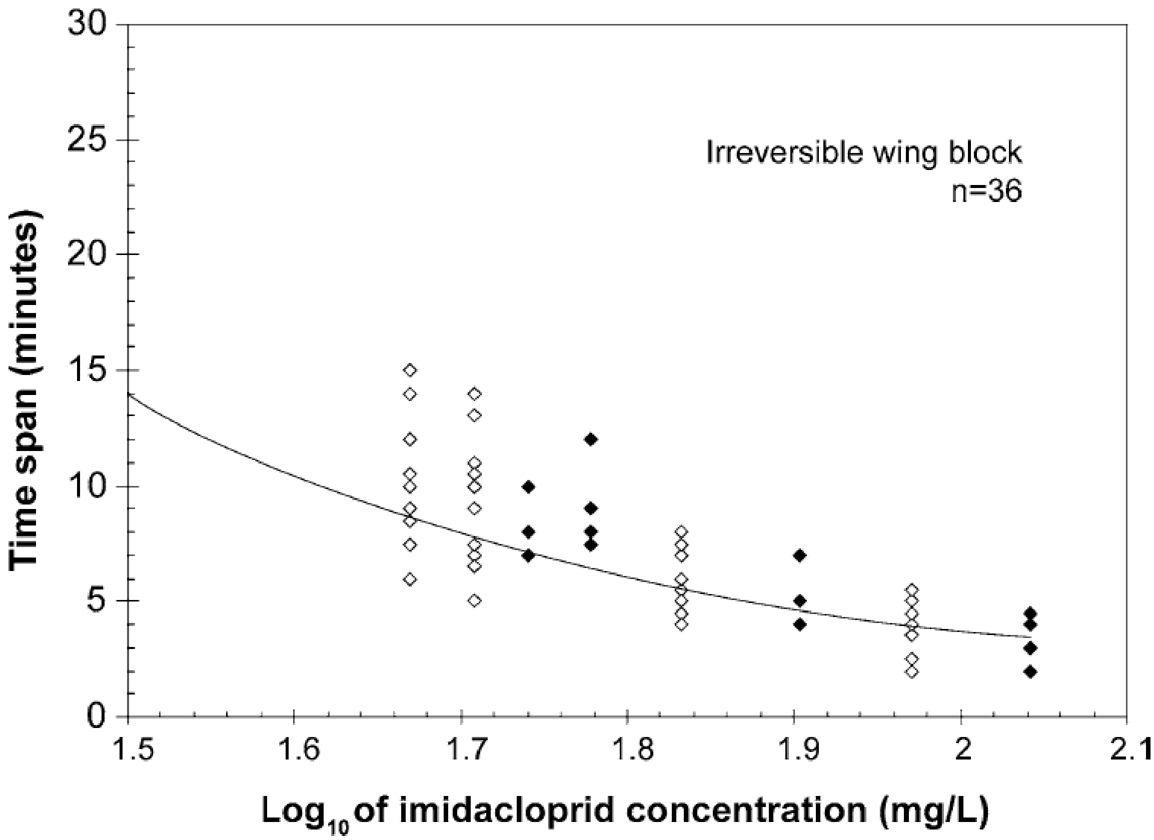

Fig. 24 Time interval between appearance irreversible wing-block of single caged bees and ingestion of guttation drops collected from leaf of potted (1-20-d-old) corn seedlings from imidacloprid-coated seeds. Concentration was determined by HPLC analysis. The curve corresponds to that shown in Fig. 3A for pure imidacloprid at the higher doses. Black symbols, pure guttation; white symbols, guttation with 15% honey. Concentration data (milligrams per liter) are transformed in log_{10}. From Girolami, Mazzon, et al. (2009)

"Bees showed a different response to the three neonicotinoids. For clothianidin and thiamethoxam, at the lowest concentrations of 1.5 mg/liter (log10 _ 0.18), the chosen symptoms (abdomen bending and wing paralysis) manifested before 1 h. For imidacloprid, the same could be observed at concentrations _6.25 mg/ liter (log10 _ 0.8), indicating a lower toxicity toward bees (Fig. 3). Increasing the dosage, the interval between abdomen bending and wing block decreased progressively, becoming nearly null at 100 mg/liter (log10 _ 2) for all neonicotinoids tested (Fig. 3). When using doses lower than the doses reported (Fig. 3), either the symptoms did not occur or they did sometimes in reversible manner and in a time exceeding 1 h. Those bees, when fed, would normally survive for at least 24 h. It must be noticed that, as it makes use of a single event of uptake, the test is less severe than those in use to evaluate the median lethal concentration (LC50), for which poisoning solutions are kept available for longer time. Results are in agreement with Yang, Chuang, et al. (2008) who reported that the imidacloprid concentration _3 mg/liter in a sugar solution is the threshold preventing bees to return to foraging. This value is close to the one (6 mg/liter) at which we observe a wing paralysis on all insects tested in 1 h. Within each given neonicotinoid concentration, no clear relationship between the actual intake volume and time of appearance of the symptoms was noticed, presumably due to individual response variability and to the frequent regurgitation events that can bias the dose-response dependency."Girolami, Mazzon, et al. (2009)

6.9. Summary on Pesticides, Fungicides and Herbicides

Overall comments: the pesticide question should not be dramatized for several reasons:

1. It depends all on the local situation and the application habits, concentrations reaching the bees vary strongly – as well as the impact of pesticide sprays.
2. The pesticide question should be seen in context: Not all pesticides show sub-lethal impact and there might be other reasons causing detrimental effects.
3. Mitigation is possible, keeping in mind the diversity and impact of pesticides in a given region.
4. From the last paper of Pettis it is allowed to conclude, that Bt maize is the preferred crop related to bee populations with much less perforations caused by pest herbivores and thus showing a clearly lower infection rate with parasitic fungi producing toxic mycotoxins Desjardins and Proctor (2007, Logrieco, Bottalico, et al. (2004, Munkvold (2004, Papst, Utz, et al. (2005, Pietri, Bertuzzi, et al. (2004, Wu (2007) and Bruce Chassy in email.
5. Fungicides might be more important having detrimental effects on bee health.

After having screened a lot of literature, the author comes to positive overall conclusions related to bees and neonicotinoids *under realistic conditions*
This is also the conclusion of a DEFRA report from the UK also gives more or less green light for the neonicotinoids, if used properly: DEFRA (20130327):

DEFRA. (20130327). An assessment of key evidence about Neonicotinoids and bees. *Ref: PB13937*, pp. 9. https://www.gov.uk/government/publications/an-assessment-of-key-evidence-about-neonicotinoids-and-bees AND http://www.ask-force.org/web/Bees/DEFRA-Assessment-key-evidence-Neonicotinoids-bees-201303.pdf AND ANNEX http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/defraBumbleBeeReportPS2371V4a.pdf AND http://fera.co.uk/

"Conclusion: While this assessment cannot exclude rare effects of neonicotinoids on bees in the field, it suggests that effects on bees do not occur under normal circumstances. This assessment also suggests that laboratory based studies demonstrating sub-lethal effects on bees from neonicotinoids did not replicate realistic conditions, but extreme scenarios. Consequently, it supports the view that the risk to bee populations from neonicotinoids, as they are currently used, is low. From DEFRA (20130327)

The two reports from the Royal Society of the United Kingdom: Godfray, Blacquière, et al. (2014, Godfray, Blacquière, et al. (2015), an attempt to come to a final assessment based on an extensive literature review, it's conclusions are generally positive. There are certainly questions on long term effects which merit continuing research.

Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Petrokofsky, G., Potts, S. G., Raine, N. E., Vanbergen, A. J., & McLean, A. R. (2014). A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society of London B: Biological Sciences, 281*(1786), pp. http://rspb.royalsocietypublishing.org/royprsb/281/1786/20140558.full.pdf AND

10.1098/rspb.2014.0558 AND http://rspb.royalsocietypublishing.org/content/281/1786/20140558 AND http://www.ask-force.org/web/Bees/Godfray-Restatement-recent-advances-evidence-neonicotinoids-pollinators-20140507.pdf

"There is evidence that in Europe and North America many species of pollinators are in decline, both in abundance and distribution. Although there is a long list of potential causes of this decline, there is concern that neonicotinoid insecticides, in particular through their use as seed treatments are, at least in part, responsible. This paper describes a project that set out to summarize the natural science evidence base relevant to neonicotinoid insecticides and insect pollinators in as policy-neutral terms as possible. A series of evidence statements are listed and categorized according to the nature of the underlying information. The evidence summary forms the appendix to this paper and an annotated bibliography is provided in the electronic supplementary material."4

And conclusions of first volume 2014:

"Neonicotinoids are efficient plant protection compounds and if their use is restricted farmers may switch to other pest-management strategies (for example, different insecticides applied in different ways or non-chemical control measures) that may have effects on pollinator populations that could overall be more or less damaging than neonicotinoids. Alternatively, they may choose not to grow the crops concerned, which will reduce exposure of pollinators to neonicotinoids but also reduce the total flowers available to pollinators.

(46) Summary. To understand the consequences of changing neonicotinoid use, it is important to consider pollinator colony-level and population processes, the likely effect on pollination ecosystem services, as well as how farmers might change their agronomic practices in response to restrictions on neonicotinoid use. While all these areas are currently being researched there is at present a limited evidence base to guide policy-makers." From Godfray, Blacquière, et al. (2014)

Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Potts, S. G., Raine, N. E., Vanbergen, A. J., & McLean, A. R. (2015). A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society of London B: Biological Sciences, 282*(1818), pp. http://rspb.royalsocietypublishing.org/royprsb/282/1818/20151821.full.pdf AND http://www.ask-force.org/web/Bees/Godfray-Restatement-recent-advances-evidence-neonicotinoids-pollinators-20150924.pdf

"A summary is provided of recent advances in the natural science evidence base concerning the effects of neonicotinoid insecticides on insect pollinators in a format (a 'restatement') intended to be accessible to informed but not expert policymakers and stakeholders. Important new studies have been published since our recent review of this field (Godfray et al. 2014 Proc. R. Soc. B 281, 20140558. (doi:10.1098/rspb.2014.0558)) ***And the subject continues to be an area of very active research and high policy relevance****."*

And:

"3. Results
the update to the restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators is given in appendix A, with an annotated bibliography provided as the electronic supplementary material.
4. Discussion
The new evidence and evidence syntheses that have been published in the last 18 months (between February 2014 and August 2015) significantly advance our understanding of the effects of neonicotinoids on insect pollinators. Nevertheless, major gaps in our understanding remain, and different policy conclusions can be drawn depending on the weight one accords to important (but not definitive) science findings and the weightings given to the economic and other interests of different stakeholders. The natural science evidence base places constraints on policies that claim to be consistent with the science, but does not specify a single course of action.
We also raise an issue here that arises from our original study but is not directly relevant to the evidence base on the effects of neonicotinoids on pollinators. In introducing the subject we wrote 'Neonicotinoid insecticides are a highly effective tool to reduce crop yield losses due to insect pests', and in the re-statement itself listed a small number of papers in the scientific literature to support this statement Godfray, Blacquière, et al. (2014). It has been pointed out that some of these papers were funded by industry and that there are other studies that have recorded no benefits of neonicotinoid use (e.g. Douglas, Rohr, et al. (2015)).
The efficacy of neonicotinoids is clearly an important issue, and we believe few would doubt that in some circumstances (combinations of crops, pests and locales) they are highly effective and in other circumstances they do not justify the costs of their purchase. We did not attempt to review this subject and should have been more careful to say we were not commenting on efficacy per se.
Though a meta-analysis of efficacy would be very informative it would also be very difficult. Efficacy studies are largely conducted by industry, the sector that benefits most from the data, and are not the type of science usually funded by public organizations. Typically, the studies are not published in the peer-reviewed literature (though they are often made available to regulators) and some are kept confidential for commercial reasons. Efficacy trials are expensive and it seems unlikely that they will ever be publicly funded at scale. It is an interesting topic for debate whether industry would benefit in the long run from placing more of its data in the public domain as well as putting in place measures to increase public confidence in studies they fund themselves. The recent movement in the pharmaceutical sector to set up trial registries (see https://clinicaltrials. gov/ct2/home and https://www.clinicaltrialsregister.eu) provides a model for how the latter might be achieved."

Bibliography on Pesticides, Fungicides and Herbicides and their impact on Honeybees:
Ammann, K. (20161126) Pesticides, Imidacloprin, Fungicides, Herbicides and Bees: Bibliography 1640 references Web of Science and other sources Ammann, K. Neuchâtel 162 pp
http://www.ask-force.org/web/Bees/Ammann-Bibliography-Bees-Pesticides-Fungicides-Herbicides-20161126.pdf

7. Negative influence of landscape structure and biodiversity through modern agriculture?

Two publications 2008 and 2009 sum up the concerns, whether we will have an acute shortage of pollinators with the vanishing bee populations. According to Aizen, Garibaldi, et al. (2008, Aizen and Harder (2009) there is no acute shortage of pollinators, and the predictions that we will in agriculture run out of pollinators and that this would have catastrophic impact, are not justifiable. Temporal trends were similar between pollinator-dependent and nondependent crops in both the developed and developing world, in evidence not supporting the view that pollinator shortages are affecting crop yield at the global scale. However, agriculture has become more pollinator dependent because of a disproportionate increase in the area cultivated with pollinator-dependent crops. If the trend toward favoring cultivation of pollinator-dependent crops continues, the need for the service provided by declining pollinators will greatly increase in the near future. There is a future need to support bee pollination with specific measures related to pollinator-dependent crops and also native species in the future.

Aizen, M. A., L. A. Garibaldi, et al. (2008). "Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency." Current Biology **18**(20): 1572-1575.
http://www.sciencedirect.com/science/article/B6VRT-4TSMC9G-T/2/36b1fa2ed5bd5030846e0c092b3d6744 AND http://www.ask-force.org/web/Bees/Aizen-Long-Term-Pollinator-Dependency-2008.pdf

*"Summary: There is evidence that pollinators are declining as a result of local and global environmental degradation [1], [2], [3] and [4]. Because a sizable proportion of the human diet depends directly or indirectly on animal pollination [5], the issue of how decreases in pollinator stocks could affect global crop production is of paramount importance [6], [7] and [8]. Using the extensive FAO data set [9], we compared 45 year series (1961-2006) in yield, and total production and cultivated area of pollinator-dependent and nondependent crops [5]. We investigated temporal trends separately for the developed and developing world because differences in agricultural intensification, and socioeconomic and environmental conditions might affect yield and pollinators [10], [11], [12] and [13]. Since 1961, crop yield (Mt/ha) has increased consistently at average annual growth rates of ~1.5%. **Temporal trends were similar between pollinator-dependent and nondependent crops in both the developed and developing world, thus not supporting the view that pollinator shortages are affecting crop yield at the global scale. We further report, however, that agriculture has become more pollinator dependent because of a disproportionate increase in the area cultivated with pollinator-dependent crops. If the trend toward favoring cultivation of pollinator-dependent crops continues, the need for the service provided by declining pollinators will greatly increase in the near future."** Aizen, Garibaldi, et al. (2008).*

Aizen, M. A. and L. D. Harder (2009). "The Global Stock of Domesticated Honeybees Is Growing Slower Than Agricultural Demand for Pollination." Current Biology **19**(11): 915-918.
http://www.sciencedirect.com/science/article/B6VRT-4W7JNHK-3/2/4d4a22402ec95321ff2d39de1dde9902 AND http://www.ask-force.org/web/Bees/Aizen-Global-Stock-Domesticated-2009.pdf
*"Summary The prospect that a global pollination crisis currently threatens agricultural productivity has drawn intense recent interest among scientists, politicians, and the general public [1], [2], [3], [4] and [5]. To date, evidence for a global crisis has been drawn from regional or local declines in pollinators themselves [6], [7], [8] and [9] or insufficient pollination for particular crops [9] and [10]. In contrast, our analysis of Food and Agriculture Organization (FAO) [11] data reveals that the global population of managed Honeybee hives has increased ~45% during the last half century and suggests that economic globalization, rather than biological factors, drives both the dynamics of the global managed Honeybee population and increasing demands for agricultural pollination services [12]. Nevertheless, available data also reveal a much more rapid (>300%) increase in the fraction of agriculture that depends on animal pollination during the last half century, which may be stressing global pollination capacity. **Although the primary cause of the accelerating increase of the pollinator dependence of commercial agriculture seems to be economic and political and not biological, the rapid expansion of cultivation of many pollinator-dependent crops has the potential to trigger future pollination problems for both these crops and native species in neighboring areas. Such environmental costs merit consideration during the development of agriculture and conservation policies."** Aizen and Harder (2009)*

Nevertheless, there are other opinions published:

Pettis published a review of coordinated responses to the decline: Pettis and Delaplane (2010)

Pettis, J. S. and K. S. Delaplane (2010), Coordinated responses to Honeybee decline in the USA, Apidologie, 41, 3, pp. 256-263, <Go to ISI>://WOS:000279029200004 AND http://www.ask-force.org/web/Bees/Pettis-Coordinated-Responses-Decline-2010.pdf

"Summary: In response to successive years of high Honeybee mortality, the United States Congress mandated the US Department of Agriculture (USDA) to increase funding for research and education directed at reducing Honeybee decline. The funding follows two administrative streams within USDA - one through the USDA Agricultural Research Service (ARS) and another through the USDA National Institute for Food and Agriculture (NIFA). ARS is funding an Areawide Project operated by the four ARS Honeybee labs, and NIFA is funding through a competitive grant process a Coordinated Agricultural Project (CAP) operated by scientists and educators heavily represented by state colleges of agriculture. Each project - Areawide and CAP - is characterized as a consortium of investigators working in a coordinated manner to reduce institutional redundancy and optimize the discovery and delivery of sustainable bee management practices to client beekeepers."Pettis and Delaplane (2010)

The graph is based on US-statistics with one small gap:

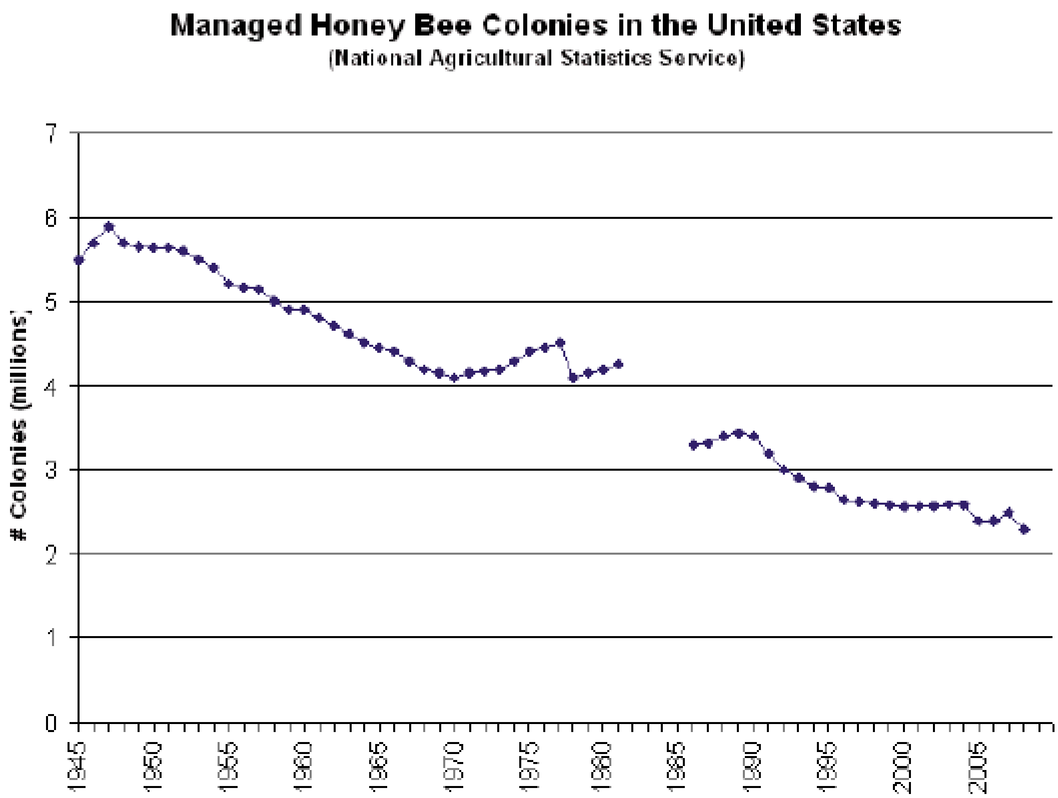

Fig. 25 The number of managed Honeybee colonies (in millions) in the United States from 1945 to 2008 as reported by the USDA National Agricultural Statistics Service. The current level of approximately 2.5 million Colonies is very low given that the US needs 1.5 million colonies in California each year to pollinate almonds. Three years of on average 30% colony losses in the US (2006–2008) threaten our ability to provide such pollination services to agriculture. No data on colony numbers were recorded for 1982– 1985. From Pettis and Delaplane (2010)

The comment of Pettis et al.:

"This reduction in managed colonies, coupled with increased colony mortality, has resulted in increased pollination fees for almonds and other crops; for example, in almonds the fee per colony has risen from $75 to $150 and in blueberries a similar doubling of pollination fees has occurred. The almond industry alone needs 1.5 million colonies annually for pollination, more than half the nation's colony reserves." Pettis and Delaplane (2010)

The paper of Kennedy et al. Kennedy, Lonsdorf, et al. (2013) provides a different, more positive message. Foremost it should be clear that wild bees also play an important role in crop pollination. The wild bee populations are also subject to decline through a constant habitat loss:

"Bees provide essential pollination services that are potentially affected both by local farm management and the surrounding landscape. To better understand these different factors, we modelled the relative effects of landscape composition (nesting and floral resources within foraging distances), landscape configuration (patch shape, interpatch connectivity and habitat aggregation) and farm management (organic vs. conventional and local-scale field diversity), and their interactions, on wild bee abundance and richness for 39 crop systems globally. Bee abundance and richness were higher in diversified and organic fields and in landscapes comprising more high-quality habitats; bee richness on conventional fields with low diversity benefited most from high-quality surrounding land cover. Landscape configuration effects were weak. Bee responses varied slightly by biome. ***Our synthesis reveals that pollinator persistence will depend on both the maintenance of high-quality habitats around farms and on local management practices that may offset impacts of intensive monoculture agriculture****."Kennedy, Lonsdorf, et al. (2013)*

*"Conclusions: Our global synthesis expands the growing body of empirical research addressing how changes in landscape structure through habitat loss, fragmentation or degradation affect pollinators and potentially pollination services. We found that the most important factors enhancing wild bee communities in agro-ecosystems were the amounts of high-quality habitats surrounding farms in combination with organic management and local-scale field diversity****. Our findings suggest that as fields become increasingly simplified (large monocultures), the amount and diversity of habitats for wild bees in the surrounding landscape become even more important. On the other hand, if farms are locally diversified then the reliance on the surrounding landscape to maintain pollinators may be less pronounced. Moreover, farms that reside within highly intensified and simplified agricultural landscapes will receive substantial benefits from on-farm diversification and organic management.*** *Safe-guarding pollinators and their services within an agricultural matrix will therefore be achieved through improved on-farm management practices coupled with the maintenance of landscape-level high-quality habitats around farms." From Kennedy, Lonsdorf, et al. (2013)*

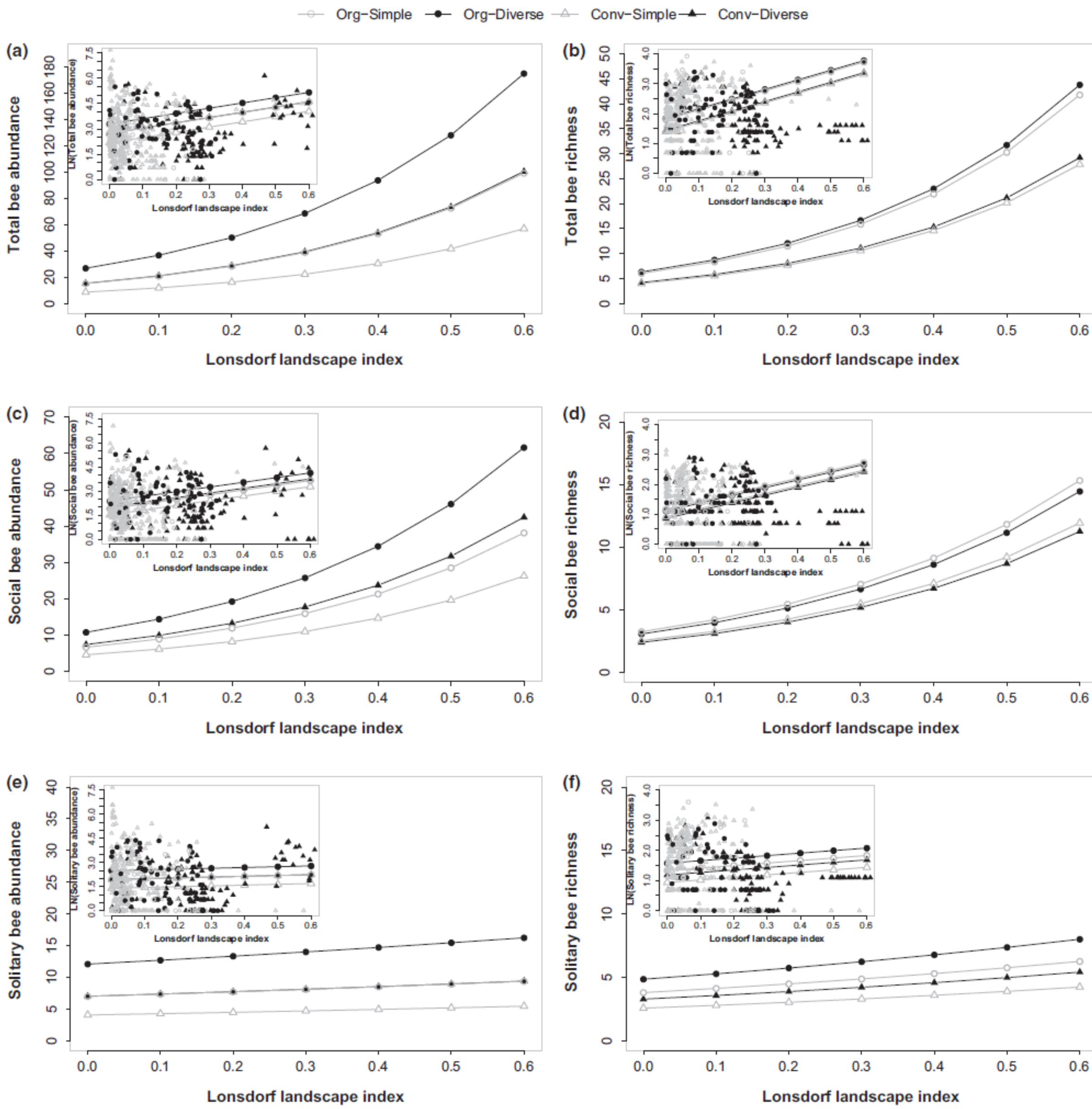

Fig. 26 Response to Lonsdorf landscape index of wild bee abundance (a) and richness (b), social bee abundance (c) and richness (d), and solitary bee abundance (e) and richness (f) in relation to field type (conventional vs. organic) and field diversity (locally simple vs. diverse). Estimates are based on model-averaged partial regression coefficients for all studies (n = 39) for important main effects [E (abundance, richness) = ƒ (LLI + FT + FD)] (Table 2). Predicted relationship based on backtransformed estimates on normal scale in the main graph (with 95% CIs in Figure S7_1) and modelled log-linear relationship with sites in the inset (based on mean values per site, varying intercepts by site and study not shown). y-axis scales vary by bee responses; predicted relationships between LLI = 0–0.60 graphed (although maximum LLI = 1.0) because 0.61 was maximum score derived for empirical landscapes. From Kennedy, Lonsdorf, et al. (2013).

The same picture in the study of Kovács-Hostyánszki, Haenke, et al. (2013), provided that wild bees are offered ample nesting opportunities.

Field margins with higher biodiversity including weeds seem to play a positive role:

Rands, S. A. and H. M. Whitney (2010), *Effects of pollinator density-dependent preferences on field margin visitations in the midst of agricultural monocultures: A modelling approach, Ecological Modelling, 221, 9, pp. 1310-1316, <Go to ISI>://WOS:000276933200008 AND* ***http://www.ask-force.org/web/Bees/Rands-Effects-Pollinator-Density-dependent-Field-Margin-2010.pdf***

"Managed field margins offer a means of reducing the impact of agricultural monocultures within intensively managed environments. By providing refuge for wild plants and the pollinators associated with them, field margins can also contribute to enhancing the pollination services within the monoculture. However, the effects of the monoculture on pollinator behaviour need to be carefully considered. It is known that pollinators may show density-dependent preferences such as neophobia (an avoidance of unfamiliar items) when different types of flower are available within their environment, and the dominance of monoculture crops within the environment may consequently have adverse effects upon the preferences shown by pollinators living in the field margins within them. In order to examine how pollinator preferences for wild flowers are affected by monocultures, we modelled the effects of density-dependent preferences, flower densities, and the geometry of field margins within a monoculture landscape using numerical simulations. This was done by considering how the placement of pollinator nests within a simple, spatially explicit landscape consisting of fields of monoculture crops separated by margins containing wild flowers affected the ratio of wild and monoculture crops experienced by the pollinator, given that it could only forage within a limited distance from its nest. Increasing field margin width and decreasing monoculture field width both led to an increase in pollinators visiting wild flowers (which levelled off as width increased). The size of the monoculture fields had little additional effect once they had passed an intermediate width. Increasing wild flower density within the margins led to a shift away from preferring monocultures. When wild flowers were at low densities compared to the monoculture, even the addition of small amounts of extra wild flowers had a large effect in shifting foraging preferences away from the monoculture. The distance which pollinators normally forage over only has an effect upon preferences for wild flowers when the travel distance is small. This suggests that careful consideration of margin design might be extremely important for those species which do not travel far. Innate preferences for density-dependence and particular crop types may also have an effect on preference behaviour. We demonstrate that the way in which resources are presented to indigenous pollinators may be extremely important in influencing where they choose to forage within agricultural landscapes. Careful margin design, as well as increasing the density of wild flowers (such as by enhancing the wild seed bank within the margins), may lead to overall improvements in ecosystem function within intensively farmed monocultures" Rands and Whitney (2010)

Fig from Rand et al. No. 27 next page.

Another paper from Clint et al. claims that Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains Clint R. V. Otto, Roth Cali L., et al. (2016).

Clint R. V. Otto, Roth Cali L., Carlson Benjamin L. and Smart Matthew D. (2016) Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains Proceedings of the National Academy of Sciences 113 37 10430-10435 pp http://www.pnas.org/content/113/37/10430.abstract AND http://www.ask-force.org/web/Bees/Otto-Land-use-change-reduces-suitability2016.pdf

"Human reliance on insect pollination services continues to increase even as pollinator populations exhibit global declines. Increased commodity crop prices and federal subsidies for biofuel crops, such as corn and soybeans, have contributed to rapid land-use change in the US Northern Great Plains (NGP), changes that may jeopardize habitat for honey bees in a part of the country that supports >40% of the US colony stock. We investigated changes in biofuel crop production and grassland land covers surrounding ∼18,000 registered commercial apiaries in North and South Dakota from 2006 to 2014. We then developed habitat selection models to identify remotely sensed land-cover and land-use features that influence apiary site selectionbyDakota beekeepers.Our study demonstrates a continual increase in biofuel crops, totaling 1.2 Mha, around registered apiary locationsinNorth and South Dakota.Such crops were avoided by commercial beekeepers when selecting apiary sites in this region. Furthermore, our analysis reveals how grasslands that beekeepers target when selecting commercial apiary locations are becomingless commonin eastern North and SouthDakota, changes that may have lastingimpact onpollinatorconservationefforts.Our study highlights how land-use change in the NGP is altering the landscape in ways that are seemingly less conducive to beekeeping. Our models can be used to guide future conservation efforts highlighted in the US national pollinator health strategy by identifying areas that support high densities of commercial apiaries and that have exhibited significant land-use changes." From Clint R. V. Otto, Roth Cali L., et al. (2016)

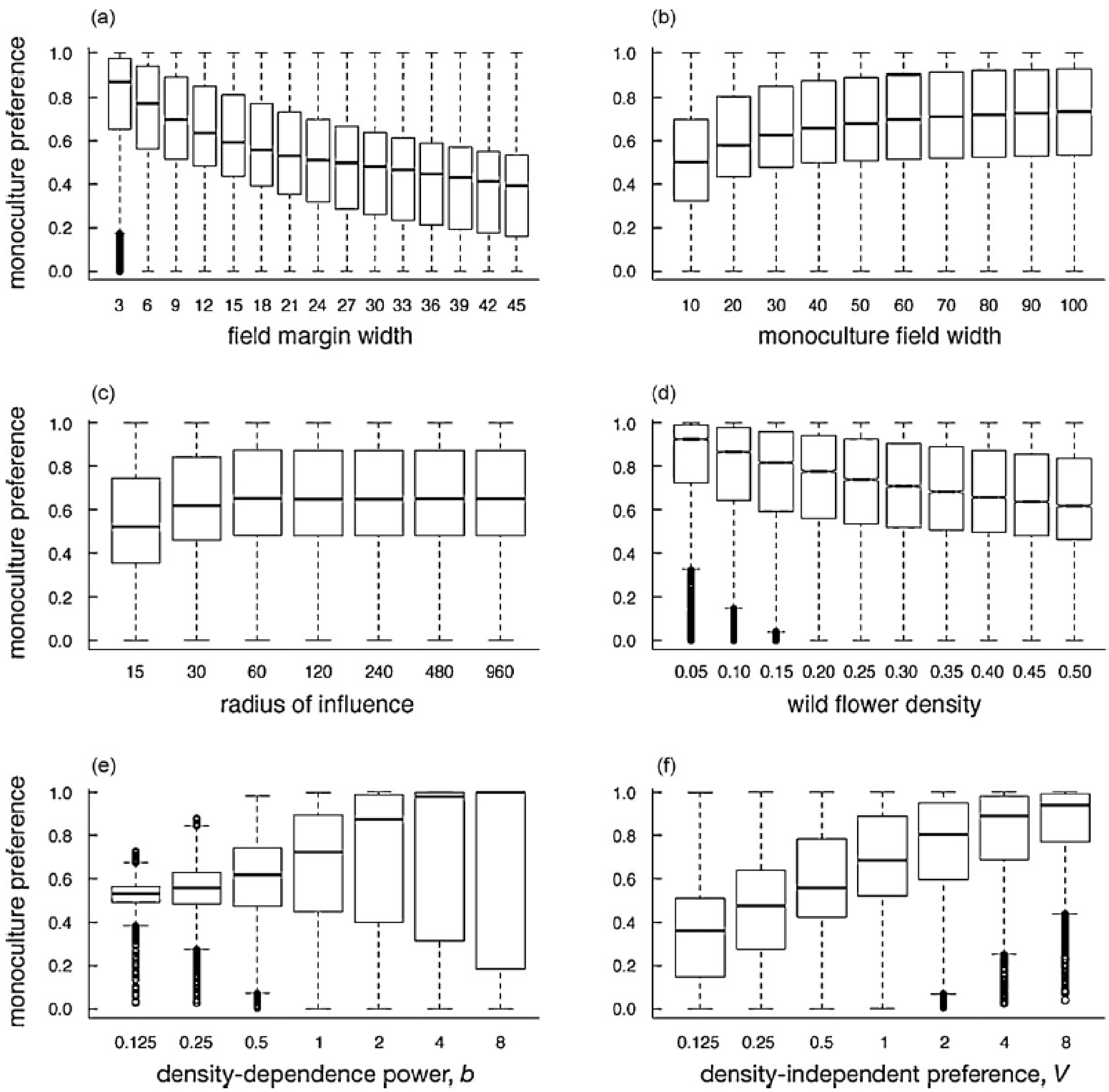

Fig. 27 Boxplots showing the effects on preference for the monoculture crop when systematically changing: (a) the width of the field margin; (b) the width of the monoculture field; (c) the radius over which pollinator choice is influenced; (d) the density of wild flowers in a unit area of the field margin relative to the density of the monoculture crop in a unit area of field; (e) b, the parameter controlling the steepness of the density-dependence function; and (f) V, the parameter controlling the density-independent preference for the monoculture crop. Note that all the figures are drawn with notches, but these are only visible in panel (d). Outliers are represented with the solid symbols. From Rands and Whitney (2010)

In another paper Girard, Chagnon, et al. (2012) it has been found that the pollen diversity collected by the bees is of importance to the colony development:

Girard, M., M. Chagnon and V. Fournier (2012), Pollen diversity collected by honey bees in the vicinity of Vaccinium spp. crops and its importance for colony development, Botany-Botanique, 90, 7, pp. 545-555, <Go to ISI>://WOS:000305951300004 AND http://www.ask-force.org/web/Bees/Girard-Pollen-Diversity-Honey-Bees-2012.pdf

"The results of this study suggest that colonies may suffer from a nutritional deficiency during their stay in large-scale blueberry crops in which weeds are intensively managed. This can affect honey bee brood colony development quite rapidly, although the effects are only observable a

few weeks later by beekeepers in the field. Our findings reflect results from a recent study by O in which the authors demonstrate that weeds are a critical source for honey bees in agrarian environments."

By the side: It should be clear that agricultural intensity and biodiversity are negatively correlated per se, independent of the production strategy.

Kleijn, D., F. Kohler, A. Baldi, P. Batary, E. D. Concepcion, Y. Clough, M. Diaz, D. Gabriel, A. Holzschuh, E. Knop, A. Kovacs, E. J. P. Marshall, T. Tscharntke and J. Verhulst (2009), On the relationship between farmland biodiversity and land-use intensity in Europe, Proceedings of the Royal Society B-Biological Sciences, 276, 1658, pp. 903-909, <Go to ISI>://WOS:000262867100014 AND http://www.ask-force.org/web/Biotech-Biodiv/Kleijn-Relationship-Biodiversity-Intensity-2009.pdf

But there are studies from England which demonstrate that the reverse is also possible: Under certain conditions conventional farming can be correlated to higher biodiversity compared to organic farming:

Gabriel, D., S. M. Sait, J. A. Hodgson, U. Schmutz, W. E. Kunin and T. G. Benton (2010), Scale matters: the impact of organic farming on biodiversity at different spatial scales, Ecology Letters, 13, 7, pp. 858-869, http://dx.doi.org/10.1111/j.1461-0248.2010.01481.x AND http://www.ask-force.org/web/Biotech-Biodiv/Gabriel-Scale-Matters-Organic.2010.pdf AND Times: http://www.ask-force.org/web/Biotech-Biodiv/Webster-Study-Spikes-organic-Times-201005.PDF

"There is increasing recognition that ecosystems and their services need to be managed in the face of environmental change. However, there is little consensus as to the optimum scale for management. This is particularly acute in the agricultural environment given the level of public investment in agri-environment schemes (AES). Using a novel multiscale hierarchical sampling design, we assess the effect of land use at multiple spatial scales (from location-within-field to regions) on farmland biodiversity. We show that on-farm biodiversity components depend on farming practices (organic vs. conventional) at farm and landscape scales, but this strongly interacts with fine- and coarse-scale variables. Different taxa respond to agricultural practice at different spatial scales and often at multiple spatial scales. Hence, AES need to target multiple spatial scales to maximize effectiveness. Novel policy levers may be needed to encourage multiple land managers within a landscape to adopt schemes that create landscape-level benefits." Gabriel, Sait, et al. (2010)

However, the multifactor complexity of the topic of Ag-Biodiversity is logically very high, therefore controversial studies are the logic consequence, the latest one clearly favors higher biodiversity correlated to organic farming:

Tuck, S. L., C. Winqvist, F. Mota, J. Ahnström, L. A. Turnbull and J. Bengtsson (2014), Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis, Journal of Applied Ecology, pp. n/a-n/a, http://dx.doi.org/10.1111/1365-2664.12219 AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-Land-use-Intensity-Effects-Organic-Farming-2014.pdf AND http:www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0001-AppendixS1-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0002-AppendixS2-20140230.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0003-TableS1-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0004-TableS2-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0005-TableS3-20140203.csv AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0005-TableS3-20140203.xla

"1. The benefits of organic farming to biodiversity in agricultural landscapes continue to be hotly debated, emphasizing the importance of precisely quantifying the effect of organic vs. conventional farming.
2. We conducted an updated hierarchical meta-analysis of studies that compared biodiversity under organic and conventional farming methods, measured as species richness. We calculated effect sizes for 184 observations garnered from 94 studies, and for each study, we obtained three standardized measures reflecting land-use intensity. We investigated the stability of effect sizes through time, publication bias due to the 'file drawer' problem, and consider whether the current literature is representative of global organic farming patterns.
3. On average, organic farming increased species richness by about 30%. This result has been robust over the last 30 years of published studies and shows no sign of diminishing.
4. Organic farming had a greater effect on biodiversity as the percentage of the landscape consisting of arable fields increased, that is, it is higher in intensively farmed regions. The average effect size and the response to agricultural intensification depend on taxonomic group, functional group and crop type.

5. There is some evidence for publication bias in the literature; however, our results are robust to its impact. Current studies are heavily biased towards northern and western Europe and North America, while other regions with large areas of organic farming remain poorly investigated.

6. Synthesis and applications. Our analysis affirms that organic farming has large positive effects on biodiversity compared with conventional farming, but that the effect size varies with the organism group and crop studied, and is greater in landscapes with higher land-use intensity. Decisions about where to site organic farms to maximize biodiversity will, however, depend on the costs as well as the potential benefits. Current studies have been heavily biased towards agricultural systems in the developed world. We recommend that future studies pay greater attention to other regions, in particular, areas with tropical, subtropical and Mediterranean climates, in which very few studies have been conducted." Tuck, Winqvist, et al. (2014)

Comment: this is not a holistic approach to a realistic comparison, since it does not deal with the factor yield, and there organic farming still has to make progress. The only really fair comparison should include yield and then the differences related to biodiversity would also even out or diminish. For a recent comparative discussion with focus on yield see Seufert, Ramankutty, et al. (2012). It demonstrates that related to yield under unfavorable conditions conventional farming clearly outcompetes organic farming heavily, under best environmental conditions the difference shrinks to 5%.

An interesting paper dealing with the British conditions in bee decline comes from Baude, Kunin, et al. (2016): Their historical nectar assessment reveals the fall and rise of floral resources in Britain

Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K., Morton, R. D., Smart, S. M., & Memmott, J. (2016). Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature, 530*(7588), pp. 85-88. http://dx.doi.org/10.1038/nature16532 AND http://www.ask-force.org/web/Bees/Baude-Historical-nectar-assessment-Britain-2016.pdf

Summary with active full-text-citations: "There is considerable concern over declines in insect pollinator communities and potential impacts on the pollination of crops and wildflowers. Among the multiple pressures facing pollinators–decreasing floral resources due to habitat loss and degradation has been suggested as a key contributing factor Potts, Biesmeijer, et al. (2010, Potts, Roberts, et al. (2010) Vanbergen, Baude, et al. (2013) Goulson (2015) Carvell, Roy, et al. (2006) Roulston and Goodell (2011) –8. However, a lack of quantitative data has hampered testing for historical changes in floral resources. Here we show that overall floral rewards can be estimated at a national scale by combining vegetation surveys and direct nectar measurements. We find evidence for substantial losses in nectar resources in England and Wales between the 1930s and 1970s; however, total nectar provision in Great Britain as a whole had stabilized by 1978, and increased from 1998 to 2007. These findings concur with trends in pollinator diversity, which declined in the mid-twentieth century 9 but stabilized more recently Carvalheiro, Kunin, et al. (2013). The diversity of nectar sources declined from 1978 to 1990 and thereafter in some habitats, with four plant species accounting for over 50% of national nectar provision in 2007. Calcareous grassland, broadleaved woodland and neutral grassland are the habitats that produce the greatest amount of nectar per unit area from the most diverse sources, whereas arable land is the poorest with respect to amount of nectar per unit area and diversity of nectar sources. Although agro-environment schemes add resources to arable landscapes, their national contribution is low. Owing to their large area, improved grasslands could add substantially to national nectar provision if they were managed to increase floral resource provision. This national-scale assessment of floral resource provision affords new insights into the links between plant and pollinator declines, and offers considerable opportunities for conservation." From Baude, Kunin, et al. (2016).

8. Another new possible cause of the Colony Collapse Disorder: Entombed Pollen?

In a recent publication a new hypothesis has been erected: Is it possible, that a newly discovered condition is the cause of the CCD: Entombed Pollen seems to contain a transmittable, up to now unknown factor as the cause for the disease:

Van Engelsdorp, D., Evans, J.D., Donovall, L., Mullin, C., Frazier, M., Frazier, J., Tarpy, D.R., Hayes, J., & Pettis, J.S. (2009)
"Entombed Pollen": A new condition in Honeybee colonies associated with increased risk of colony mortality. Journal of Invertebrate Pathology, 101, 2, pp 147-149
<Go to ISI>://WOS:000267382800012 AND http://www.ask-force.org/web/Bees/VanEngelsdorp-Entombed-Pollen-2009.pdf

"Here we describe a new phenomenon, entombed pollen, which is highly associated with increased colony mortality. Entombed pollen is sunken, capped cells amidst "normal", uncapped cells of stored pollen, and some of the pollen contained within these cells is brick red in color. There appears to be a lack of microbial agents in the pollen, and larvae and adult bees do not have an increased rate of mortality when they are fed diets supplemented with entombed pollen in vitro, suggesting that the pollen itself is not directly responsible for increased colony mortality. However, the increased incidence of entombed pollen in reused wax comb Suggests that there is a transmittable factor common to the phenomenon and colony mortality. In addition, there were elevated pesticide levels, notably of the fungicide chlorothalonil, in entombed pollen. Additional studies are needed to determine if there is a causal relationship between entombed pollen, chemical residues, and colony mortality." VanEngelsdorp, Evans, et al. (2009A)

9. Energetic stress: a possible cause of the Colony Collapse Disorder?

On another hypothesis it is infection with Nosema ceranae being responsible via energetic stress for the disease:

Mayack and Naug (2009) Energetic stress in the honeybee Apis mellifera from Nosema ceranae infection. Journal of Invertebrate Pathology, 100, 3, pp
185-188 <Go to ISI>://WOS:000264850500007 AND
http://www.ask-force.org/web/Bees/Maycack-Energetic-Stress-Nosema-2009.pdf

"Parasites are dependent on their hosts for energy to reproduce and can exert a significant nutritional stress on them. Energetic demand placed oil the host is especially high in cases where the parasite-host complex is less co-evolved. The higher virulence of the newly discovered honeybee pathogen, Nosema ceranae, which causes a higher mortality in its new host Apis mellifera, might be based on a similar the mechanism. Using Proboscis Extension Response and feeding experiments, we show that bees infected with N. ceranae have a higher hunger level that leads to a lower survival Significantly, we also demonstrate that the survival of infected bees fed ad libitum is not different from that Of uninfected bees. These results demonstrate that energetic stress is the probable cause of the shortened life span observed in infected bees. We argue that energetic stress can lead to the precocious and risky foraging observed in Nosema infected bees and discuss its relevance to colony collapse syndrome. the significance of energetic stress as a general mechanism by which infectious diseases influence host behavior and physiology is discussed." Mayack and Naug (2009)

10. A virus (IAPV, Israeli Acute Paralysis Virus) might be the cause of CCD

Cox-Foster, D., S. Conlan, E. C. Holmes, G. Palacios, A. Kalkstein, J. D. Evans, N. A. Moran, P. L. Quan, D. Geiser, T. Briese, M. Hornig, J. Hui, D. Vanengelsdorp, J. S. Pettis and W. I. Lipkin (2008). "The latest buzz about colony collapse disorder - Response." Science 319: 725-725. AND http://www.ask-force.org/web/Bees/Anderson-Cox-Foster-Controversy-2008.pdf
<Go to ISI>://WOS:000252963000016

"THE REPORT "A METAGENOMIC SURVEY OF microbes in Honeybee colony collapse disorder" (D. L. Cox-Foster et al., 12 October 2007, p. 283) identified Israeli acute paralysis virus (IAPV) as a putative marker for colony collapse disorder (CCD). It also purports to show a relationship between U.S. colony declines as early as 2004 and importations of Australian honeybees. We believe these links are tenuous for several reasons: (i) Importations of Australian honeybees to the United States did not commence until 2005. (ii) No evidence is presented for a causal link between IAPV and CCD. Koch's postulates, as modified for including IAPV, do not respect national boundaries. IAPV is not confined to the United States or Australia. It has also been found in bees in Israel and royal jelly from Manchuria. We anticipate that with the new focus on IAPV and the distribution of diagnostic reagents, we will learn that it is even more widely distributed. Nonetheless, IAPV lineages have now been found in U.S. bees; one of them correlates genetically with IAPV found in bees in Australian shipments. The presence of IAPV strains in older U.S. samples does not eliminate a role for this virus in CCD."
Cox-Foster, Conlan, et al. (2008, Cox-Foster, Conlan, et al. (2007A, Cox-Foster, Conlan, et al. (2007B)

Maori, E., N. Paldi, S. Shafir, H. Kalev, E. Tsur, E. Glick and I. Sela (2009). "IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion." Insect Molecular Biology **18**(1): 55-60.
<Go to ISI>://WOS:000262516400006 AND http://www.ask-force.org/web/Bees/Maori-IAPV-Silenced-2009.pdf

"Colony Collapse Disorder (CCD) has been associated with Israeli acute paralysis virus (IAPV). CCD poses a serious threat to apiculture and agriculture as a whole, due to the consequent inability to provide the necessary amount of bees for pollination of critical crops. Here we report on RNAi-silencing of IAPV infection by feeding bees with double-stranded RNA, as an efficient and feasible way of controlling this viral disease. The association of CCD with IAPV is discussed, as well as the potential of controlling CCD." Maori, Lavi, et al. (2007, Maori, Paldi, et al. (2009)

Palacios, G., J. Hui, P. L. Quan, A. Kalkstein, K. S. Honkavuori, A. V. Bussetti, S. Conlan, J. Evans, Y. P. Chen, D. vanEngelsdorp, H. Efrat, J. Pettis, D. Cox-Foster, E. C. Holmes, T. Briese and W. I. Lipkin (2008). "Genetic analysis of Israel acute paralysis virus: Distinct clusters are circulating in the United States." Journal of Virology 82(13): 6209-6217. <Go to ISI>://WOS:000256947300012 AND http://www.ask-force.org/web/Bees/Palacios-Genetic-Analysis-2008.pdf

"Israel acute paralysis virus (IAPV) is associated with colony collapse disorder of Honeybees. Nonetheless, its role in the pathogenesis of the disorder and its geographic distribution are unclear. Here, we report phylogenetic analysis of IAPV obtained from bees in the United States, Canada, Australia, and Israel and the establishment of diagnostic real-time PCR assays for IAPV detection. Our data indicate the existence of at least three distinct IAPV lineages, two of them circulating in the United States. Analysis of representatives from each proposed lineage suggested the possibility of recombination events and revealed differences in coding sequences that may have implications for virulence." Palacios, Hui, et al. (2008)

Ribiere, M., V. Olivier, P. Blanchard, F. Schurr, O. Celle, P. Drajnudel, J. P. Faucon, R. Thiery and M. P. Chauzat (2008). "The collapse of bee colonies: the CCD case ("Colony collapse disorder") and the IAPV virus (Israeli acute paralysis virus)." Virologie **12**(5): 319-322.
<Go to ISI>://WOS:000262852300001 AND http://www.ask-force.org/web/Bees/Ribiere-CCD-Virologie-2008.pdf

Conclusion
En conclusion, en l'absence de données quant au lien de causalité entre la présence de ces virus et les pertes aux États-Unis comme en France, des recherches restent nécessaires afin d'évaluer leur implication dans les phénomènes d'affaiblissements et de mortalités de colonies d'abeilles. Cependant, il faut garder à l'esprit que d'autres facteurs peuvent être impliqués dans ces dépérissements de colonies. Ainsi, on peut lister les différents pathogènes qui agissent seuls ou en concomitance, la compétition interspécifique entre les différentes espèces d'abeilles particulièrement sur le continent américain, l'usage de races d'abeilles nouvellement introduites dans des régions données, le morcellement des habitats qui est la conséquence du développement des grandes cultures ou de l'introduction des espèces végétales envahissantes, et l'usage des pesticides. La clé de la compréhension des phénomènes d'affaiblissements des colonies d'abeilles passe par l'approche intégrative de ces différents facteurs. Ribiere, Olivier, et al. (2008)

Teixeira, E. W., Y. P. Chen, D. Message, J. Pettis and J. D. Evans (2008). "Virus infections in Brazilian Honeybees." Journal of Invertebrate Pathology **99**(1): 117-119.
<Go to ISI>://WOS:000259131400018 AND http://www.ask-force.org/web/Bees/Weinstein-Teixeira-Virus-Brazil-2008.pdf

"This work describes the first molecular-genetic evidence for viruses in Brazilian Honeybee samples. Three different bee viruses, Acute bee paralysis virus (ABPV), Black queen cell Virus (BQCV), and Deformed wing virus (DWV) were identified during a screening of RNAs from 1920 individual adult bees collected in a region of southeastern Brazil that has recently shown unusual bee declines. ABPV was detected in 27.1% of colony samples, while BQCV and DWV were found in 37% and 20.3%, respectively. These levels are substantially lower than the frequencies found for these viruses in Surveys from other parts of the world. We also developed and validated a Multiplex RT-PCR assay for the simultaneous detection of ABPV, BQCV, and DWV in Brazil." Teixeira, Chen, et al. (2008)

Another Iridovirus got into the focus of CCD researchers, Bromenshenk, Henderson, et al. (2010):

"In 2010 Colony Collapse Disorder (CCD), again devastated Honeybee colonies in the USA, indicating that the problem is neither diminishing nor has it been resolved. Many CCD investigations, using sensitive genome-based methods, have found small RNA bee viruses and the microsporidia, Nosema apis in healthy and collapsing colonies alike with no single pathogen firmly linked to Honeybee losses.We used Mass spectrometry-based proteomics (MSP) to identify and quantify thousands of proteins from healthy and collapsing bee colonies. MSP revealed two unreported RNA viruses in North American Honeybees, Varroa destructor-1 virus and Kakugo virus, and identified an invertebrate iridescent virus (IIV) Iridoviridae associated with CCD colonies. Prevalence of IIV significantly discriminated among strong, failing, and collapsed colonies. In addition, bees in failing colonies contained not only IIV, but also Nosema. Co-occurrence of these microbes consistently marked CCD in (1) bees from commercial apiaries sampled across the U.S. in 2006 € 2007, (2) bees sequentially sampled as the disorder progressed in an observation hive colony in 2008, and (3) bees from a recurrence of CCD in Florida in 2009. The pathogen pairing was not observed in samples from colonies with no history of CCD, namely bees from Australia and a large, non-migratory beekeeping business in Montana. Laboratory cage trials with a strain of IIV type 6 and Nosema ceranae confirmed that co-infection with these two pathogens was more lethal to bees than either pathogen alone. Nosema with Honeybee colony decline, giving credence to older research pointing to IIV, interacting with Nosema and mites, as probable cause of bee losses in the USA, Europe, and Asia. We next need to characterize the IIV and Nosema that we detected and develop management practices to reduce Honeybee losses." Bromenshenk, Henderson, et al. (2010)

But there seem to lack evidence for such a connection Tokarz, Firth, et al. (2011)

"Colony collapse disorder (CCD) is characterized by the unexplained losses of large numbers of adult worker bees (Apis mellifera) from apparently healthy colonies. Although infections, toxins, and other stressors have been associated with the onset of CCD, the pathogenesis of this disorder remains obscure. Recently, a proteomics study implicated a double-stranded DNA virus, invertebrate iridescent virus (Family Iridoviridae) along with a microsporidium (Nosema sp.) as the cause of CCD.
We tested the validity of this relationship using two independent methods: (i) we surveyed healthy and CCD colonies from the United States and Israel for the presence of members of the Iridovirus genus and (ii) we reanalyzed metagenomics data previously generated from RNA pools of CCD colonies for the presence of Iridovirus-like sequences. Neither analysis revealed any evidence to suggest the presence of an Iridovirus in healthy or CCD colonie". Tokarz, Firth, et al. (2011)

Nevertheless, the discussion on viral infection causes seems to be more complex than anticipated as demonstrated by Runckel, Flenniken, et al. (2011)

*"Honeybees (Apis mellifera) play a critical role in global food production as pollinators of numerous crops. Recently, Honeybee populations in the United States, Canada, and Europe have suffered an unexplained increase in annual losses due to a phenomenon known as Colony Collapse Disorder (CCD). Epidemiological analysis of CCD is confounded by a relative dearth of bee pathogen field studies. To identify what constitutes an abnormal pathophysiological condition in a Honeybee colony, it is critical to have characterized the spectrum of exogenous infectious agents in healthy hives over time. We conducted a prospective study of a large scale migratory bee keeping operation using high-frequency sampling paired with comprehensive molecular detection methods, including a custom microarray, qPCR, and ultra deep sequencing. We established seasonal incidence and abundance of known viruses, Nosema sp., Crithidia mellificae, and bacteria. Ultra deep sequence analysis further identified four novel RNA viruses, two of which were the most abundant observed components of the Honeybee microbiome (,1011 viruses per Honeybee**). Our results demonstrate episodic viral incidence and distinct pathogen patterns between summer and winter time-points. Peak infection of common Honeybee viruses and Nosema occurred in the summer, whereas levels of the trypanosomatid Crithidia mellificae and Lake Sinai virus 2, a novel virus, peaked in January**." Runckel, Flenniken, et al. (2011).*

11. Varroa mite infection, a major threat to honeybees

Varroa mite on a honey bee larva

Varroa mite on Honey Bee

An overview from Wikipedia: Varroa destructor

Varroa destructor and *Varroa jacobsoni* are parasitic mites that feed on the bodily fluids of adult, pupal and larval bees. *Varroa* mites can be seen with the naked eye as a small red or brown spot on the bee's thorax. *Varroa* mites are carriers for a virus that is particularly damaging to the bees. Bees infected with this virus during their development will often have visibly deformed wings.

Honey Bee with deformed wings

Varroa mites have led to the virtual elimination of feral bee colonies in many areas, and are a major problem for kept bees in apiaries. Some feral populations are now recovering—it appears they have been naturally selected for *Varroa* resistance.

Varroa mites were first discovered in Southeast Asia in about 1904, but are now present on all continents except Australia. They were discovered in the United States in 1987, in New Zealand in 2000, and in Devon, United Kingdom in 1992.

These mites are generally not a problem for a strongly growing hive. When the hive population growth is reduced in preparation for winter or due to poor late summer forage, the mite population growth can overtake that of the bees and can then destroy the hive. Often a colony will simply abscond (leave as in a swarm, but leaving no population behind) under such conditions.

Varroa in combination with deformed wing virus and bacteria have been related to one of the major causes in colony collapse disorder.

Standard methods in the study of the Varroa mite are given by Dietemann, Nazzi, et al. (2013):

Dietemann, V., F. Nazzi, S. J. Martin, D. L. Anderson, B. Locke, K. S. Delaplane, Q. Wauquiez, C. Tannahill, E. Frey, B. Ziegelmann, P. Rosenkranz and J. D. Ellis (2013), Standard methods for varroa research, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300009 AND http://www.ask-force.org/web/Bees/Dietemann-Standard-Varroa-20130901.pdf

"Very rapidly after Varroa destructor invaded apiaries of Apis mellifera, the devastating effect of this mite prompted an active research effort to understand and control this parasite. Over a few decades, varroa has spread to most countries exploiting A. mellifera. As a consequence, a large number of teams have worked with this organism, developing a diversity of research methods. Often different approaches have been followed to achieve the same goal. The diversity of methods made the results difficult to compare, thus hindering our understanding of this parasite. In this paper, we provide easy to use protocols for the collection, identification, diagnosis, rearing, breeding, marking and measurement of infestation rates and fertility of V. destructor. We also describe experimental protocols to study orientation and feeding of the mite, to infest colonies or cells and measure the mite's susceptibility to acaricides. Where relevant, we describe which mite should be used for bioassays since their behaviour is influenced by their physiological state. We also give a method to determine the damage threshold above which varroa damages colonies. This tool is fundamental to be able to implement integrated control concepts. We have described pros and cons for all methods for the user to know which method to use under which circumstances. These methods could be embraced as standards by the community when designing and performing research on V. destructor." Dietemann, Nazzi, et al. (2013)

An excellent review on the varroa mite problems is given by

Rosenkranz, P., P. Aumeier and B. Ziegelmann (2010), Biology and control of Varroa destructor, Journal of Invertebrate Pathology, 103, pp. S96-S119, <Go to ISI>://WOS:000273993100011 AND http://www.ask-force.org/web/Bees/Rosenkranz-Biology-Control-Varroa-2010.pdf

"The ectoparasitic honey bee mite Varroa destructor was originally confined to the Eastern honey bee Apis cerana. After a shift to the new host Apis mellifera during the first half of the last century, the parasite dispersed world wide and is currently considered the major threat for apiculture. The damage caused by Varroosis is thought to be a crucial driver for the periodical colony losses in Europe and the USA and regular Varroa treatments are essential in these countries. Therefore, Varroa research not only deals with a fascinating host–parasite relationship but also has a responsibility to find sustainable solutions for the beekeeping.
This review provides a survey of the current knowledge in the main fields of Varroa research including the biology of the mite, damage to the host, host tolerance, tolerance breeding and Varroa treatment. We first present a general view on the functional morphology and on the biology of the Varroa mite with special emphasis on host–parasite interactions during reproduction of the female mite. The pathology section describes host damage at the individual and colony level including the problem of transmission of secondary infections by the mite. Knowledge of both the biology and the pathology of Varroa mites is essential for understanding possible tolerance mechanisms in the honey bee host. We comment on the few examples of natural tolerance in A. mellifera and evaluate recent approaches to the selection of Varroa tolerant honey bees. Finally, an extensive listing and critical evaluation of chemical and biological methods of Varroa treatments is given.
This compilation of present-day knowledge on Varroa honey bee interactions emphasizes that we are still far from a solution for Varroa infestation and that, therefore, further research on mite biology, tolerance breeding, and Varroa treatment is urgently needed" Rosenkranz, Aumeier, et al. (2010)

Schoening, Gisder, et al. (2012) demonstrate evidence for damage-dependent hygienic behaviour towards Varroa destructor-parasitized brood:

"The ectoparasitic mite Varroa destructor and honey bee pathogenic viruses have been implicated in the recent demise of honey bee colonies. Several studies have shown that the combination of V. destructor and deformed wing virus (DWV) poses an especially serious threat to honey bee health. Mites transmitting virulent forms of DWV may cause fatal DWV infections in the developing bee, while pupae parasitized by mites not inducing or activating overt DWV infections may develop normally. Adult bees respond to brood diseases by removing affected brood. This hygienic behaviour is an essential part of the bees' immune response repertoire and is also shown towards mite-parasitized brood. However, it is still unclear whether the bees react towards the mite in the brood cell or rather towards the damage done to the brood. We hypothesized that the extent of mite-associated damage rather than the mere presence of parasitizing mites triggers hygienic behaviour. Hygienic behaviour assays performed with mites differing in their potential to transmit overt DWV infections revealed that brood parasitized by 'virulent' mites (i.e. mites with a high potential to induce fatal DWV infections in parasitized pupae) were removed significantly more often than brood parasitised by 'less virulent' mites (i.e. mites with a very low potential to induce overt DWV infections) or non-parasitized brood.
Chemical analyses of brood odor profiles suggested that the bees recognize severely affected brood by olfactory cues. Our results suggest that bees show selective, damage-dependent hygienic behaviour, which may be an economic way for colonies to cope with mite infestation." Schoening, Gisder, et al. (2012)

The same phenomenon is independently described also by Locke, Le Conte, et al. (2012) with obvious geographical variations:

Locke, B., Y. Le Conte, D. Crauser and I. Fries (2012), Host adaptations reduce the reproductive success of Varroa destructor in two distinct European honey bee populations, Ecology and Evolution, 2, 6, pp. 1144-1150, <Go to ISI>://WOS:000312447900005 AND
http://www.ask-force.org/web/Bees/Locke-Host-Adaptations-reduce-reproductive-Success-Varroa-2012.pdf

"Honey bee societies (Apis mellifera), the ectoparasitic mite Varroa destructor, and honey bee viruses that are vectored by the mite, form a complex system of host–parasite interactions. Coevolution by natural selection in this system has been hindered for European honey bee hosts since apicultural practices remove the mite and consequently the selective pressures required for such a process. An increasing mite population means increasing transmission opportunities for viruses that can quickly develop into severe infections, killing a bee colony. Remarkably, a few subpopulations in Europe have survived mite infestation for extended periods of over 10 years without management by beekeepers and offer the possibility to study their natural host–parasite coevolution. Our study shows that two of these "natural" honey bee populations, in Avignon, France and Gotland, Sweden, have in fact evolved resistant traits that reduce the fitness of the mite (measured as the reproductive success), thereby reducing the parasitic load within the colony to evade the development of overt viral infections. Mite reproductive success was reduced by about 30% in both populations. ***Detailed examinations of mite reproductive parameters suggest these geographically and genetically distinct populations favor different mechanisms of resistance, even though they have experienced similar selection pressures of mite infestation. Compared to unrelated control colonies in the same location, mites in the Avignon population had high levels of infertility while in Gotland there was a higher proportions of mites that delayed initiation of egg-laying. Possible explanations for the observed rapid coevolution are discussed." Locke, Le Conte, et al. (2012)***

Neumann, Yanez, et al. (2012). propose new rules with decision alternatives to cope with the invasion dynamics of Varroa:

Neumann, P., O. Yanez, I. Fries and J. R. de Miranda (2012), Varroa invasion and virus adaptation, Trends in Parasitology, 28, 9, pp. 353-354, <Go to ISI>://WOS:000308777800001 AND http://www.ask-force.org/web/Bees/Neumann-Varroa-Invasion-Virus-Adaption-2012.pdf

''The progressively reduced tolerance of honey bee colonies to mite infestations following new Varroa invasion is primarily a consequence of. . .
(i) . . .the cumulative quantitative effects of DWV transmission involving V. destructor;
(ii) . . .increased virulence of the DWV quasi-species due to adaptations to a new transmission route (qualitative effects).''
These alternative hypotheses predict the following changes as the Varroa front passes through a region:
1. lower mite infestation rates at colony death (i or ii);
2. the proportion of DWV-replicating mites increases
(ii) or not (i);
3. the DWV titre of symptomatic bees decreases (ii) or not (i);
4. the DWV titre of asymptomatic bees increases (i) or not (ii);
5. the ahead-of-front DWV quasi-species is retained unchanged (i), changes genetically (ii) or is replaced by the incoming quasi-species (i or ii). Neumann, Yanez, et al. (2012)

Guzman-Novoa, Emsen, et al. (2012) explain how Genotypic variability influences relationships between mite infestation levels, mite damage, grooming intensity, and removal of Varroa destructor mites in selected strains of worker honey bees:

Guzman-Novoa, E., B. Emsen, P. Unger, L. G. Espinosa-Montano and T. Petukhova (2012), Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of Varroa destructor mites in selected strains of worker honey bees (Apis mellifera L.), Journal of Invertebrate Pathology, 110, 3, pp. 314-320, <Go to ISI>://WOS:000305362700007 AND http://www.ask-force.org/web/Bees/Guzman-Novoa-Genotypic-Variability-Varroa-2012.pdf

The objective of this study was to demonstrate genotypic variability and analyze the relationships between the infestation levels of the parasitic mite Varroa destructor in honey bee (Apis mellifera) colonies, the rate of damage of fallen mites, and the intensity with which bees of different genotypes groom themselves to remove mites from their bodies. Sets of paired genotypes that are presumably susceptible and resistant to the varroa mite were compared at the colony level for number of mites falling on sticky papers and for proportion of damaged mites. They were also compared at the individual level for intensity of grooming and mite removal success. Bees from the ''resistant'' colonies had lower mite population rates (up to 15 fold) and higher percentages of damaged mites (up to 9 fold) than bees from the ''susceptible'' genotypes. At the individual level, bees from the ''resistant'' genotypes performed significantly more instances of intense grooming (up to 4 fold), and a significantly higher number of mites were dislodged from the bees' bodies by intense grooming than by light grooming (up to 7 fold) in all genotypes. ***The odds of mite removal were high and significant for all ''resistant'' genotypes when compared with the ''susceptible'' genotypes. The results of this study strongly suggest that grooming behavior and the intensity with which bees perform it, is an important component in the resistance of some honey bee genotypes to the growth of varroa mite populations. The implications of these results are discussed****. Guzman-Novoa, Emsen, et al. (2012)*

11.1 Treatment of Varroa infections: (from Wikipedia)

A variety of treatments are currently marketed or practiced to attempt to control these mites. The treatments are generally segregated into chemical and mechanical controls.

Common chemical controls include "hard" chemicals such as such as Amitraz (marketed as Apivar), fluvalinate (marketed as Apistan), and coumaphos (marketed as CheckMite). "Soft" chemical chemical controls include thymol (marketed as ApiLife-VAR and Apiguard), sucrose octanoate esters (marketed as Sucrocide), oxalic acid and formic acid (sold in gel packs as Mite-Away, but also used in other formulations). According to the U.S. Environmental Protection Agency, when used in beehives as directed, these treatments kill a large proportion of the mites while not substantially disrupting bee behavior or life span. Use of chemical controls is generally regulated and varies from country to country. With few exceptions, they are not intended for use during production of marketable honey.

Common mechanical controls generally rely on disruption of some aspect of the mites' lifecycle. These controls are generally intended not to eliminate all mites, but merely to maintain the infestation at a level which the colony can tolerate. Examples of mechanical controls include drone brood sacrifice (varroa mites are preferentially attracted to the drone brood), powdered sugar dusting (which encourages cleaning behavior and dislodges some mites), screened bottom boards (so any dislodged mites fall through the bottom and away from the colony), brood interruption and, perhaps, downsizing of the brood cell size. A device called the varroa mite control entrance (VMCE) is under development as of 2008. The VMCE works in conjunction with a screened bottom board, by dislodging varroa mites from bees as they enter and exit a hive.

Mahmood, Wagchoure, et al. (2012) show details about control of ectoparasitic mites in honeybee (Apis mellifera L.) colonies by using Thymol and Oxalic Acid:

Mahmood, R., E. S. Wagchoure, S. Raja and G. Sarwar (2012), Control of Varroa destructor Using Oxalic Acid, Formic Acid and Bayvarol Strip in Apis mellifera (Hymenoptera: Apidae) Colonies, Pakistan Journal of Zoology, 44, 6, pp. 1473-1477, <Go to ISI>://WOS:000313462300002 AND http://www.ask-force.org/web/Bees/Mahmood-Control-Ectoparasitic-Mites-Thymol-Oxalic-2012.pdf

A study was carried out to determine the effects of thymol (powdered form) with 3.2% oxalic acid (OA) on two ectoparasitic mites, Varroa destructor Anderson and Trueman (Acari:Varroidae) and Tropilaelaps clareae Delfinado and Baker (Laelapidae: Acrina) populations in honeybee Apis mellifera linguistica (Hymenoptera: Apidae) colonies in the fall. Thymol 2, 4 and 6 g with 3.2% OA applied to twenty honeybee colonies thrice on different dates, showed 26, 41, 36% mortality in T. clareae and 93, 99 and 94% mortality in V. destructor, respectively. **The results showed that 3.2% OA with 4g thymol was the best treatment for controlling these mites. No queens were lost, and there was no adult honeybee mortality in any of the colonies during the experiment.** Mahmood, Wagchoure, et al. (2012)

12. Natural infection by Nosema ceranae (including Nosema apis) the cause of CCD?

A great number of papers (in my bibliography ca. 450 publications) show that Nosema is part of the CCD problem: The Nosema disease was known already in 1912 as the "Isle of Wight disease" Fantham and Porter (1912).

Nosema ceranae was formerly classified as a microsporidian, today reclassified as a fungus, a small, unicellular parasite that mainly affects Honeybees. It causes **nosemosis**, also called **nosema**, which is the most common and widespread of adult Honeybee diseases. The dormant stage of *Nosema ceranae (including Nosema apis)* is a long lived spore which is resistant to temperature extremes and dehydration and cannot be killed by freezing the contaminated comb. Nosemosis is a listed disease with the Office International des Epizooties (OIE).

In 1996 Malone et al. Malone and Giacon (1996) pointed again with repeated infection experiments to the connection between reduced aging and Nosema in some populations:

*"Inbred bees (Apis mellifera ligustica) from seven different colonies were dosed individually with spores of Nosema apis and kept in cages. Longevity and spores carried at the time of death were recorded for each bee. The experiment was repeated on three different dates. Control bee longevity varied with experiment date, although the pattern of this response varied from colony to colony. Dosing significantly reduced the lifespans of bees in all but three colonies**. Results suggest that bees with superior ability to survive in cages may also withstand Nosema apis infection better. Each cage of dosed bees produced a number of dead bees without detectable spore loads. Survival data comparisons suggest that dosing newly-emerged bees with Nosema apis may result in a relatively fast death for some bees and a slower death for the majority. Spore loads were very variable with no clear relationship to survival time**." Malone and Giacon (1996)*

Higes, M., R. Martin-Hernandez, C. Botias, E. G. Bailon, A. V. Gonzalez-Porto, L. Barrios, M. J. del Nozal, J. L. Bernal, J. J. Jimenez, P. G. Palencia and A. Meana (2008). "How natural infection by Nosema ceranae causes honeybee colony collapse." Environmental Microbiology 10(10): 2659-2669.
<Go to ISI>://WOS:000259147900017 AND http://www.ask-force.org/web/Bees/Higes-Natural-Infection-Nosema-2008.pdf

*"In recent years, honeybees (Apis mellifera) have been strangely disappearing from their hives, and strong colonies have suddenly become weak and died. The precise aetiology underlying the disappearance of the bees remains a mystery. However, during the same period, Nosema ceranae, a microsporidium of the Asian bee Apis cerana, seems to have colonized A. mellifera, and it's now frequently detected all over the world in both healthy and weak honeybee colonies. For first time, we show that natural N. ceranae infection can cause the sudden collapse of bee colonies, establishing a direct correlation between N. ceranae infection and the death of honeybee colonies under field conditions. Signs of colony weakness were not evident until the queen could no longer replace the loss of the infected bees. The long asymptomatic incubation period can explain the absence of evident symptoms prior to colony collapse. **Furthermore, our results demonstrate that healthy colonies near to an infected one can also become infected, and that N. ceranae infection can be controlled with a specific antibiotic, fumagillin. Moreover, the administration of 120 mg of fumagillin has proven to eliminate the infection, but it cannot avoid reinfection after 6 months. We provide Koch's postulates between N. ceranae infection and a syndrome with a long incubation period involving continuous death of adult bees, non-stop brood rearing by the bees and colony loss in winter or early spring despite the presence of sufficient remaining pollen and honey."** Higes, Martin-Hernandez, et al. (2008A, Higes, Martin-Hernandez, et al. (2008B)*

Pajuelo, A. G., C. Torres and F. J. O. Bermejo (2008). "Colony losses: a double blind trial on the influence of supplementary protein nutrition and preventative treatment with fumagillin against Nosema ceranae." Journal of Apicultural Research **47**(1): 84-86.<Go to ISI>://WOS:000254014000014 AND http://www.ask-force.org/web/Bees/Pajuelo-Colony-Losses-2008.pdf

Serious losses of Honeybee colonies have been commanding the attention of the Spanish beekeeping sector over the last few years. It is thought that the problem has been caused by the joint action of a series of factors that could be provoking an immunosuppressive reaction in bees, making them more susceptible to previously known diseases such as: European Foul Brood (Melissococcus pluton); American Foul Brood (Paenibacil/us larvae); chalk brood (Ascosphaem apis); viruses and Varroa destructor, and new emerging diseases such as Nosema ceronae. *These factors are thought to include: climatologically difficult years with a consequential nutritional impact on colonies, the effect of* *neonicotinoid insecticides, and unsuitable management practices. Nutritional problems caused by climatic conditions are not new to apiculture. In Australia, there were similar occurrences at the end of the 1970s Kleinschmidt and Kondos (1979) cand in the USA, Sanford (1987, Sanford (2007, Savoy, Lupan, et al. (1997) cites the so-called "Stress Accelerated Decline". The Iberian Peninsula has recently been experiencing the hottest years since temperature was first recorded, two of the four hottest years being 2003 and 2004 (European Environmental Agency. www.eea.eu.intlmain <http://www.eea.eu.intlmain>). Pajuelo, Torres, et al. (2008)*.

McMullan, J. B. and M. J. F. Brown (2009). "A qualitative model of mortality in Honeybee (Apis mellifera) colonies infested with tracheal mites (Acarapis woodi)." Experimental and Applied Acarology **47**(3): 225-234.
<Go to ISI>://WOS:000262972100005 AND http://www.ask-force.org/web/Bees/McMullan-Qualitative-Model-Mortality-2009.pdf

"The tracheal mite has been associated with colony deaths worldwide since the mite was first discovered in 1919. Yet controversy about its role in Honeybee colony mortality has existed since that time. Other pathogens such as bacteria and viruses have been suggested as the cause of colony deaths as well as degenerative changes in individual Honeybees. Using data from published work we developed a qualitative mortality model to explain colony mortality due to tracheal mite infestation in the field. Our model suggests that colonies of tracheal-mite infested Honeybees, with no other pathogens present, can die out in the late winter/early spring period due to their inability to thermoregulate. An accumulation of factors conspire to cause colony death including reduced brood/bee population, loose winter clusters, reduced flight muscle function and increasing mite infestation. In essence a cascade effect results in the colony losing its cohesion and leading to its ultimate collapse." McMullan and Brown (2009)

There is also a correlation between Nosema infection degrees and pesticide exposure of the bee colonies Pettis, vanEngelsdorp, et al. (2012)

"Global pollinator declines have been attributed to habitat destruction, pesticide use, and climate change or some combination of these factors, and managed Honeybees, Apis mellifera, are part of worldwide pollinator declines. Here we exposed Honeybee colonies during three brood generations to sub-lethal doses of a widely used pesticide, imidacloprid, and then subsequently challenged newly emerged bees with the gut parasite, Nosema spp. The pesticide dosages used were below levels demonstrated to cause effects on longevity or foraging in adult Honeybees. Nosema infections increased significantly in the bees from pesticide-treated hives when compared to bees from control hives demonstrating an indirect effect of pesticides on pathogen growth in Honeybees. ***We clearly demonstrate an increase in pathogen growth within individual bees reared in colonies exposed to one of the most widely used pesticides worldwide, imidacloprid, at below levels considered harmful to bees. The finding that individual bees with undetectable levels of the target pesticide, after being reared in a sub-lethal pesticide environment within the colony, had higher Nosema is significant. Interactions between pesticides and pathogens could be a major contributor to increased mortality of Honeybee colonies, including colony collapse disorder, and other pollinator declines worldwide****." Pettis, vanEngelsdorp, et al. (2012)*

Generally, there is a growing prevalence of Nosema ceranae cases in bee colonies reported from Spain:Botias, Martin-Hernandez, et al. (2012A)

"Microsporidiosis of adult honeybees caused by Nosema apis and Nosema ceranae is a common worldwide disease with negative impacts on colony strength and productivity. Few options are available to control the disease at present. The role of the queen in bee population renewal and the replacement of bee losses due to Nosema infection is vital to maintain colony homeostasis. ***Younger queens have a greater egg laying potential and they produce a greater proportion of uninfected newly eclosed bees to compensate for adult bee losses; hence, a field study was performed to determine the effect of induced queen replacement on Nosema infection in Honeybee colonies, focusing on colony strength and honey production. In addition, the impact of long-term Nosema infection of a colony on the ovaries and ventriculus of the queen was evaluated. Queen replacement resulted in a remarkable decrease in the rates of Nosema infection, comparable with that induced by fumagillin treatment. However, detrimental effects on the overall colony state were observed due to the combined effects of stressors such as the queenless condition, lack of brood and high infection rates. The ovaries and ventriculi of queens in infected colonies revealed no signs of Nosema infection and there were no lesions in ovarioles or epithelial ventricular cells.****" Botias, Martin-Hernandez, et al. (2012A)*

About the precise location of Nosema spp. spores Ptaszynska, Borsuk, et al. (2012) published details:

"Nosemosis is a serious honeybee disease linked to Colony Collapse Disorder (CCD). It cause many changes at the individual bee level, which also affects the health of the entire bee colony. N. ceranae and N. apis are not tissue specific as was previously thought and besides the ventriculus epithelium their spore are also present in other tissue, such as Malpighian tubules, hypopharyngeal glands, salivary glands, and fat bodies. Emplacement of Nosema infection in honeybee glands interferes with the production of the royal jelly, honey, bee bread. Moreover spores remaining in the honeybee glands are a potential reservoir of infection.

The aim of the research was to determine the correlation among the number of Nosema spores in whole bees, as well as in their ventriculus and hypopharyngeal glands. Nosema-infected Honeybees were collected in the spring, when there should be a comparable degree of Nosema infection level in all tissues. Three independent experiments were conducted. In these studies the number of spores in the hypopharyngeal glands was the lowest and the highest results were observed for ventriculus samples. A large number of spores in the hypopharyngeal glands was also observed. This can be the cause of a reduction or loss of these gland's function; moreover, it may increase the horizontal transmission of the infection within a hive as well as to a queen bee." Ptaszynska, Borsuk, et al. (2012)

Villa, Bourgeois, et al. (2013) made it clear that there is no clear genomic correlation between Nosema ceranae and different Honeybee populations, but the Nosema infection causes reduction of bee populations.

Villa, J. D., A. L. Bourgeois and R. G. Danka (2013), Negative evidence for effects of genetic origin of bees on Nosema ceranae, positive evidence for effects of Nosema ceranae on bees, Apidologie, 44, 5, pp. 511-518, <Go to ISI>://WOS:000323246700003 AND http://www.ask-force.org/web/Bees/Villa-Negative-Evidence-Origin-Positive-Effects-Bees-2013.pdf

"In two tests, Honeybee colonies of different origins were sampled monthly to detect possible differential infection with Nosema ceranae; colony sizes and queen status were monitored quarterly. One experiment used queens crossed with drones of the same type obtained from colonies which had previously exhibited high and low infections. A second experiment used queens from ten commercial sources. No clear genotypic (P00.682) or phenotypic (P00.623) differences in infection were evident. Colony deaths and supersedures did not relate significantly with infection except for deaths of colonies in the autumn (P00.02).
Significant effects on colony growth were found in all seasons: average 3-month decreases in population ranged from 0.4 to 1.4 frames of bees per million N. ceranae per bee. These results confirm that N. ceranae can be involved in weakening of colonies even in warm climates and suggest that breeding for resistance may require more intense selection, larger base populations, or different screening methods." Villa, Bourgeois, et al. (2013)

Roudel, Aufauvre, et al. (2013) found that Nosema ceranae has a variable virulence because of genetic polymorphism and/or its polyphenism responding to environmental pressure.

Roudel, M., J. Aufauvre, B. Corbara, F. Delbac and N. Blot (2013), New insights on the genetic diversity of the honeybee parasite Nosema ceranae based on multilocus sequence analysis, Parasitology, 140, 11, pp. 1346-1356, <Go to ISI>://WOS:000323383200004 AND http://www.ask-force.org/web/Bees/Roudel-genetic-diversity-Nosema-ceranae-2013.pdf

"The microsporidian parasite Nosema ceranae is a common pathogen of the Western honeybee (Apis mellifera) whose variable virulence could be related to its genetic polymorphism and/or its polyphenism responding to environmental cues.
Since the genotyping of N. ceranae based on unique marker sequences had been unsuccessful, we tested whether a multilocus approach, assessing the diversity of ten genetic markers – encoding nine proteins and the small ribosomal RNA subunit – allowed the discrimination between N. ceranae variants isolated from single A. mellifera individuals in four distant locations. High nucleotide diversity and allele content were observed for all genes. Most importantly, the diversity was mainly present within parasite populations isolated from single honeybee individuals. In contrast the absence of isolate differentiation precluded any taxa discrimination, even through a multilocus approach, but suggested that similar populations of parasites seem to infect honeybees in distant locations. As statistical evolutionary analyses showed that the allele frequency is under selective pressure, we discuss the origin and consequences of N. ceranae heterozygosity in a single host and lack of population divergence in the context of the parasite natural and evolutionary history." Roudel, Aufauvre, et al. (2013)

Flight behavior of Apis mellifera is altered by Nosema infections, Dosselli, Grassl, et al. (2016):

Dosselli, R., Grassl, J., Carson, A., Simmons, L. W. and Baer, B. (2016) Flight behaviour of honey bee (Apis mellifera) workers is altered by initial infections of the fungal parasite Nosema apis Scientific Reports 6 36649 pp 10.1038/srep36649 AND http://www.nature.com/articles/srep36649#supplementary-information AND http://www.ask-force.org/web/Bees/Dosselli-Flight-behaviour-honey-bee-Nosema-2016.pdf

"Honey bees (Apis mellifera) host a wide range of parasites, some being known contributors towards dramatic colony losses as reported over recent years. To counter parasitic threats, honey bees possess effective immune systems. Because immune responses are predicted to cause substantial physiological costs for infected individuals, they are expected to trade off with other life history traits that ultimately affect the performance and fitness of the entire colony. Here, we tested whether the initial onset of an infection negatively impacts the flight behaviour of honey bee workers, which is an energetically demanding behaviour and a key component of foraging activities. To do this, we infected workers with the widespread fungal pathogen Nosema apis, which is recognised and killed by the honey bee immune system. We compared their survival

and flight behaviour with non-infected individuals from the same cohort and colony using radio frequency identification tags (RFID). ***We found that over a time frame of four days post infection, Nosema did not increase mortality but workers quickly altered their flight behaviour and performed more flights of shorter duration. We conclude that parasitic infections influence foraging activities, which could reduce foraging ranges of colonies and impact their ability to provide pollination services."*** *From Dosselli, Grassl, et al. (2016)*

13. Chalkbrood and stonebrood: two fungal diseases affecting Honeybees

Chalkbrood (and to a lesser degree) stonebrood can result into severe population reduction in Honeybees. Chalkbrood as a common and widespread disease is caused by Ascosphaera apis, Chalkbrood is not yet well understood and is infected by a rarely observed Aspergillus sp.

Jensen, A. B., K. Aronstein, J. M. Flores, S. Vojvodic, M. A. Palacio and M. Spivak (2013), Standard methods for fungal brood disease research, Journal of Apicultural Research, 52, 1, pp. <Go to ISI>://WOS:000315730300013 AND http://www.ask-force.org/web/Bees/Jensen-Standard-Fungal-Brood-Disease-20130901.pdf

"Chalkbrood and stonebrood are two fungal diseases associated with Honeybee brood. Chalkbrood, caused by Ascosphaera apis, is a common and widespread disease that can result in severe reduction of emerging worker bees and thus overall colony productivity. Stonebrood is caused by Aspergillus spp. that are rarely observed, so the impact on colony health is not very well understood. A major concern with the presence of Aspergillus in Honeybees is the production of airborne conidia, which can lead to allergic bronchopulmonary aspergillosis, pulmonary aspergilloma, or even invasive aspergillosis in lung tissues upon inhalation by humans. In the current chapter we describe the Honeybee disease symptoms of these fungal pathogens. In addition, we provide research methodologies and protocols for isolating and culturing, in vivo and in vitro assays that are commonly used to study these host pathogen interactions. We give guidelines on the preferred methods used in current research and the application of molecular techniques. We have added photographs, drawings and illustrations to assist bee-extension personnel and bee scientists in the control of these two diseases." Jensen, Aronstein, et al. (2013)

Vojvodic, Boomsma, et al. (2012) demonstrate that the infection with various fungal pathogens is highly complex and not yet really understood. But it is clear, that fungal infections play worldwide an important role in worldwide Honeybee health declines.

Vojvodic, S., J. J. Boomsma, J. Eilenberg and A. B. Jensen (2012), Virulence of mixed fungal infections in Honeybee brood, Frontiers in Zoology, 9, pp. <Go to ISI>://WOS:000305830800001 AND http://www.ask-force.org/web/Bees/Vojvodic-Virulence-Mixed-Fungal-Infections-2012.pdf

*"**Introduction**: Honeybees, Apis mellifera, have a diverse community of pathogens. Previous research has mostly focused on bacterial brood diseases of high virulence, but milder diseases caused by fungal pathogens have recently attracted more attention. This interest has been triggered by partial evidence that co-infection with multiple pathogens has the potential to accelerate Honeybee mortality. In the present study we tested whether co-infection with closely related fungal brood-pathogen species that are either specialists or non-specialist results in higher host mortality than infections with a single specialist. We used a specially designed laboratory assay to expose Honeybee larvae to controlled infections with spores of three Ascosphaera species: A. apis, the specialist pathogen that causes chalkbrood disease in Honeybees, A. proliperda, a specialist pathogen that causes chalkbrood disease in solitary bees, and A. atra, a saprophytic fungus growing typically on pollen brood-provision masses of solitary bees.*
***Results:** We show for the first time that single infection with a pollen fungus A. atra may induce some mortality and that co-infection with A. atra and A. apis resulted in higher mortality of Honeybees compared to single infections with A. apis. However, similar single and mixed infections with A. proliperda did not increase brood mortality.*
Conclusion: Our results show that co-infection with a closely related fungal species can either increase or have no effect on host mortality, depending on the identity of the second species. Together with other studies suggesting that multiple interacting pathogens may be contributing to worldwide Honeybee health declines, our results highlight the importance of studying effects of multiple infections, even when all interacting species are not known to be specialist pathogens." Vojvodic, Boomsma, et al. (2012)

14. A new threat to the honeybees: the parasitic Phorid fly Apocephalus borealis

Core, A., C. Runckel, et al. (2012). "A New Threat to Honeybees, the Parasitic Phorid Fly <italic>Apocephalus borealis</italic>." PLoS ONE **7**(1): e29639.
http://dx.doi.org/10.1371%2Fjournal.pone.0029639 AND http://www.ask-force.org/web/Bees/Core-New-Threat-Honeybees-2012.pdf

"Honeybee colonies are subject to numerous pathogens and parasites. Interaction among multiple pathogens and parasites is the proposed cause for Colony Collapse Disorder (CCD), a syndrome characterized by worker bees abandoning their hive. Here we provide the first documentation that the phorid fly <italic>Apocephalus borealis</italic>, previously known to parasitize bumble bees, also infects and eventually kills Honeybees and may pose an emerging threat to North American apiculture. Parasitized Honeybees show hive abandonment behavior, leaving their hives at night and dying shortly thereafter. On average, seven days later up to 13 phorid larvae emerge from each dead bee and pupate away from the bee. Using DNA barcoding, we confirmed that phorids that emerged from Honeybees and bumble bees were the same species. Microarray analyses of Honeybees from infected hives revealed that these bees are often infected with deformed wing virus and <italic>Nosema ceranae</italic>. Larvae and adult phorids also tested positive for these pathogens, implicating the fly as a potential vector or reservoir of these Honeybee pathogens. Phorid parasitism may affect hive viability since 77% of sites sampled in the San Francisco Bay Area were infected by the fly and microarray analyses detected phorids in commercial hives in South Dakota and California's Central Valley. Understanding details of phorid infection may shed light on similar hive abandonment behaviors seen in CCD." Core, Runckel, et al. (2012)

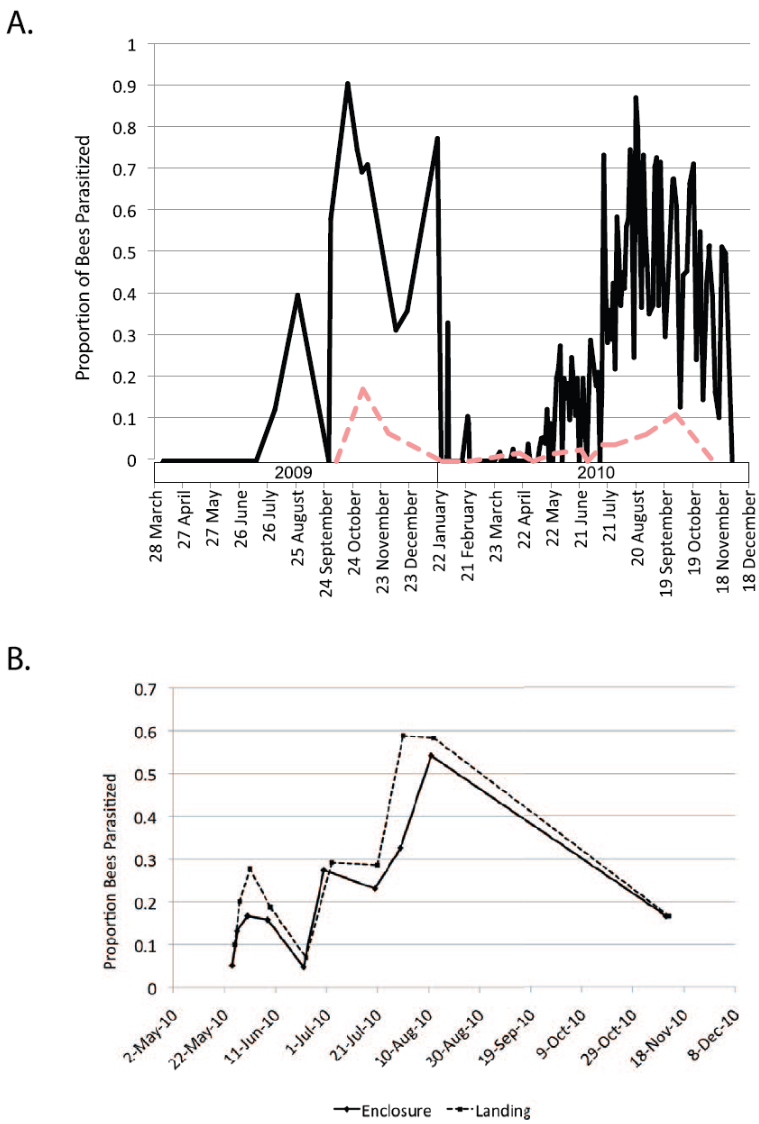

Fig. 28 Rates of phorid parasitism in Honeybees. (A) Rates of parasitism for bees sampled from April 2009 through November 2010. Black solid line shows rates in stranded bees from under lights on the San Francisco State University campus, while the pink dashed line shows

rates in foraging bees. Stranded bees found under lights were sampled at irregular intervals during 2009 and sampled every two days in 2010. Foragers were sampled monthly from our main study hive. A rate of zero indicates that samples from that period contained no parasitized bees. We compared rates of parasitism in stranded and active foraging bees collected at San Francisco State University from October 2009 through January 2010 and from July
2010 to December 2010 (when parasitism rates peaked). 2009–2010 peak rates of parasitism in samples of stranded bees (Mean = 60%, n = 276) were significantly higher than peak rates of parasitism in active foragers from our main study hive (Mean = 6%, n = 164) (x2 = 126.7, d.f. 1, p,0.0001). This pattern repeated in 2010 when peak rates of parasitism in samples of stranded bees (Mean = 50%, n = 860) were again significantly higher than rates of parasitism in active foragers (Mean = 4%, n = 422) (x2 = 255.3, d.f. 1, p,0.0001). (B) Proportion of Honeybees parasitized by phorids in samples from stranded bees collected from the Hensill Hall landing under lights (dotted line) and from samples of bees collected from overnight hive enclosures on adjacent nights (solid line). Parasitism rates of bees trapped in the enclosures closely track rates in stranded bees found under lights during the same period and the number of bees found under lights significantly declined when the enclosure was in place (Welch's t-test p,0.0001) indicating that stranded bees came from our main study hive and were parasitized prior to abandoning the hive. doi:10.1371/journal.pone.0029639.g003 Core, Runckel, et al. (2012)

15. Could climate change contribute to the colony collapse of Honeybees?

Le Conte, Y. and M. Navajas (2008). "Climate change: impact on Honeybee populations and diseases." Revue Scientifique Et Technique-Office International Des Epizooties **27**(2): 499-510.
<Go to ISI>://WOS:000259353700017 AND http://www.ask-force.org/web/Bees/Leconte-Climate-Change-Bees-2008.pdf

"The European Honeybee, Apis mellifera, is the most economically valuable pollinator of agricultural crops worldwide. Bees are also crucial in maintaining biodiversity by pollinating numerous plant species whose fertilisation requires an obligatory pollinator. Apis mellifera is a species that has shown great adaptive potential, as it is found almost everywhere in the world and in highly diverse climates. In a context of climate change, the variability of the Honeybee's life-history traits as regards temperature and the environment shows that the species possesses such plasticity and genetic variability that this could give rise to the selection of development cycles suited to new environmental conditions. Although we do not know the precise impact of potential environmental changes on Honeybees as a result of climate change, there is a large body of data at our disposal indicating that environmental changes have a direct influence on Honeybee development. In this article, the authors examine the potential impact of climate change on Honeybee behaviour, physiology and distribution, as well as on the evolution of the Honeybee's interaction with diseases. Conservation measures will be needed to prevent the loss of this rich genetic diversity of Honeybees and to preserve ecotypes that are so valuable for world biodiversity." Le Conte and Navajas (2008A, Le Conte and Navajas (2008B)

16. Inbreeding may cause bee populations more susceptible to infections?

This is a relatively old controversy with lots of papers contradicting each other, but there is no real solution yet presented. A recent dispute may be typical for the issue:

It is B.A. Harpur Harpur, Kent, et al. (2012, Harpur, Minaei, et al. (2012, Harpur, Minaei, et al. (2013, Harpur, Sobhani, et al. (2013) with extensive argumentation stating that the inbreeding question should be dismissed, but De la Rua et al. contradict clearly: De la Rua, Jaffe, et al. (2013). As usual, straightforward statements should be avoided because bee populations are managed and are situated in highly diverse situations and conditions, generalizations should be avoided.

B. A. Harpur, S. Minaei, C. F. Kent and A. Zayed (2012)
Management increases genetic diversity of Honeybees via admixture, Molecular Ecology, 21, 18, 4414-4421

"The process of domestication often brings about profound changes in levels of genetic variation in animals and plants. The Honeybee, Apis mellifera, has been managed by humans for centuries for both honey and wax production and crop pollination. Human management and selective breeding are believed to have caused reductions in genetic diversity in Honeybee populations, thereby contributing to the global declines threatening this ecologically and economically important insect. However, previous studies supporting this claim mostly relied on population genetic comparisons of European and African (or Africanized) Honeybee races; such conclusions require reassessment given recent evidence demonstrating that the Honeybee originated in Africa and colonized Europe via two independent expansions. We sampled Honeybee workers from two managed populations in North America and Europe as well as several old-world progenitor populations in Africa, East and West Europe. Managed bees had highly introgressed genomes representing admixture between East and West European progenitor populations. ***We found that managed Honeybees actually have higher levels of genetic diversity compared with their progenitors in East and West Europe, providing an unusual example whereby human management increases genetic diversity by promoting admixture. The relationship between genetic diversity and Honeybee declines is tenuous given that managed bees have more genetic diversity than their progenitors and many viable domesticated animals."*** *Harpur, Minaei, et al. (2012)*
http://dx.doi.org/10.1111/j.1365-294X.2012.05614.x AND http://www.force.org/web/Bees/Harpur-Management-Increases-Genetic-Diversity-2012.pdf

P. De la Rua, R. Jaffe, I. Munoz, J. Serrano, R. F. A. Moritz and F. B. Kraus (2013)
Conserving genetic diversity in the honeybee: Comments on Harpur et al. (2012), Molecular Ecology, 22, 12, 3208-3210 (sic) 4414-4421
The article by Harpur et al. (2012) Management increases genetic diversity of Honeybees via admixture' concludes that ...Honeybees do not suffer from reduced genetic diversity caused by management and, consequently, that reduced genetic diversity is probably not contributing to declines of managed Apis mellifera populations'. In the light of current honeybee and beekeeping declines and their consequences for honeybee conservation and the pollination services they provide, we would like to express our concern about the conclusions drawn from the results of Harpur etal. (2012). While many honeybee management practices do not imply admixture, we are convinced that the large-scale genetic homogenization of admixed populations could drive the loss of valuable local adaptations. We also point out that the authors did not account for the extensive gene flow that occurs between managed and wild/feral honeybee populations and raise concerns about the data set used. Finally, we caution against underestimating the importance of genetic diversity for honeybee colonies and highlight the importance of promoting the use of endemic honeybee subspecies in apiculture.De la Rua, Jaffe, et al. (2013)
<Go to ISI>://WOS:000320396100004 AND http://www.ask-force.org/web/Bees/DelaRua-Conserving-Genetic-Diversity-comment-Harpur-2013.pdf

More literature on the case, which according to the author of this review, is not really solved:

B. A. Harpur, C. Kent and A. Zayed (2012)
Variation in differentiation across the Honeybee, Apis mellifera, genome: signs of adaptive selection, Genome, 55, 10, 719-719
<Go to ISI>://WOS:000311829100017 AND NEBIS: BEIM BUCHBINDER, later check again
http://opac.nebis.ch/F?func=direct&local_base=NEBIS&doc_number=000037816

B. A. Harpur, S. Minaei, C. F. Kent and A. Zayed (2013)
Admixture increases diversity in managed Honeybees: Reply to De la Rua etal. (2013), Molecular Ecology, 22, 12, 3211-3215

"De la Rua etal. (2013) express some concerns about the conclusions of our recent study showing that management increases genetic diversity of Honeybees (Apis mellifera) by promoting admixture (Harpur etal. 2012). We provide a brief review of the literature on the population genetics of A.mellifera and show that we utilized appropriate sampling methods to estimate genetic diversity in the focal populations. Our finding of higher genetic diversity in two managed A.mellifera populations on two different continents is expected to be the norm given the large number of studies documenting admixture in Honeybees. ***Our study focused on elucidating how management affects genetic diversity in Honeybees, not on how to best manage bee colonies. We do not endorse the intentional admixture of Honeybee populations, and we agree with De la Rua etal. (2013) that native Honeybee subspecies should be conserved."*** *Harpur, Minaei, et al. (2013)*
<Go to ISI>://WOS:000320396100005 AND http://www.ask-force.org/web/Bees/Harpur-Admixture-Increases-Reply-Rua-2013.pdf

B. A. Harpur, M. Sobhani and A. Zayed (2013)
A review of the consequences of complementary sex determination and diploid male production on mating failures in the Hymenoptera, Entomologia Experimentalis Et Applicata, 146, 1, 156-164

"Complementary sex determination is the ancestral sex-determination mechanism in the Hymenoptera. Under this system, diploid individuals develop into females if they are heterozygous at an autosomal sex-determining locus or loci, whereas haploid individuals develop into males because they are hemizygous at the sex-determining locus or loci. However, diploid males can still arise from fertilized eggs if such individuals are homozygous at the sex-determining locus or loci. Diploid males are often viable but sire few daughters, thereby representing a substantial genetic load in hymenopteran populations. Here, we review the effects of complementary sex determination and diploid male production from the perspective of female hymenopterans. Because female hymenopterans need not mate to produce haploid sons, complementary sex determination can cause special forms of mating failures by preventing some females from controlling the sex ratio of their brood and producing the desired number of daughters. Under some circumstances, complementary sex determination can cause complete mating failure by preventing females from producing daughters altogether. ***Although we outline serious gaps of knowledge in the field, the data at hand suggest that diploid male production can substantially increase mating failures in small populations of economically and ecologically important hymenopterans****." Harpur, Minaei, et al. (2013)*
<Go to ISI>://WOS:000312222600016 AND http://www.ask-force.org/web/Be es/Harpur-Review-Consequences-Complementary-Sex-2013.pdf

And there is much more recent and older literature on genomics and inbreeding of bee populations which should be included in this debate:

Abrahamovich, Atela, et al. (2007, Botias, Martin-Hernandez, et al. (2012B, Bouga, Alaux, et al. (2011, Canovas, De la Rua, et al. (2008, Canovas, de la Rua, et al. (2011, Chahbar, Munoz, et al. (2013, de la Rua, Galian, et al. (2006, De la Rua, Galian, et al. (2003, de la Rua, Hernandez-Garcia, et al. (2005, De la Rua, Jaffe, et al. (2009, De la Rua, Serrano, et al. (1998, Hernandez-Garcia, De la Rua, et al. (2009, Jaffe, Dietemann, et al. (2010, Jara, Cepero, et al. (2012, Kent, Minaei, et al. (2012, Kent, Minaei, et al. (2013, May-Itza, Quezada-Euan, et al. (2012, Munoz, Dall'Olio, et al. (2009, Munoz and De la Rua (2012, Munoz, Stevanovic, et al. (2012, Pinto, Henriques, et al. (2013, Radloff, Hepburn, et al. (2001, Ruiz, May-Itza, et al. (2013, Seeley and Tarpy (2007, Shaibi, Munoz, et al. (2009, Sherman, Seeley, et al. (1998, Simon, Cenis, et al. (2007)

And more:

Baudry, Solignac, et al. (1998, Harpur, Kent, et al. (2012, Harpur, Minaei, et al. (2012, Harpur, Minaei, et al. (2013, Jostins Luke (20110429, Kent, Minaei, et al. (2012, Kent, Minaei, et al. (2013, Loper, Wolf, et al. (1992)

17. Could Electro-smog be the cause of the Colony Collapse Disorder?

The latest hypothesis on CCD is supported by an Indian experiment Sharma and Kumar (2009), which postulates a connection between electrosmog caused by mobile phones and a reduced orientation of bees.

Sharma, V. P. and N. R. Kumar (2009). "Changes in honeybee behaviour and biology under the influence of cellphone radiations." Current Science **98**(10): 1376-1378.
<Go to ISI>://WOS:000268550400621 AND
http://www.ask-force.org/web/Bees/Sharma-Changes-Honeybee-Cellphone-2010.pdf AND an alarmist newspaper flash
http://www.ask-force.org/web/Bees/Lean-CCD-Cellphones-The-Independent-20070415.PDF

Increase in the usage of electronic gadgets has led to electropollution of the environment. Honeybee behaviour and biology has been affected by electrosmog since these insects have magnetite in their bodies which helps them in navigation. There are reports of sudden disappearance of bee populations from honeybee colonies. The reason is still not clear. We have compared the performance of honeybees in cellphone radiation exposed and unexposed colonies. A significant ($p < 0.05$) decline in colony strength and in the egg laying rate of the queen was observed. The behaviour of exposed foragers was negatively influenced by the exposure, there was neither honey nor pollen in the colony at the end of the experiment Sharma and Kumar (2009).

The caveat is that basically it is a n=1 experiment which needs to be confirmed properly.

18. Diesel exhaust rapidly degrades floral odours used by honeybees

Proboscis movement and degree of Diesel exhaust are correlated after Girling, Lusebrink, et al. (2013) and suggest hampered recognition of flower odors:

Girling, R. D., I. Lusebrink, E. Farthing, T. A. Newman and G. M. Poppy (2013), Diesel exhaust rapidly degrades floral odours used by honeybees, Sci. Rep., 3, pp. http://dx.doi.org/10.1038/srep02779 AND http://www.ask-force.org/web/Bees/Gierling-Diesel-Exaust-degrades-odours-2013.pdf

Honeybees utilise floral odours when foraging for flowers; we investigated whether diesel exhaust pollution could interrupt these floral odor stimuli. A synthetic blend of eight floral chemicals, identified from oilseed rape, was exposed to diesel exhaust pollution. Within one minute of exposure the abundances of four of the chemicals were significantly lowered, with two components rendered undetectable. Honeybees were trained to recognize the full synthetic odor mix; altering the blend, by removing the two chemicals rendered undetectable, significantly reduced the ability of the trained honeybees to recognize the altered odor. Furthermore, we found that at environmentally relevant levels the mono-nitrogen oxide (NO_X) fraction of the exhaust gases was a key facilitator of this odor degradation. Such changes in recognition may impact upon a honeybee's foraging efficiency and therefore the pollination services that they provide. From Girling, Lusebrink, et al. (2013).

19. Monsanto proposes a solution

Monsanto (2012), Honeybee Health The Challenge, publ: Monsanto http://www.monsanto.com/improvingagriculture/pages/Honeybee-health.aspx AND http://www.ask-force.org/web/Bees/Monsanto-Honeybee-Health-2012.pdf

The Challenge

Bees play a vital role in agriculture as natural pollinators. Pollination is a necessary part of some plants' fertilization processes, because it allows for the development of fruits and seeds.

One-third of the food you eat depends upon pollination, including almonds, apples, berries, cucumbers and melons. Honeybees have an important role in contributing a service that helps provide us with variety and more nutritious food.

Farmers are facing the challenge of providing more food for a growing population. And, the Honeybee population has been facing its own problems. Colony Collapse Disorder (CCD) - is a phenomenon in which bees are disappearing abruptly from an otherwise healthy colony. The USDA report confirms that there are many causes that compromise bee health, including pathogens or diseases, poor nutrition and pesticides.

What Monsanto is Doing to Help

As a company solely focused on agriculture, we recognize the importance of bees to both our business and growers. We are committed to supporting Honeybee health and researching solutions for these complex issues.

PAm (Project Apis m.) Collaboration

Monsanto is collaborating with PAm to assist in forage projects in order to provide more nutritious food for bees. Beekeepers are often concerned with whether there is enough forage or food available during pollination seasons. The availability of natural pollen equates to healthier bees, and healthier bees are better able to tolerate stressors. By planting a variety of plants to contribute to the available pollen, this collaboration will help enable around 1.6 million Honeybee colonies, which are annually transported to the California almond fields, to have access to increased nutrition sources during their work in California.

Honeybee Advisory Council (HBAC)

The Honeybee Advisory Council is comprised of members of the beekeeping industry, experts and academia. We have learned a great deal about the complicated challenges facing beekeepers. With this council as a guiding force, our bee health research and development efforts are focused on the leading challenges.

Beeologics

In 2011, Monsanto acquired the Israel-based company,Beeologics. Beeologics research focuses on testing biological products to provide targeted control of pests and diseases in order to provide safe, effective ways to protect the Honeybee. For example, a major factor of Colony Collapse Disorder is credited to the parasitic varroa mite. This mite weakens bees' immune system and spreads viruses.

Currently, BioDirect - our first biological technology platform - is in discovery phase, but has shown promising results in testing that it could be effective against specific insects, such as varroa mites, while leaving beneficial insects unaffected. To put it simply, research is being done to control a problem insect on a beneficial insect.

Additionally, field trials are creating tremendous datasets that will be helpful to continuing research and the beekeeping community.

Honeybees are essential for productive agriculture and the environment. The collaboration with PAm, the Honeybee Advisory Council and Monsanto provides a strong foundation to help to find sustainable solutions to bee health.

20. The Issue of multiple factors

It is well known in the science of environmental impacts on organisms that the reality is often a package of multiple factors acting. In the research of air pollution there is a rich literature on this matter, the author has himself with a group of researchers worked for years about this problem with the means of biomonitoring. A set of Lichens (well known for their susceptibility towards air pollutants due to their epiphytic life) has been tested against a set of air pollutants, and the multifactorial analysis has revealed that those organisms living on tree bark react to a multiple set of air pollutants. Ammann, Herzig, et al. (1987, Herzig, Liebendorfer, et al. (1989, Herzig, Urech, et al. (1990, Liebendorfer, Herzig, et al. (1988, Wanner, Ammann, et al. (1986)

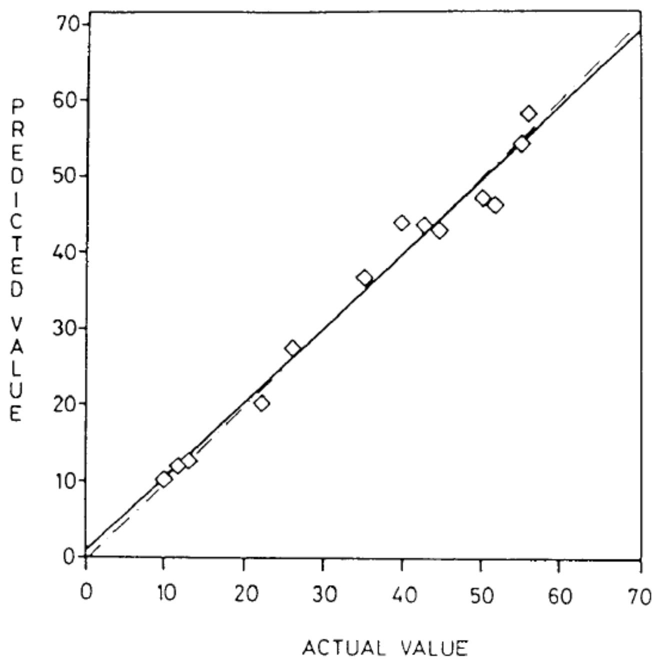

Fig. 29 Overal-1 model obtained by multiple linear regression including S02, N03, Cl, Dust, Pb, Cu, Zn, Cd from Ammann, Herzig, et al. (1987)

Also in the discussion of impact of various environmental factors on bees (including infection cases) there are papers dealing with multivariate statistics, but not really targeting the multifactor impact on CCD of all possible leads.

Papers deal with multifactor analysis of in vitro studies on bee populations:Williams, Alaux, et al. (2013) and mainly under the standard methods:

"Adult honey bees are maintained in vitro in laboratory cages for a variety of purposes. For example, researchers may wish to perform experiments on honey bees caged individually or in groups to study aspects of parasitology, toxicology, or physiology under highly controlled conditions, or they may cage whole frames to obtain newly emerged workers of known age cohorts. ***Regardless of purpose, researchers must manage a number of variables, ranging from selection of study subjects (e.g. honey bee subspecies) to experimental environment (e.g. temperature and relative humidity).*** *Although decisions made by researchers may not necessarily jeopardize the scientific rigor of an experiment, they may profoundly affect results, and may make comparisons with similar, but independent, studies difficult. Focusing primarily on workers, we provide recommendations for maintaining adults under in vitro laboratory conditions, whilst acknowledging gaps in our understanding that require further attention. We specifically describe how to properly obtain honey bees, and how to choose appropriate cages,*

incubator conditions, and food to obtain biologically relevant and comparable experimental results. Additionally, we provide broad recommendations for experimental design and statistical analyses of data that arises from experiments using caged honey bees. ***The ultimate goal of this, and of all COLOSS BEEBOOK papers, is not to stifle science with restrictions, but rather to provide researchers with the appropriate tools to generate comparable data that will build upon our current understanding of honey bees.*** *From Williams, Alaux, et al. (2013)*

On another topic, but methodologically interesting is a paper from Panchin, debunking the (in)famous papers of Séralini and others from a rigorous and professional statistical point of view: Panchin and Tuzhikov (2016), it is certainly worth reading.

21. Legal Aspects, EU Court of Justice Decisions, EU Labelling Rules, Misunderstandings among European Bee Keepers

Unfortunately, the European Court has decided on September 2012 to force honey producers to label pollen in honey and thus cause additional marketing costs.GMO Compass (20121022), GMO Safety (20120505) GMO Compass (20130916):

GMO Compass (20130916), Possible application of gene technology: Labelling Pollen from GM source plants (rapeseed, maize, luzerne/alfalfa), published: GMO Compass Genius GmbH, http://www.gmo-compass.org/eng/database/food/238.honey.html AND http://www.ask-force.org/web/Bees/Labeling-Rules-Honey-European-Court-Justice-GMO-compass-20130916.pdf

Labelling
Until now, honey containing pollen from GM plants did not legally have to be labelled as such. The ECJ ruling has changed this.
Pollen from GM plants is now regarded as an ingredient, for which – as for all other food ingredients – there is a labelling requirement.
Pollen must be mentioned as an ingredient on honey labels if the amount of GM pollen is higher than 0.9 per cent (labelling threshold) of the total pollen in the honey (this applies only to authorized GMOs). It is not technically possible to measure the exact GMO proportion of the pollen contained in honey.
It is not yet clear how long the ECJ ruling will remain valid. In September 2012, the European Commission adopted a proposal to amend the rules on honey, which would reclassify pollen as a "natural constituent" of honey, instead of as an ingredient as in the ECJ ruling. However, nothing is likely to change where GM pollen is concerned: pollen from GM plants would be permitted only if the plants are authorized for food use in the EU. The Commission's proposal needs the majority approval of the European Parliament and member states before it can be made law.

Negative comments with a clear analysis of the unacceptable Situation is given by Jany (2012)

Jany, K. D. (2012), Auswirkungen und Herausforderungen des EuGH-Urteils zu Pollen im Honig, Journal Für Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 7, 3, pp. 175-177, <Go to ISI>://WOS:000307766100001 AND http://www.ask-force.org/web/Bees/Jany-Auswirkungen-Herausforderungen-Pollen-Honig-2012.pdf

Compare also the comment of Davison John (20121029) before the new decision of the European Court.

See the fact sheet of Scienceindustries of Switzerland: Scienceindustries (20130618) with the legal and regulatory situation in Switzerland.

The decision of the European Parliament followed with a majority of 430 against 224 votes the reasonable decision of European Commission: (see the bold part of the summary of Davison and Kershen below)

http://www.europarl.europa.eu/sides/getDoc.do?type=REPORT&reference=A7-2013-0440&language=DE
http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+REPORT+A7-2013-0440+0+DOC+XML+V0//EN

After the decision of the European Court several comments have been published:
Davison John and Kershen Drew (2014)

Davison John and Kershen Drew (2014), Honey containing GM-pollen in Europe, in: CropGen, pp. 1-4, CropGen London, London, http://www.cropgen.org/article_513.html AND
http://www.ask-force.org/web/Bees/Davison-Kershen-Honey-Containing-GM-Pollen-Europe-2014.pdf

"In 2011, the European Court of Justice (ECJ) issued an opinion in Bablok v. Freistaat Bayern (1). The ECJ ruled that pollen, under EU law, must be considered an "ingredient" rather than a "constituent" of honey. As a consequence of this ECJ ruling, European honey producers and their international counterparts faced many complications in complying with ingredient labelling regulations and with international standards defining honey as a single ingredient, all-natural substance, i.e., simply honey. The ECJ ruling had the potential of destroying the European honey industry and to block imports from third countries (2, 3). Although urgent European Commission (EC) action was thus required, it took more than 2 years before it happened.

In January 2014, the European Parliament (EP) voted 430 to 224 to adopt an EC proposal amending the EU Honey Directive 2001/110/EC so that pollen is a constituent, not an ingredient, of honey (4). According to EU legislation, there is a huge difference between an ingredient and a constituent. An ingredient is part of a mixture and, as far as GMOs are concerned, would be subject to GMO labelling regulations. Thus, GMO maize pollen, as an ingredient, would require labelling if present at greater than 0.9% of the total maize pollen. In contrast, if honey is a natural entity – and pollen is a constituent of honey – then the GMO labelling requirement is calculated as a % of total honey. Pollen comprises between 0.005 and 0.05% of honey and thus labelling would never be required. After this vote, the EC still must take additional steps to finalize their proposal, now endorsed by the Parliament, into EU law. Assuredly the EC will take these additional steps. Thus, the EJC decision has been overturned by statutory and regulatory revision. The European honey industry has been saved from most legal complications and from potential destruction.
Despite the clarification that, in the EU, pollen is a constituent of honey, not an ingredient, there are several issues that remain unresolved about honey and pollen. This article identifies and discusses these unresolved issues." *Davison John and Kershen Drew (2014)*

Comments on the new judgement of the European Court of Justice turning around the absurd situation caused by fanatic opponents are published by Hoefer Eberhard and Jany Klaus (2014):

Hoefer Eberhard and Jany Klaus (2014), Honig-Urteil: Außer Spesen nichts gewesen, in: NOVO Argumente, pp. 1-6, NOVO, München, http://novoargumente.com/magazin.php/novo_notizen/artikel/0001520#comments AND http://www.ask-force.org/web/Bees/Hoefer-Jany-Honig-Urteil-Ausser-Spesen-nichts-gewesen-201402.pdf AND English translation: http://www.ask-force.org/web/Bees/Hoefer-Jany-Honey-Judgement-NOVO-201402.pdf

English summary:
"With the "honey-judgment" of the EU-CJ there was concern that green resentment against genetic engineering would paralyze apiculture. Now the nonsensical sentence was neutralized by the European Parliament – applauded by the chemists Eberhard Hofer and Klaus-Dieter Jany.
Most likely, on 15 January some judges of the European Court of Justice (ECJ) took deep breaths of relieve. On this day, the European Parliament has neutralized a strange verdict of the chamber and turned the highly controversial "Honey judgment" from September 6, 2011 into waste.

And:
Joel Schiro, president of the French professional beekeepers who are not really friends of green genetic engineering stated, on the occasion of an international workshop of the German Federal Department of Agriculture in December 2011 in Berlin: "The judgment of the ECJ of 6 September 2011 is the most terrible disaster that has ever befallen the profession of beekeepers. For all involved stakeholders and intelligent interest groups, whether trained beekeepers, scientists, bottlers or industrial honey customers, this is quite evident. Honey must not become the hostage of a political and ideological fight against genetically modified organisms (GMOs), which has nothing to do with the basic technical facts and does not concern us. "
Schiro approaches thus the actual core of the debate: The declared aim of the initiators is to prevent by any means the introduction of genetic engineering in Germany and pleaded consumer protection. The beekeepers were mercilessly abused as a political instrument."Hoefer Eberhard and Jany Klaus (2014).

A detailed chronology has been drafted by Höfer and Jany in German, in the course of finishing including appendices 2-4 soon translated into English, appendix 1 will be a summary of the CCD report by Klaus Ammann, including the original link.

Jany Klaus and Hoefer Eberhard (20140130)

Jany Klaus and Hoefer Eberhard (20140130), Die deutsche Imkerschaft und die Grüne Gentechnik. Ein chronologischer Überblick zum Kampf der deutschen Imkerschaft gegen die Grüne Gen- technik zum Schutz von Honig und anderen Bienenprodukten. With Annexes 2-4, No. pp. http://www.ask-force.org/web/Bees/Jany-Hoefer-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-2-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-4-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-3-Chronologie-Deutsche-Imker-2014.pdf AND Summary in English http://www.ask-force.org/web/Bees/Jany-Hoefer-Chronology-English-Summary-2014.pdf

"Summary:
The report gives an overview of the efforts of German apiculture to enforce a ban on the green genetic engineering in Germany and shows the developments which led to the amendment of Honey Directive 2001/110/EC.
The German beekeeper association claims gene technology to be a sustained and existential threat for beekeepers and they fear that the entry of pollen from genetically modified plants in honey will reduce the acceptance of such produce by the German honey consumers and as a consequence the market viability of GM pollen honey is lost. Like other commercial groups bee-keeper associations organized an intensive lobbying together with other GMO opponents and eco-groups against GMOs, however they actually disregarded their own interests as beekeepers, instead they work for the interests of consumers and farmers.
By the end of 2006 the beekeeper organization Mellifera e.V. called for an Alliance against agricultural genetic engineering, and to sue against the cultivation of MON 810 and the release of GM crops for research purposes. The court action costs will be borne by the Alliance. Since 2007 all applications against the cultivation of GM corn MON 810 were rejected by the administrative courts and as a consequence court cases were started. The procedures aim at a ban on the cultivation of GM crops in a safety distance not less than 6 km from the bee hives in order to enforce the implementation of measures for the protection of honey from entry of GM pollen and also for the enforcement of compensation claims in case of commercialization losses. None of the numerous processes up to the Federal Administrative Court was successful. In none of the court procedures it was possible to enforce a ban on cultivation of GM maize MON 810, nor did they succeed to reach court injunctions on protective

measures, which prevent the entry of GM maize pollen into honey. The courts also did not see any existential threat to the beekeepers with the cultivation of GM maize. Rather, they have imposed to the beekeepers to avoid fields of GM maize and to move their beehives accordingly. 2006 individual beekeepers and associations of beekeepers called publicly for (GENDRECK WEG = AWAY WITH GENE DIRT") field destructions and proceeded to action. Whereas politics distanced itself from field destructions as a means to enforce interests, the beekeeping associations tacitly tolerated such action. The German professional commercial beekeepers association on the contrary even awarded a price (the "Golden Sting") to the activist initiator of "AWAY WITH GENE DIRT.

The European Court of Justice (ECJ) clarified in 2011 the legal status of GM pollen in honey. GM pollen in honey is no GMO, but pollen is now generally classified as an ingredient in honey, consequently GM pollen required also a safety assessment and authorization as GM food. Honey with GM pollen, which has not been approved, loses with a deadline of 06.09.2011 its marketability. Honey with pollen from approved GM crops can be marked up to a threshold of 0.9% GM pollen. European Court of Justice gave no indication on the comparative basis of the threshold. Honey should now be accompanied by a list of the ingredients including details on pollen. This is unfortunately in contradiction with the guideline 2001 / 110 / EC on honey stating clearly that honey should not contain any ingredients. The EU Commission was ordered by the ECJ to amend the honey guideline. In the draft amendment of the guideline presented by the EU Commission, EU Parliament (15.01.2014) has agreed to this, it now considers pollen as a natural constituent of honey whatever source may be. Thus, pollen is not an ingredient and this eliminates the previously prescribed list of ingredients for honey. Honey with GM pollen must only be labeled as a GM product, if the thresold is above 0.9% GMO, the 0.9% refer clearly to weight percent. ***German beekeepers have reached thus in a democratic vote in the European Parliament to label honey as a GM product only in very rare cases. The EU parliamentarians have done a big favor to the German beekeepers: their existence is now secured and they keep their privileges.***

The future will show whether German beekeepers Association's efforts will be successful with their aim to ban of the green gene technology and discriminate imported honey as a GMO product. The future will show whether this policy leads to benefits for German's reputation of hobby and professional beekeepers reputation or whether it finally will have adverse effects." Jany Klaus and Hoefer Eberhard (20140130)

In the meanwhile, the book has been printed and is available as a hard copy: Jany Klaus Dieter and Eberhard Höfer (2014)

Jany Klaus Dieter and Eberhard Höfer (2014), Die deutsche Imkerschaft und Grüne Gentechnik Volume 57 Verlag Dr. Kovac, IS: SBN 978-3-8300-8038-1. pp. 210, http://www.verlagdrkovac.de/978-3-8300-8038-1.htm AND for personal use: http://www.ask-force.org/web/Bees/Jany-Hoefer-Deutsche-Imkerschaft-Gentechnik-2014.pdf

The English version has been published one year later: Jany Klaus-Dieter and Eberhard Höfer (2015)

Jany Klaus-Dieter, & Eberhard Höfer. (2015). *The German Bee Keepers and Modern Plant Biotechnology*. pp. 192 Hamburg: Wissenschaftsverlag Dr. Kovac Hamburg. ISBN 978-3-8300-8224-8 http://www.verlagdrkovac.de/3-8300-8224-X.htm?lang=english AND for personal use only: http://www.ask-force.org/web/Bees/Beekeeper-Jany-Hoefer-2015a.pdf

22. Summary: Colony Collapse Disorder: Many suspects, no smoking gun, except the Varroa mite, other parasites and the Deformed Viral Wing disease, systemic pesticides (imidacloprids) focus on regional differences

A major European report by OPERA OPERA, Dr. Ettore Capri, et al. (2013) with 64 pages, a summary of the main report on the Colony Collapse Disorder, based on the previous findings of OPERA, Dr. Anne Alix Ministry of Agriculture France, et al. (2011,).

OPERA, O. R. C. Dr. Ettore Capri, L. o. B. P. o. C. A. J. Dr. Mariano Higes, Spain,, B. P. I. Dr. Konstantinos Kasiotis, Greece,, G. Dr. Kyriaki Machera Benaki Phytopathological Institute, O. R. C. Alexandru Marchis, S. U. Dr Stephen J. Martin, UK,, J. K.-I. Jens Pistorius, Germany,, E. P. A. Thomas Steeger, U.S.A, and N. B. U. Dr. Helen Thompson, UK and Selwyn Wilkins, National Bee Unit, UK, (2013), Bee health in Europe - Facts & figures 2013, Compendium of the latest information on bee health in Europe, Universita Cattolica del Sacro Cuore, Piacenza, Italia, No. pp. 64, http://www.ask-force.org/web/Bees/OPERA-Capri-Bea-Health-Europe-Facts-Figures-2013.pdf

CONCLUSIONS

According to FAO data for the period 1992 - 2010, in Europe, the number of beehives has remained fairly constant while the causes for the fluctuations between years are not easily identifiable.

COLOSS reports that between 2008 and 2012, winter losses ranged from 7 to 30% with variations between countries and between years for the same country. No clear overarching trend can be highlighted.Beekeeping practices and the materials used, such as the type of hive, can be of high importance for the well-being of bees.
A number of pests and diseases have been demonstrated as being implicated with colony losses. The major pests/diseases are Varroa destructor, American foulbrood, European foulbrood, Nosema spp., Honeybee viruses, and Acarine mite (Acarapis woodi). Varroa has irreversibly changed the Deformed Wing Virus (DWV) viral landscape across the world. DWV is now considered one of the key players in colony losses in Europe. Future threats and non-native invasive species are also of high interest, like the Small Hive Beetle (Aethina tumida), Tropilaelaps spp. (another parasitic mite) and the Asian Hornet (Vespa velutina).
Overall, pesticide-related bee monitoring activities can be a helpful tool to assess potential side effects to bees on a large-scale level and under realistic field conditions, which can be relevant where the regular risk assessment still contains uncertainties.
International organizations like FAO, OECD, and ICPPR have developed a series of activities to address issues related to Bee health. The European Commission has designated a European reference laboratory for bee health; is co-funding national programs to support beekeeping and to collect data on bee health as well as revising risk assessment procedures for pesticides.

RECOMMENDATIONS

- *Due to the multi factorial nature behind the causes of colony collaborative work between the various disciplines is necessary to resolve the issues.*
- *An analysis of the factors influencing the number of colonies in each country is necessary as trends vary between them.*
- *Focus on improved beekeeping practices and the implementation of risk mitigation practices.*
- *Promote the communication and training of good beekeeping practices and programs co-financed by the EU to support the apiculture sector should be continued.*
- *Continue research on pathogens, diseases, pests and veterinary products.*
- *Continue to develop risk mitigation methods for the safe use of pesticides and education of pesticide users to understand the approved conditions of use.*
- *Promote landscape management practices that are proven to be effective to promote bee health.*
- *Promote the research on the genetics of managed and feral Honeybees*

Watanabe, M. E. (2008). "Colony collapse disorder: Many suspects, no smoking gun." Bioscience **58**(5): 384-388.
<Go to ISI>://WOS:000255971600005, http://www.ask-force.org/web/Bees/Watanabe-CCD-Many-Suspects-2008.pdf

"From the text:
The evidence to date Honeybees (Apis mellifera) can be loaded with parasites. Varroa mites (Varroa destructor) are relatively large ectoparasites that feed on bee hemolymph (insect "blood") and wreak havoc in hives. Tracheal mites (Acarapis woodi [Rennie]) attach to the bees' breathing apparatus and suck out hemolymph, injecting the bees with bacteria and weakening and killing adult bees. And two species of microsporidia, Nosema Apis and Nosema ceranae, can infect a bee's gut, damaging its digestive tract, exposing it to numerous bacteria and viruses, and shortening its lifespan. Bees are also subject to all sorts of chemical insults, especially environmental and in-hive insecticides and in -hive antibiotics, as well as to stress.
And about the virus hypothesis: The most pressing question at present, however, is whether a virus is causing the die-off. Cox-Foster, Conlan, et al. (2008, Cox-Foster, Conlan, et al. (2007A) led a study, published last fall (12 October 2007 Science), to identify microbial species associated with CCD affected migratory bee operations. Sequences from at least eight species of bacteria (some uncultured), two species of fungi, the two Nosema microsporidians, one trypanosome, the varroa mite, and seven virus species were found in the affected bees. Cox-Foster and colleagues concluded that Israeli acute paralysis virus (IAPV), which was identified only recently; is a marker for CCD but not necessarily the cause. W Ian Lipkin, from the Mailman School of Public Health at Columbia University in New York, who did much of the genetic work for the article, says that his group is now studying the distribution of IAPV. First described in Israel in 2004, IAPV has been present in the United States since before 2006. It was identified in material found in the US Department of agriculture's freezers dating from 2002. (Hackenberg remarks that there were similar-appearing die-offs in 2004 and 2005, though on a lesser scale than in 2006.) Cox-Foster explains that Lipkin's group has identified three complete viral genomes: one found in Honeybees from Australia, another from Israel, and a third in affected bee operations in the eastern United States and from two sites in Canada (New Brunswick and British Columbia). The Australian virus sequence matches sequences identified in bee operations in California and other states in the western United States. This makes sense, because beginning in 2005, under pressure from almond growers, the US Congress passed an exemption to the Honeybee Act of 1922, which forbade all importation of Honeybees to prevent the spread of disease to US bee colonies. At the time the act was passed, Isle of Wight disease (caused by tracheal mites) was ravaging bees in Europe, and Congress wanted to make sure the disease did not enter the United States." Watanabe (2008).

A recent publication of Krupke, Hunt, et al. (2012) confirms the view of multiple causes of CCD, but focuses nevertheless on pesticides, the summary may well serve as a final comment:

"Populations of Honeybees and other pollinators have declined worldwide in recent years. A variety of stressors have been implicated as potential causes, including agricultural pesticides. Neonicotinoid insecticides, which are widely used and highly toxic to Honeybees, have been found in previous analyses of Honeybee pollen and comb material. However, the routes of exposure have remained largely undefined. We used LC/MS-MS to analyze samples of Honeybees, pollen stored in the hive and several potential exposure routes associated with plantings of neonicotinoid treated maize. Our results demonstrate that bees are exposed to these compounds and several other agricultural pesticides in several ways throughout the foraging period. During spring, extremely high levels of clothianidin and thiamethoxam were found in planter

exhaust material produced during the planting of treated maize seed. We also found neonicotinoids in the soil of each field we sampled, including unplanted fields. Plants visited by foraging bees (dandelions) growing near these fields were found to contain neonicotinoids as well. This indicates deposition of neonicotinoids on the flowers, uptake by the root system, or both. Dead bees collected near hive entrances during the spring sampling period were found to contain clothianidin as well, although whether exposure was oral (consuming pollen) or by contact (soil/planter dust) is unclear. We also detected the insecticide clothianidin in pollen collected by bees and stored in the hive. When maize plants in our field reached anthesis, maize pollen from treated seed was found to contain clothianidin and other pesticides; and Honeybees in our study readily collected maize pollen. These findings clarify some of the mechanisms by which Honeybees may be exposed to agricultural pesticides throughout the growing season. These results have implications for a wide range of large-scale annual cropping systems that utilize neonicotinoid seed treatments."Krupke, Hunt, et al. (2012)

The same conclusions come from Dainat, Evans, et al. (2012), summarizing predicting factors for population declines in the hives:

B. Dainat, J. D. Evans, Y. P. Chen, L. Gauthier and P. Neumann (2012)
Predictive markers of Honeybee colony collapse, PLoS ONE, 7, 2, e32151

"Across the Northern hemisphere, managed Honeybee colonies, Apis mellifera, are currently affected by abrupt depopulation during winter and many factors are suspected to be involved, either alone or in combination. Parasites and pathogens are considered as principal actors, in particular the ectoparasitic mite Varroa destructor, associated viruses and the microsporidian Nosema ceranae. Here we used long term monitoring of colonies and screening for eleven disease agents and genes involved in bee immunity and physiology to identify predictive markers of honeybee colony losses during winter. The data show that DWV, Nosema ceranae, Varroa destructor and Vitellogenin can be predictive markers for winter colony losses, but their predictive power strongly depends on the season. In particular, the data support that V. destructor is a key player for losses, arguably in line with its specific impact on the health of individual bees and colonies". Dainat, Evans, et al. (2012)
<Go to ISI>://MEDLINE:22384162 AND http://www.ask-force.org/web/Bees/Dainat-Predictive-Markers-CCD-2012.pdf

A new systems approach for the understanding of honeybee decline is proposed by Becher, Osborne, et al. (2013): following a stocktaking and synthesis of existing models they propose:

1. The health of managed and wild honeybee colonies appears to have declined substantially in Europe and the United States over the last decade. Sustainability of honeybee colonies is important not only for honey production, but also for pollination of crops and wild plants alongside other insect pollinators. A combination of causal factors, including parasites, pathogens, land use changes and pesticide usage, are cited as responsible for the increased colony mortality.
2. However, despite detailed knowledge of the behavior of honeybees and their colonies, there are no suitable tools to explore the resilience mechanisms of this complex system under stress. Empirically testing all combinations of stressors in a systematic fashion is not feasible. We therefore suggest a cross-level systems approach, based on mechanistic modelling, to investigate the impacts of (and interactions between) colony and land management.
3. We review existing honeybee models that are relevant to examining the effects of different stressors on colony growth and survival. Most of these models describe honeybee colony dynamics, foraging behavior or honeybee – varroa mite – virus interactions.
4. We found that many, but not all, processes within honeybee colonies, epidemiology and foraging are well understood and described in the models, but there is no model that couples in-hive dynamics and pathology with foraging dynamics in realistic landscapes.
5. Synthesis and applications. We describe how a new integrated model could be built to simulate multifactorial impacts on the honeybee colony system, using building blocks from the reviewed models. The development of such a tool would not only highlight empirical research priorities but also provide an important forecasting tool for policy makers and beekeepers, and we list examples of relevant applications to bee disease and landscape management decisions.Becher, Osborne, et al. (2013)

Note also the Wikipedia contribution on a list of diseases of the honey-bees with some additional items not mentioned in this report:
http://en.wikipedia.org/wiki/List_of_diseases_of_the_honey_bee#cite_note-mite-away-2

Final comments of the reviewer:
The Varroa mite seems to be in the center of all detrimental effects, and it needs still lots of field experimental work to determine which factors are really reducing the resistance of bees against the mite, but this might be futile in the light of the Monsanto project, which takes into

account the lowering of immune levels by the Varroa mite, as well as other external factors having the same effect.

Monsanto (2012), Honeybee Health The Challenge, published by Monsanto http://www.monsanto.com/improvingagriculture/pages/Honeybee-health.aspx AND http://www.ask-force.org/web/Bees/Monsanto-Honeybee-Health-2012.pdf

"In 2011, Monsanto acquired the Israel-based company, Beeologics. Beeologics research focuses on testing biological products to provide targeted control of pests and diseases in order to provide safe, effective ways to protect the Honeybee. For example, a major factor of Colony Collapse Disorder is credited to the parasitic Varroa mite. This mite weakens bees' immune system and spreads viruses.
Currently, BioDirect - our first biological technology platform - is in discovery phase, but has shown promising results in testing that it could be effective against specific insects, such as Varroa mites, while leaving beneficial insects unaffected. To put it simply, research is being done to control a problem insect on a beneficial insect". Monsanto (2012)

23. Bibliography Scientific Literature and Reports

Bibliography on bees and CCD with a total of 7604 items from December 2016
From the Web of Sciences and other sources, Klaus Ammann Ammann Klaus (20161203)

Ammann Klaus (20161203) Bibliography Bees and Colony Collapse Disease, WOS and other sources, 7604 refereces Neuchatel 724 pp http://www.ask-force.org/web/Bees/Ammann-Bibliography-Bees-CCD-WOS-etc-20161203.pdf

24. Literature cited

Abrahamovich, A. H., Atela, O., De la Rua, P. and Galian, J. (2007) Assessment of the mitochondrial origin of honey bees from Argentina Journal of Apicultural Research 46 3 191-194 pp ISBN/0021-8839 <Go to ISI>://WOS:000250023400010

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A. and Klein, A. M. (2008) Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency Current Biology 18 20 1572-1575 pp ISBN/0960-9822 http://www.sciencedirect.com/science/article/B6VRT-4TSMC9G-T/2/36b1fa2ed5bd5030846e0c092b3d6744 AND http://www.ask-force.org/web/Bees/Aizen-Long-Term-Pollinator-Dependency-2008.pdf

Aizen, M. A. and Harder, L. D. (2009) The Global Stock of Domesticated Honey Bees Is Growing Slower Than Agricultural Demand for Pollination Current Biology 19 11 915-918 pp ISBN/0960-9822 http://www.sciencedirect.com/science/article/B6VRT-4W7JNHK-3/2/4d4a22402ec95321ff2d39de1dde9902 AND http://www.ask-force.org/web/Bees/Aizen-Global-Stock-Domesticated-2009.pdf

Akimov, I. A., Zaloznaya, L. M., Yefimov, V. M. and Galaktionov, Y. K. (1990) SEASONAL AND GEOGRAPHICAL VARIATION OF THE MORPHOLOGICAL CHARACTERS OF VARROA JACOBSONI (PARASITIFORMES, VARROIDAE) - VARIATION OF MEAN-VALUES, STANDARD DEVIATIONS AND COEFFICIENTS OF THE FLUCTUATING ASYMMETRY Zoologichesky Zhurnal 69 9 27-38 pp ISBN/0044-5134 <Go to ISI>://WOS:A1990EH65300003 NOT IN NEBIS

Alaux, C., Brunet, J. L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L. P. and Le Conte, Y. (2010) Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera) Environmental Microbiology 12 3 774-782 pp ISBN/1462-2912 <Go to ISI>://WOS:000274942300019 AND http://www.ask-force.org/web/Bees/Alaux-Interaction-Neonics-Nosema-2010.pdf

Allen, M. F. and Ball, B. V. (1996) The incidence and world distribution of honey bee viruses Bee World 77 147-162 pp

Ammann, K., Herzig, R., Liebendörfer, L. and Urech, M. (1987) Multivariate Correlation of Deposition Data of 8 different Air Pollutants to Lichen Data in a Small Town in Switzerland Proc. 3rd Int. Congr. on Aerobiology International Association of Aerobiology Basel 401-406 pp http://www.ask-force.org/web/Flechten/Ammann-Multivariate-Corr-1986.PDF

Ammann Klaus (20161203) Bibliography Bees and Colony Collapse Disease, WOS and other sources, 7604 refereces Neuchatel 724 pp http://www.ask-force.org/web/Bees/Ammann-Bibliography-Bees-CCD-WOS-etc-20161203.pdf

Anderson, D. L. and Roberts, J. M. K. (2013) Standard methods for Tropilaelaps mites research Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800019 AND http://www.ask-force.org/web/Bees/Anderson-methods-Tropilaelaps-mites-research-2015.pdf

Anonymous (1869) Report of the Commissioner of Agriculture for the year 1868, Statistics of beekeeping U.S. Government Printing Office Washington 1-688, 278-280 pp https://play.google.com/books/reader?printsec=frontcover&output=reader&id=nFndee5KJN8C&pg=GBS.PR5 AND http://www.ask-force.org/web/Bees/Foul-Brood-Disease-1868-Ag-Report-1869.docx

Anonymous (1874) Report of the Commissioner of Agriculture for the year 1872, Report from Iowa U.S. Government Printing Office Washington 479-480. pp https://play.google.com/books/reader?id=5GZQ5J0s430C&printsec=frontcover&output=reader&authuser=0&hl=en_GB&pg=GBS.PA220

Anonymous (2011) 7th COLOSS Workshop, Belgrade, SERBIA, August 25 -26, 2011 Mellifera 11 21-22 10-46 pp ISBN/1302-5821 <Go to ISI>://BIOSIS:PREV201200460543

Aston, D. (2010) Honey bee winter loss survey for England, 2007-8 Journal of Apicultural Research 49 1 111-112 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500021

Bailey, L. (2002) The Isle of Wight Disease Poole, UK 11 pp
Bailey, L. and Ball, B. V. (1991) Honey Bee Pathology Academic Press London, UK
Bal, R., Erdogan, S., Theophilidis, G., Baydas, G. and Naziroglu, M. (2010) Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording Neurotoxicology 31 1 113-120 pp ISBN/0161-813X <Go to ISI>://WOS:000274611000012

Bal, R., Naziroglu, M., Turk, G., Yilmaz, O., Kuloglu, T., Etem, E. and Baydas, G. (2012) Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats Cell Biochemistry and Function 30 6 492-499 pp ISBN/0263-6484 <Go to ISI>://WOS:000307787200009

Bal, R., Turk, G., Tuzcu, M., Yilmaz, O., Kuloglu, T., Baydas, G., Naziroglu, M., Yener, Z., Etem, E. and Tuzcu, Z. (2013) Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats Drug and Chemical Toxicology 36 4 421-429 pp ISBN/0148-0545 <Go to ISI>://WOS:000323931300005 AND http://www.ask-force.org/web/Pesticides/Bal-Effects-Neonicoinoid-Insects-2013.pdf

Bal, R., Turk, G., Tuzcu, M., Yilmaz, O., Kuloglu, T., Gundogdu, R., Gur, S., Agca, A., Ulas, M., Cambay, Z., Tuzcu, Z., Gencoglu, H., Guvenc, M., Ozsahin, A. D., Kocaman, N., Aslan, A. and Etem, E. (2012a) Assessment of imidacloprid toxicity on reproductive organ system of adult male rats Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 47 5 434-444 pp ISBN/0360-1234 <Go to ISI>://WOS:000303579200010

Bal, R., Turk, G., Yilmaz, O., Etem, E., Kuloglu, T., Baydas, G. and Naziroglu, M. (2012b) Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats Cell Biology and Toxicology 28 3 187-200 pp ISBN/0742-2091 <Go to ISI>://WOS:000303401600006

Baude, M., Kunin, W. E., Boatman, N. D., Conyers, S., Davies, N., Gillespie, M. A. K., Morton, R. D., Smart, S. M. and Memmott, J. (2016) Historical nectar assessment reveals the fall and rise of floral resources in Britain Nature 530 7588 85-88 pp ISBN/0028-0836 http://dx.doi.org/10.1038/nature16532 AND http://www.ask-force.org/web/Bees/Baude-Historical-nectar-assessment-Britain-2016.pdf

Baudry, E., Solignac, M., Garnery, L., Gries, M., Cornuet, J. and Koeniger, N. (1998) Relatedness among honeybees (Apis mellifera) of a drone congregation Proceedings of the Royal Society of London. Series B: Biological Sciences 265 1409 2009-2014 pp http://rspb.royalsocietypublishing.org/content/265/1409/2009.abstract AND http://www.ask-force.org/web/Bees/Baudry-Relatedness-among-honeybee-1998.pdf

Becher, M. A., Osborne, J. L., Thorbek, P., Kennedy, P. J. and Grimm, V. (2013) REVIEW: Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models Journal of Applied Ecology 50 4 868-880 pp ISBN/0021-8901 <Go to ISI>://WOS:000321762900007 and http://www.ask-force.org/web/Golden-Rice/Becher-Towards-Systems-Approach-CCD-2013.pdf

Becher Matthias A., Volker Grimm, Pernille Thorbek, Juliane Horn, Peter J. Kennedy and Osborne, J. L. (2014) BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure 470-482 pp DOI: 10.1111/1365-2664.12222 AND http://onlinelibrary.wiley.com/doi/10.1111/1365-2664.12222/epdf AND http://www.ask-force.org/web/Bees/Becher-BEEHAVE-systems-model-honeybee-colony-dynamics-2014.pdf

Beckert M., Y. D., C. Charlier, H. Darmency, C. Richard, I. Savini, A. Tibi, CNRS, INRA, (2011) Les variétés végétales tolérantes aux herbicides. Effets agronomiques, environnementaux, socio-économiques Expertise scientifique collective, synthèse du rapport, CNRS-INRA (France) France pp http://inra.dam.front.pad.brainsonic.com/ressources/afile/223293-076bc-resource-expertise-vth-synthese.html AND http://www.ask-force.org/web/HerbizideTol/Beckert-Varietes-Vegetales-Tolerantes-Herbicides-2011.pdf

Behle, R. W. and Popham, H. J. R. (2012) Laboratory and field evaluations of the efficacy of a fast-killing baculovirus isolate from Spodoptera frugiperda Journal of Invertebrate Pathology 109 2 194-200 pp ISBN/0022-2011 <Go to ISI>://WOS:000299857500004

Berthoud, H., Imdorf, A., Haueter, M., Radloff, S. and Neumann, P. (2010) Virus infections and winter losses of honey bee colonies (Apis mellifera) Journal of Apicultural Research 49 1 60-65 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500008

Beuhne, R. (1910) Bee mortality. J. Dept. of Agr. of Victoria 7 149 - 151 pp
Beuhne, R. (1916) Bee keeping in Victoria. Department of Agriculture, Victoria Australia Victoria Australia 149 - 151 pp
Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J. and Kunin, W. E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands Science 313 5785 351-354 pp ISBN/0036-8075 <Go to ISI>://WOS:000239154300044 AND http://www.ask-force.org/web/Bees/Biesmeijer-Parallel-Declines-Pollinators-2006.pdf

Blacquiere, T., Smagghe, G., van Gestel, C. A. M. and Mommaerts, V. (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment Ecotoxicology 21 4 973-992 pp ISBN/0963-9292 <Go to ISI>://WOS:000302800900002 AND http://www.ask-force.org/web/Bees/Blacquiere-Neonicotinoids-Bees-Concentrations-2012.pdf

Borsuk, G., Olszewski, K., Strachecka, A., Paleolog, J. and Kasperek, K. (2012) Genetic and morphometric variation of the Varroa destructor developing in standard and small comb cells Medycyna Weterynaryjna 68 10 599-602 pp ISBN/0025-8628 <Go to ISI>://WOS:000309377600005 AND http://www.ask-force.org/web/Bees/Borsuk-Genetic-morphometric-variation-Varroa-destructor-standard-comb-cells-2012.pdf

Botias, C., Martin-Hernandez, R., Dias, J., Garcia-Palencia, P., Matabuena, M., Juarranz, A., Barrios, L., Meana, A., Nanetti, A. and Higes, M. (2012a) The effect of induced queen replacement on Nosema spp. infection in honey bee (Apis mellifera iberiensis) colonies Environmental Microbiology 14 4 845-859 pp ISBN/1462-2912 <Go to ISI>://WOS:000302540200002 AND http://www.ask-force.org/web/Bees/Botias-Effect-Induced-Queen-Replacement-2012.pdf

Botias, C., Martin-Hernandez, R., Garrido-Bailon, E., Gonzalez-Porto, A., Martinez-Salvador, A., De La Rua, P., Meana, A. and Higes, M. (2012b) The growing prevalence of Nosema ceranae in honey bees in Spain, an emerging problem for the last decade Research in Veterinary Science 93 1 150-155 pp ISBN/0034-5288 <Go to ISI>://WOS:000305852200030 AND http://www.ask-force.org/web/Bees/Botias-Growing-Prevalence-Nosema-Bees-Spain-2012.pdf

Bouga, M., Alaux, C., Bienkowska, M., Buchler, R., Carreck, N. L., Cauia, E., Chlebo, R., Dahle, B., Dall'Olio, R., De la Rua, P., Gregorc, A., Ivanova, E., Kence, A., Kence, M., Kezic, N., Kiprijanovska, H., Kozmus, P., Kryger, P., Le Conte, Y., Lodesani, M., Murilhas, A. M., Siceanu, A., Soland, G., Uzunov, A. and Wilde, J. (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping Journal of Apicultural Research 50 1 51-84 pp ISBN/0021-8839 <Go to ISI>://WOS:000287335500006 AND http://www.ask-force.org/web/Bees/Bouga-review-methods-discrimination-bee-populations-2015.pdf

Brodschneider, R., Moosbeckhofer, R. and Crailsheim, K. (2010) Surveys as a tool to record winter losses of honey bee colonies: a two year case study in Austria and South Tyrol Journal of Apicultural Research 49 1 23-30 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500004

Bromenshenk, J. J., Henderson, C. B., Wick, C. H., Stanford, M. F., Zulich, A. W., Jabbour, R. E., Deshpande, S. V., McCubbin, P. E., Seccomb, R. A., Welch, P. M., Williams, T., Firth, D. R., Skowronski, E., Lehmann, M. M., Bilimoria, S. L., Gress, J., Wanner, K. W. and Cramer, R. A., Jr. (2010) Iridovirus and Microsporidian Linked to Honey Bee Colony Decline PLoS ONE 5 10 e13181 pp http://dx.doi.org/10.1371%2Fjournal.pone.0013181 AND http://www.ask-force.org/web/Bees/Bromenshenk-Iridovirus-Microsporidian-Bee-2010.pdf

Buchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., Kryger, P., Spivak, M., Uzunov, A. and Wilde, J. (2013) Standard methods for rearing and selection of Apis mellifera queens Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300007 AND http://www.ask-force.org/web/Bees/Buechler-Standard-Rearing-Selection-20130901.pdf

Canovas, F., De la Rua, P., Serrano, J. and Galian, J. (2008) Geographical patterns of mitochondrial DNA variation in Apis mellifera iberiensis (Hymenoptera : Apidae) Journal of Zoological Systematics and Evolutionary Research 46 1 24-30 pp ISBN/0947-5745 <Go to ISI>://WOS:000252323700003

Canovas, F., de la Rua, P., Serrano, J. and Galian, J. (2011) Microsatellite variability reveals beekeeping influences on Iberian honeybee populations Apidologie 42 3 235-251 pp ISBN/0044-8435 <Go to ISI>://WOS:000292612900001

Carreck, N. L. (2016) Bees, Britain and the EU … Bee World 93 1 1-1 pp ISBN/0005-772X http://dx.doi.org/10.1080/0005772X.2016.1222791 AND http://www.ask-force.org/web/Bees/Carreck-Bees-Britain-and-EU-2016.pdf

Carreck, N. L., Ball, B. V. and Martin, S. J. (2010) The epidemiology of cloudy wing virus infections in honey bee colonies in the UK Journal of Apicultural Research 49 1 66-71 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500009

Carreck, N. L., Bell, B. V. and Martin, S. J. (2010) Honey bee colony collapse and changes in viral prevalence associated with Varroa destructor Journal of Apicultural Research 49 1 93-94 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500013

Carvalheiro, L. G., Kunin, W. E., Keil, P., Aguirre-Gutierrez, J., Ellis, W. N., Fox, R., Groom, Q., Hennekens, S., Van Landuyt, W., Maes, D., Van de Meutter, F., Michez, D., Rasmont, P., Ode, B., Potts, S. G., Reemer, M., Roberts, S. P. M., Schaminee, J., WallisDeVries, M. F. and Biesmeijer, J. C. (2013) Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants Ecology Letters 16 7 870-878 pp ISBN/1461-023X <Go to ISI>://WOS:000320275100005 AND http://www.ask-force.org/web/Bees/Carvalheiro-Species-richness-declines-NW-European-pollinators-plants-2013.pdf

Carvell, C., Roy, D. B., Smart, S. M., Pywell, R. F., Preston, C. D. and Goulson, D. (2006) Declines in forage availability for bumblebees at a national scale Biological Conservation 132 4 481-489 pp ISBN/0006-3207 <Go to ISI>://WOS:000241348900007 AND http://www.ask-force.org/web/Bees/Carvell-Declines-forage-availability-bumblebees-2006.pdf

Chahbar, N., Munoz, I., Dall'Olio, R., De la Rua, P., Serrano, J. and Doumandji, S. (2013) Population structure of North African honey bees is influenced by both biological and anthropogenic factors Journal of Insect Conservation 17 2 385-392 pp ISBN/1366-638X <Go to ISI>://WOS:000316335900016

Charriere, J.-D. and Neumann, P. (2010) Surveys to estimate winter losses in Switzerland Journal of Apicultural Research 49 1 132-133 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500029

Chauzat, M.-P., Carpentier, P., Madec, F., Bougeard, S., Cougoule, N., Drajnudel, P., Clement, M.-C., Aubert, M. and Faucon, J.-P. (2010) The role of infectious agents and parasites in the health of honey bee colonies in France Journal of Apicultural Research 49 1 31-39 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500005

Chauzat, M.-P., Martel, A.-C., Blanchard, P., Clement, M.-C., Schurr, F., Lair, C., Ribiere, M., Wallner, K., Rosenkranz, P. and Faucon, J.-P. (2010a) A case report of a honey bee colony poisoning Journal of Apicultural Research 49 1 113-115 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500022

Chauzat, M.-P., Martel, A.-C., Zeggane, S., Drajnudel, P., Schurr, F., Clement, M.-C., Ribiere-Chabert, M., Aubert, M. and Faucon, J.-P. (2010b) A case control study and a survey on mortalities of honey bee colonies (Apis mellifera) in France during the winter of 2005-6 Journal of Apicultural Research 49 1 40-51 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500006

Chen, M., Collins, E. M., Tao, L. and Lu, C. S. (2013) Simultaneous determination of residues in pollen and high-fructose corn syrup from eight neonicotinoid insecticides by liquid chromatography-tandem mass spectrometry Analytical and Bioanalytical Chemistry 405 28 9251-9264 pp ISBN/1618-2642 <Go to ISI>://WOS:000326374100027 AND http://www.ask-force.org/web/Bees/Chen-Simultaneous-Determination-2013.pdf

Clairmont, F. R., Milne, R. E., Pham, V. T., Carriere, M. B. and Kaplan, H. (1998) Role of DNA in the activation of the Cry1A insecticidal crystal protein from Bacillus thuringiensis Journal of Biological Chemistry 273 15 9292-9296 pp <Go to ISI>://WOS:000072990800100

Clint R. V. Otto, Roth Cali L., Carlson Benjamin L. and Smart Matthew D. (2016) Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains Proceedings of the National Academy of Sciences 113 37 10430-10435 pp http://www.pnas.org/content/113/37/10430.abstract AND http://www.ask-force.org/web/Bees/Otto-Land-use-change-reduces-suitability2016.pdf

Core, A., Runckel, C., Ivers, J., Quock, C., Siapno, T., DeNault, S., Brown, B., DeRisi, J., Smith, C. D. and Hafernik, J. (2012) A New Threat to Honey Bees, the Parasitic Phorid Fly <italic>Apocephalus borealis</italic> PLoS ONE 7 1 e29639 pp http://dx.doi.org/10.1371%2Fjournal.pone.0029639 AND http://www.ask-force.org/web/Bees/Core-New-Threat-Honey-Bees-2012.pdf

Couty, A., Down, R. E., Gatehouse, A. M. R., Kaiser, L., Pham-Delegue, M. H. and Poppy, G. M. (2001) Effects of artificial diet containing GNA and GNA-expressing potatoes on the development of the aphid parasitoid Aphidius ervi Haliday (Hymenoptera : Aphidiidae) Journal of Insect Physiology 47 12 1357-1366 pp ISBN/0022-1910 <Go to ISI>://WOS:000172427300002

Cox-Foster, D., Conlan, S., Holmes, E. C., Palacios, G., Kalkstein, A., Evans, J. D., Moran, N. A., Quan, P. L., Geiser, D., Briese, T., Hornig, M., Hui, J., Vanengelsdorp, D., Pettis, J. S. and Lipkin, W. I. (2008) The latest buzz about colony collapse disorder - Response Science 319 725-725 pp <Go to ISI>://WOS:000252963000016 AND http://www.ask-force.org/web/Bees/Anderson-Cox-Foster-Controversy-2008.pdf

Cox-Foster, D. and Vanengelsdorp, D. (2009) Saving the HONEYBEE, Solving the Mystery of the Vanishing Bees Scientific American 300 4 40-+ pp ISBN/0036-8733 <Go to ISI>://WOS:000264456700029 AND http://www.scientificamerican.com/article.cfm?id=saving-the-honeybee&print=true AND http://www.ask-force.org/web/Bees/Cox-Foster-Solving-the-Mystery1-2009.pdf

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S. K., Simons, J. F., Egholm, M., Pettis, J. S. and Lipkin, W. I. (2007a) A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder Science, electronic prepring Science Express 1-4 pp http://www.sciencemag.org/cgi/content/abstract/1146498v1 AND http://www.ask-force.org/web/Bees/Cox-Foster-Metagenomic-Survey-2007.pdf

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P. L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J. H., Cui, L. W., Hutchison, S. K., Simons, J. F., Egholm, M., Pettis, J. S. and Lipkin, W. I. (2007b) A metagenomic survey of microbes in honey bee colony collapse disorder Science 318 283-287 pp ISBN/0036-8075 <Go to ISI>://WOS:000250086100050

Crabtree Bill and Wager Robert (20160810) Are bees in peril from neonicotinoids? Farmer evidence challenges doomsayers and 197 comments glp Washington DC 4 text and 37 commentss pp https://www.geneticliteracyproject.org/2016/08/10/bees-peril-neonicotinoids-farmer-evidence-challenges-doomsayers/ AND http://www.ask-force.org/web/Bees/Crabtree-Are-bees-in-peril-neonicotinoids-20160810.pdf

Crailsheim, K., Brodschneider, R., Aupinel, P., Behrens, D., Genersch, E., Vollmann, J. and Riessberger-Galle, U. (2013) Standard methods for artificial rearing of Apis mellifera larvae Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300005 AND http://www.ask-force.org/web/Bees/Crailsheim-Standard-Artificial-Raring-20130901.pdf

Crailsheim, K., Brodschneider, R. and Neuman, P. (2009) The COLOSS puzzle: filling in the gaps Proceedings of the 4th COLOSS Conference, 3-4 March 2009. Zagreb, Croatia 46-47 pp

Currie, R. W., Pernal, S. F. and Guzman-Novoa, E. (2010) Honey bee colony losses in Canada Journal of Apicultural Research 49 1 104-106 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500018

Dahle, B. (2010) The role of Varroa destructor for honey bee colony losses in Norway Journal of Apicultural Research 49 1 124-125 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500026

Dai, P.-L., Zhou, W., Zhang, J., Lang, Z.-H., Zhou, T., Wang, Q., Cui, H.-J., Jiang, W.-Y. and Wu, Y.-Y. (2015) Effects of Bt cry1Ah corn pollen on immature worker survival and development of Apis cerana cerana Journal of Apicultural Research 54 1 72-76 pp ISBN/0021-8839 http://dx.doi.org/10.1080/00218839.2015.1035075 AND http://www.ask-force.org/web/Bees/Dai-Effects-Bt-Cry1Ah-corn-pollen-immature-worker-survival-2015.pdf

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L. and Neumann, P. (2012) Predictive markers of honey bee colony collapse PLoS ONE 7 2 e32151 pp ISBN/1932-6203 <Go to ISI>://MEDLINE:22384162 AND http://www.ask-force.org/web/Bees/Dainat-Predictive-Markers-CCD-2012.pdf

Davison John and Kershen Drew (2014) Honey containing GM-pollen in Europe CropGen London London 1-4 pp http://www.cropgen.org/article_513.html AND http://www.ask-force.org/web/Bees/Davison-Kershen-Honey-Containing-GM-Pollen-Europe-2014.pdf

de Graaf, D. C., Alippi, A. M., Antunez, K., Aronstein, K. A., Budge, G., De Koker, D., De Smet, L., Dingman, D. W., Evans, J. D., Foster, L. J., Funfhaus, A., Garcia-Gonzalez, E., Gregorc, A., Human, H., Murray, K. D., Nguyen, B. K., Poppinga, L., Spivak, M., vanEngelsdorp, D., Wilkins, S. and Genersch, E. (2013) Standard methods for American foulbrood research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300011 AND http://www.ask-force.org/web/Bees/deGraaf-Standard-American-Foulbrood-20130901.pdf

de la Rua, P., Galian, J., Pedersen, B. V. and Serrano, J. (2006) Molecular characterization and population structure of Apis mellifera from Madeira and the Azores Apidologie 37 6 699-708 pp ISBN/0044-8435 <Go to ISI>://WOS:000243036100007

De la Rua, P., Galian, J., Serrano, J. and Moritz, R. F. A. (2003) Genetic structure of Balearic honeybee populations based on microsatellite polymorphism Genetics Selection Evolution 35 3 339-350 pp ISBN/0999-193X <Go to ISI>://WOS:000182656900005

de la Rua, P., Hernandez-Garcia, R., Jimenez, Y., Galian, J. and Serrano, J. (2005) Biodiversity of Apis mellifera iberica (Hymenoptera : Apidae) from northeastern Spain assessed by mitochondrial analysis Insect Systematics & Evolution 36 1 21-28 pp ISBN/1399-560X <Go to ISI>://WOS:000229064200002

De la Rua, P., Jaffe, R., Dall'Olio, R., Munoz, I. and Serrano, J. (2009) Biodiversity, conservation and current threats to European honeybees Apidologie 40 3 263-284 pp ISBN/0044-8435 <Go to ISI>://WOS:000267832600005

De la Rua, P., Jaffe, R., Munoz, I., Serrano, J., Moritz, R. F. A. and Kraus, F. B. (2013) Conserving genetic diversity in the honeybee: Comments on Harpur etal. (2012) Molecular Ecology 22 12 3208-3210 pp ISBN/0962-1083 <Go to ISI>://WOS:000320396100004 AND http://www.ask-force.org/web/Bees/DelaRua-Conserving-Genetic-Diversity-comment-Harpur-2013.pdf

De la Rua, P., Serrano, J. and Galian, J. (1998) Mitochondrial DNA variability in the Canary Islands honeybees (Apis mellifera L.) Molecular Ecology 7 11 1543-1547 pp ISBN/0962-1083 <Go to ISI>://WOS:000076933000010

DEFRA (20130327) An assessment of key evidence about Neonicotinoids and bees Department for Environment, Food and Rural Affairs UK London 9 pp https://www.gov.uk/government/publications/an-assessment-of-key-evidence-about-neonicotinoids-and-bees AND http://www.ask-force.org/web/Bees/DEFRA-Assessment-key-evidence-Neonicotinoids-bees-201303.pdf AND ANNEX http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/defraBumbleBeeReportPS2371V4a.pdf AND http://fera.co.uk/

Delaplane, K. S., van der Steen, J. and Guzman-Novoa, E. (2013) Standard methods for estimating strength parameters of Apis mellifera colonies Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300003 AND http://www.ask-force.org/web/Bees/Delaplane-Standard-Estimating-Parameters-Apis-Cologies-20130901.pdf

Desjardins, A. E. and Proctor, R. H. (2007) Molecular biology of Fusarium mycotoxins International Journal of Food Microbiology 119 1-2 47-50 pp ISBN/0168-1605 http://www.sciencedirect.com/science/article/pii/S0168160507003856 AND http://www.ask-force.org/web/HerbizideTol/Desjardins-Molecular-Biology-Fusarium-2007.pdf

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P. and Ellis, J. D. (2013) Standard methods for varroa research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300009 AND http://www.ask-force.org/web/Bees/Dietemann-Standard-Varroa-20130901.pdf

Dosselli, R., Grassl, J., Carson, A., Simmons, L. W. and Baer, B. (2016) Flight behaviour of honey bee (Apis mellifera) workers is altered by initial infections of the fungal parasite Nosema apis Scientific Reports 6 36649 pp 10.1038/srep36649 AND http://www.nature.com/articles/srep36649#supplementary-information AND http://www.ask-force.org/web/Bees/Dosselli-Flight-behaviour-honey-bee-Nosema-2016.pdf

Doucet-Personeni C., MP. Halm, F. Touffet, A. Rortais and G. Arnold (20030918) Imidaclopride utilisé en enrobage de semences (Gaucho®) et troubles des abeilles Rapport final Comité Scientifique et Technique de l'Etude Multifactorielle des Troubles des Abeilles (CST), CNRS 91198

Gif-sur- Yvette, 106 pp http://agriculture.gouv.fr/IMG/pdf/rapportfin.pdf AND http://www.ask-force.org/web/Bees/Doucet-Personeni-Imidacloprin-Troubles-Abeilles-Rapport-Finale-20030918.pdf

Douglas, M. R., Rohr, J. R. and Tooker, J. F. (2015) Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield Journal of Applied Ecology 52 1 250-260 pp ISBN/0021-8901 <Go to ISI>://WOS:000348740800027 AND http://www.ask-force.org/web/Bees/Douglas-Neonicotinoid-insecticide-travels-through-soil-food-chain-2015.pdf

Duan, J. J., Marvier, M., Huesing, J., Dively, G. and Huang, Z. Y. (2008) A Meta-Analysis of Effects of Bt Crops on Honey Bees (Hymenoptera: Apidae) PLoS ONE 3 1 e1415 pp http://dx.doi.org/10.1371%2Fjournal.pone.0001415 AND http://www.ask-force.org/web/Bt/Duan-Meta-Analysis-Effects-Bees-2008.pdf

EASAC (201504) Ecosystem services, agriculture and neonicotinoids European Academies Halle Germany ISBN 978-3-8047-3437-1/ISBN 978-3-8047-3437-1 http://www.easac.eu/home/reports-and-statements/detail-view/article/ecosystem-se.html AND http://www.ask-force.org/web/Bees/EASAC-Policy-Report-26-Neonicotinoid-201504.pdf

Easton, A. H. and Goulson, D. (2013) The Neonicotinoid Insecticide Imidacloprid Repels Pollinating Flies and Beetles at Field-Realistic Concentrations Plos One 8 1 ISBN/1932-6203 <Go to ISI>://WOS:000315211500032 AND http://www.ask-force.org/web/Bees/Easton-Neonicotinoid-Insecticide-Repels-Pollinating-2013.pdf

EFSA-Opinion (2005a) Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds, Publication date: 03/03/2005, Last updated: 06/07/2006, Adopted date: 19/01/2005 EFSA Parma 1-22 pp reference EFSA-GMO-NL-2004-02), Question number: EFSA-Q-2004-087/reference EFSA-GMO-NL-2004-02), Question number: EFSA-Q-2004-087 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772104.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-1507-food-2005.pdf

EFSA-Opinion (2005b) Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA GMO BE 2004 07) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x MON810 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto, Publication date: 12/07/2005, Last updated: 06/07/2006 Adopted date: 06/07/2005 EFSA Parma 1-25 pp Reference EFSA-GMO-BE-2004-07, Question number: EFSA-Q-2004-159/Reference EFSA-GMO-BE-2004-07, Question number: EFSA-Q-2004-159 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770218.htm, AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-MON863xMON810xNK603-2005.pdf

EFSA-Opinion (2005c) Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA GMO DE 2004 03) for the placing on the market of insect protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto Publication date: 12/07/2005, Last updated: 06/07/2006 Adopted date: 08/06/2005 EFSA Parma 1-25 pp Reference EFSA-GMO-DE-2004-03, Question number: EFSA-Q-2004-112/Reference EFSA-GMO-DE-2004-03, Question number: EFSA-Q-2004-112 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770743.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-MON863xMON810-food-feed-2005.pdf

EFSA-Opinion (2005d) Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA GMO UK 2004 06) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto Publication date: 12/07/2005, Last updated: 06/07/2006, Adopted date: 06/07/2005 EFSA Parma 1-21 pp Reference EFSA GMO UK 2004 06, Question number: EFSA-Q-2004-154/Reference EFSA GMO UK 2004 06, Question number: EFSA-Q-2004-154 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770806.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-MON863xNK603-Ref-EFSA-GMO-UK-2004-06-2005.pdf

EFSA-Opinion (2005e) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation, under Part C of Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds Publication date: 03/03/2005, Last updated: 17/11/2006 Adopted date: 19/01/2005 EFSA Parma 1-33 pp Reference C/ES/01/01, Question number: EFSA-Q-2004-072/Reference C/ES/01/01, Question number: EFSA-Q-2004-072 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620771053.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-M1507-Imp-feed-indust-2005.pdf

EFSA-Opinion (2005f) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification (Reference C/GB/02/M3/3) for the placing on the market of glyphosate-tolerant and insect-resistant genetically modified maize NK603 x MON810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto Publication date: 21/12/2005, Last updated: 21/12/2005, Adopted date: 13/10/2005 EFSA Parma 1-22 pp Reference C/GB/02/M3/3), Question number: EFSA-Q-2005-056/Reference C/GB/02/M3/3), Question number: EFSA-Q-2005-056 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770094.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-1507xNK603-Ref-C-GB-02-M2-3-2005.pdf

EFSA-Opinion (2005g) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the Notification for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. Publication date: 12/07/2005, Last updated: 14/07/2005 Adopted date: 08/06/2005 EFSA Parma 1-22 pp

Reference C/DE/02/9) Question number: EFSA-Q-2003-089/Reference C/DE/02/9) Question number: EFSA-Q-2003-089 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770857.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-MON863xMON810-Ref-C-DE-09-9-2005.pdf

EFSA-Opinion (2005h) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the notification for the placing on the market of insect resistant genetically modified maize Bt11, for cultivation, feed and industrial processing Publication date: 19/05/2005, Last updated: 17/11/2006 Adopted date: 20/04/2005 EFSA Parma 1-33 pp Reference C/F/96/05.10, Question number: EFSA-Q-2004-012/Reference C/F/96/05.10, Question number: EFSA-Q-2004-012 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620771029.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-Bt11cult-feed-industr-2005.pdf

EFSA-Opinion (2005i) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC, Publication date: 25/07/2005, Last updated: 06/07/2006 Adopted date: 08/06/2005 EFSA Parma 1-22 pp Question number: EFSA-Q-2005-055/Question number: EFSA-Q-2005-055 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620770150.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-invoced-Hungary-2005.pdf

EFSA-Opinion (2006a) Opinion of the Scientific Panel on genetically modified organisms [GMO] on an application (Reference EFSA-GMO-UK-2004-05) for the placing on the market of insect-protected and glufosinate and glyphosate-tolerant genetically modified maize 1507 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred and Mycogen Seeds EFSA Parma 1-23 pp Reference EFSA-GMO-UK-2004-05, Question number: EFSA-Q-2004-139/Reference EFSA-GMO-UK-2004-05, Question number: EFSA-Q-2004-139 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769379.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-1507xNK603-2006.pdf

EFSA-Opinion (2006b) Opinion of the Scientific Panel on genetically modified organisms [GMO] related to genetically modified crops (Bt176 maize, MON810 maize, T25 maize, Topas 19/2 oilseed rape and Ms1xRf1 oilseed rape) subject to safeguard clauses invoked according to Article 16 of Directive 90/220/EEC Opinion adopted on 29 March 2006, Publication date: 11/04/2006 Last updated: 27/07/2006 EFSA Parma 1-15 pp Reference EFSA-GMO-UK-2004-05, Question number: EFSA-Q-2005-294/Reference EFSA-GMO-UK-2004-05, Question number: EFSA-Q-2005-294 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769622.htm AND http://www.ask-force.org/web/Regulation/EFSA-Opinion-Maize-Bt176-MON810-t25-2006.pdf

EFSA-Opinion (2008a) Request from the European Commission related to the safeguard clause invoked by Austria on maize MON810 and T25 according to Article 23 of Directive 2001/18/EC, Scientific Opinion of the Panel on Genetically Modified Organisms Question No EFSA-Q-2008-314 The EFSA Journal 891 2-64 pp http://www.ask-force.org/web/EFSA-Scientific-Opinion-MON810-T25-2008.pdf

EFSA-Opinion (2008b) Request from the European Commission related to the safeguard clause invoked by France on maize MON810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003, Scientific Opinion of the Panel on Genetically Modified Organisms The EFSA Journal 850 1-45 pp http://www.ask-force.org/web/Bt/EFSA-French-Safeguard-ClauseMON810-2008.pdf

EFSA-Opinion (2008c) Request from the European Commission related to the safeguard clause invoked by Greece on maize MON810 according to Article 23 of Directive 2001/18/EC Scientific Opinion of the Panel on Genetically Modified Organisms (Question No EFSA-Q-2008-313) The EFSA Journal 757 1-12 pp http://www.ask-force.org/web/Bt/EFSA-Request-Opinjion-Greece-MON810-EFSA-Q-2008-313-2008.pdf

EFSA-Opinion (2008d) Request from the European Commission related to the safeguard clause invoked by Hungary on maize MON810 according to Article 23 of Directive 2001/18/EC1, Scientific Opinion of the Panel on Genetically Modified Organisms (Question No EFSA-Q-2008-316) The EFSA Journal 756 1-18 pp http://www.ask-force.org/web/Bt/EFSA-Request-and-Opinion-EFSA-Q-2008-316-2008.pdf

EFSA-Opinion (2009a) Applications (EFSA-GMO-RX-MON810) for renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON810; (2) feed consisting of and/or containing maize MON810, including the use of seed for cultivation; and of (3) food and feed additives, and feed materials produced from maize MON810, all under Regulation (EC) No 1829/2003 from Monsanto The EFSA Journal 1149 1-85 pp http://www.ask-force.org/web/Bt/EFSA-Opinion-MON810-finalopinion_en_rev-2009.pdf AND http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902628240.htm AND http://www.ask-force.org/web/Bt/EFSA-Technical-Meeting-MON81-final-2009.pdf

EFSA-Opinion (2009b) Opinion on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta Scientific Opinion of the Panel on Genetically Modified Organisms The EFSA Journal 977 1-13 pp http://www.ask-force.org/web/Bt/EFSA-Opinion-EFSA-GMO-RX-Bt11-2009.pdf

EFSA-Report (20140224) Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations EFSA Journal 12 3, 3594 102 pp doi:10.2903/j.efsa.2014.3594 AND www.efsa.europa.eu/efsajournal AND http://www.ask-force.org/web/EFSA/EFSA-Report-Integrated-Environmental-Risk-Bees-2014.pdf

EFSA Conclusion (20130314) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin EFSA Journal 11 1 http://www.efsa.europa.eu/en/efsajournal/doc/3066.pdf AND http://www.ask-force.org/web/Bees/EFSA-Conclusion-Clothianidin-Bees-20130314.pdf

EFSA conclusion (20130322) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance fipronil EFSA Journal 2013 11 5 3158 pp 10.2903/j.efsa.2013.3158 AND www.efsa.europa.eu/efsajournal AND http://www.ask-force.org/web/EFSA/EFSA-Conclusion-Pesticide-Fipronil-20130323.pdf

El Hassani, A. K., Dacher, M., Gary, V., Lambin, M., Gauthier, M. and Armengaud, C. (2008) Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (Apis mellifera) Archives of Environmental Contamination and Toxicology 54 4 653-661 pp ISBN/0090-4341 <Go to ISI>://WOS:000254749700011 AND http://www.ask-force.org/web/Bees/El-Hassani-Effects-Lethal-Sublethal-Acetamiprid-2008.pdf

Ellis, J. D., Graham, J. R. and Mortensen, A. (2013) Standard methods for wax moth research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300010 AND http://www.ask-force.org/web/Bees/Ellis-Standard-Wax-Moth-Research-20130901.pdf

Ellis, J. D. and Munn, P. A. (2005) The worldwide health status of honey bees. Bee World 85 88-101 pp
Entine Jon (20130430) The Politics of Bees Turns Science on its Head -- Europe Bans Neonics While Local Beekeepers, Scientists Say Action is Precipitous FORBES FORBES New York 9 pp http://www.forbes.com/sites/jonentine/2013/04/30/the-politics-of-bees-turns-science-on-its-head-europe-bans-neonics-while-local-beekeepers-scientists-say-action-is-precipitous/ AND http://www.ask-force.org/web/Bees/Entine-Politics-Bees-Turns-Science-20130430.pdf

Entine Jon (20141125) Bee deaths and neonics: Inside story of Colony Collapse Disorder, Harvard's Chensheng Lu's crusade Genetic Literacy Project Arlington VA, USA http://www.geneticliteracyproject.org/2014/11/25/bee-deaths-neonics-inside-story-of-colony-collapse-disorder-harvards-chensheng-lus-crusade/ AND http://www.ask-force.org/web/Bees/Entine-Bee-Deaths-Neonics-Inside-Story-20141125.pdf

Evans, J. D., Schwarz, R. S., Chen, Y. P., Budge, G., Cornman, R. S., De la Rua, P., de Miranda, J. R., Foret, S., Foster, L., Gauthier, L., Genersch, E., Gisder, S., Jarosch, A., Kucharski, R., Lopez, D., Lun, C. M., Moritz, R. F. A., Maleszka, R., Munoz, I. and Alice Pinto, M. (2013) Standard methods for molecular research in Apis mellifera Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800009 AND http://www.ask-force.org/web/Bees/Evans-Standard-methods-molecular-research-Apis-mellifera-2015.pdf

Fantham, H. B. and Porter, A. (1912) The morphology and life history of Nosema apis and the significance of its various stages in the socalled 'Isle of Wight Disease' in bees (Microsporidiosis) Annals of Tropical Medicine and Parasitology 6 163-195 pp http://www.ask-force.org/web/Bees/Fantham-Morphology-Life-History-Nosema-apis-1912.pdf

Feltham, H., Park, K. and Goulson, D. (2014) Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency Ecotoxicology 1-7 pp ISBN/0963-9292 http://dx.doi.org/10.1007/s10646-014-1189-7 AND http://www.ask-force.org/web/Pesticides/Feltham-Field-realistic-doses-Imidacloprid-2014.pdf AND http://www.ask-force.org/web/Pesticides/Feltham-Field-Release-Imidacloprid-Press-2014.pdf

Ferroglio, E., Zanet, S., Peraldo, N., Tachis, E., Trisciuoglio, A., Laurino, D. and Porporato, M. (2013) Nosema ceranae has been infecting honey bees Apis mellifera in Italy since at least 1993 Journal of Apicultural Research 52 2 60-61 pp ISBN/0021-8839 <Go to ISI>://WOS:000317041800011 AND http://www.ask-force.org/web/Bees/Ferroglio-Nosema-Italy-at-least-1993.pdf

Finley, J., Camazine, S. and Frazier, M. (1996) The epidemic of honey bee colony losses during the 1995-1996 season American Bee Journal 136 11 805-808 pp ISBN/0002-7626 <Go to ISI>://WOS:A1996VR28100022 AND Year 1996 not in NEBIS, not in Google Scholar

Foote, L. H. (1966) The mystery of the disappearing bees Gleanings in Bee Culture, 94 152-153 pp
Forsgren, E., Budge, G. E., Charriere, J. D. and Hornitzky, M. A. Z. (2013) Standard methods for European foulbrood research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300012 AND http://www.ask-force.org/web/Bees/Forsgren-Standard-European-Foulbrood-20130901.pdf

Fremuth, W. (1984) EXPERIMENTAL-DATA ON THE DEVELOPMENT OF VARROA-JACOBSONI IN STANDARDIZED COLONIES Apidologie 15 3 250-251 NOT IN LIST pp ISBN/0044-8435 <Go to ISI>://WOS:A1984TQ05900008 NOT IN WEBSITE, NOT IN NEBIS

Fries, I. Nosema ceranae in European honey bees (Apis mellifera) Journal of Invertebrate Pathology 103 Supplement 1 S73-S79 pp ISBN/0022-2011 http://www.sciencedirect.com/science/article/B6WJV-4XNMC3B-9/2/062b53a66b814938bc8181f0deafaac9 AND http://www.botanischergarten.ch/Bees/Fries-Nosema-Ceranae-Europe-2010.pdf

Fries, I., Chauzat, M. P., Chen, Y. P., Doublet, V., Genersch, E., Gisder, S., Higes, M., McMahon, D. P., Martin-Hernandez, R., Natsopoulou, M., Paxton, R. J., Tanner, G., Webster, T. C. and Williams, G. R. (2013) Standard methods for Nosema research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300014 AND http://www.ask-force.org/web/Bees/Fries-Standard-Nosema-Research-20130901.pdf

Gabriel, D., Sait, S. M., Hodgson, J. A., Schmutz, U., Kunin, W. E. and Benton, T. G. (2010) Scale matters: the impact of organic farming on biodiversity at different spatial scales Ecology Letters 13 7 858-869 pp ISBN/1461-0248 http://dx.doi.org/10.1111/j.1461-0248.2010.01481.x AND http://www.ask-force.org/web/Biotech-Biodiv/Gabriel-Scale-Matters-Organic.2010.pdf AND Times: http://www.ask-force.org/web/Biotech-Biodiv/Webster-Study-Spikes-organic-Times-201005.PDF

Gajger, I. T., Tomljanovic, Z. and Petrinec, Z. (2010) Monitoring health status of Croatian honey bee colonies and possible reasons for winter losses Journal of Apicultural Research 49 1 107-108 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500019

Gajger, I. T., Vugrek, O., Petrinec, Z., Grilec, D. and Tomljanovic, Z. (2010) Detection of Nosema ceranae in honey bees from Croatia Journal of Apicultural Research 49 4 340-341 pp ISBN/0021-8839 <Go to ISI>://WOS:000283013700008

Garrido, P. M., Martin, M. L., Negri, P. and Eguaras, M. J. (2013) A standardized method to extract and store haemolymph from Apis mellifera and the ectoparasite Varroa destructor for protein analysis Journal of Apicultural Research 52 2 ISBN/0021-8839 <Go to ISI>://WOS:000317041800013 AND http://www.ask-force.org/web/Bees/Garrido-Standardized-method-extract-store-haemolymph-2015.pdf

Genersch, E., Gisder, S., Hedtke, K., Hunter, W. B., Mockel, N. and Muller, U. (2013) Standard methods for cell cultures in Apis mellifera research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300002 AND http://www.ask-force.org/web/Bees/Genersch-Standard-Cell-Cultures-Apis-Mellifera-20130901.pdf

Georg, G. (20151005) Neonicotinoids and Bees: Separating Fact From Fiction. THE SEED INDUSTRY SPEAKS OUT ON THE BAN OF NEONICOTINOIDS SEED TREATMENTS AS THE FULL IMPACT OF THE BAN IS FELT ON EU CROP PRODUCTION europeanseed 2 3 5 pp http://european-seed.com/neonicotinoids-and-bees-separating-fact-from-fiction/ AND http://www.ask-force.org/web/Bees/Goeres-Neonicotinoids-and-Bees-Separating-Fact-From-Fiction-20151005.docx AND http://www.ask-force.org/web/Bees/Goeres-Neonicotinoids-and-Bees-Separating-Fact-From-Fiction-20151005.pdf

Gibbons, D., Morrissey, C. and Mineau, P. (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife Environmental Science and Pollution Research 1-16 pp ISBN/0944-1344 http://www.ask-force.org/web/Bees/Gibbons-Review-Direct-Indirect-Effects-Neonics-Vertebrates-2014.pdf

Girard, M., Chagnon, M. and Fournier, V. (2012) Pollen diversity collected by honey bees in the vicinity of Vaccinium spp. crops and its importance for colony development Botany-Botanique 90 7 545-555 pp ISBN/1916-2790 <Go to ISI>://WOS:000305951300004 AND http://www.ask-force.org/web/Bees/Girard-Pollen-Diversity-Honey-Bees-2012.pdf

Girling, R. D., Lusebrink, I., Farthing, E., Newman, T. A. and Poppy, G. M. (2013) Diesel exhaust rapidly degrades floral odours used by honeybees Sci. Rep. 3 http://dx.doi.org/10.1038/srep02779 AND http://www.ask-force.org/web/Bees/Gierling-Diesel-Exaust-degrades-odours-2013.pdf

Girolami, V., Marzaro, M., Vivan, L., Mazzon, L., Giorio, C., Marton, D. and Tapparo, A. (2013) Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers Journal of Applied Entomology 137 1-2 35-44 pp ISBN/0931-2048 <Go to ISI>://WOS:000313517400004 AND http://www.ask-force.org/web/Bees/Girolami-Aerial-Powdering-Bees-Neonicotinoid-2013.pdf

Girolami, V., Mazzon, L., Squartini, A., Mori, N., Marzaro, M., Di Bernardo, A., Greatti, M., Giorio, C. and Tapparo, A. (2009) Translocation of Neonicotinoid Insecticides From Coated Seeds to Seedling Guttation Drops: A Novel Way of Intoxication for Bees Journal of Economic Entomology 102 5 1808-1815 pp ISBN/0022-0493 <Go to ISI>://WOS:000270605700011 AND http://www.ask-force.org/web/Bees/Girolami-Translocation-Neonicotinoid-Insecticides-2009.pdf

Gitlin Boris (20090924) Is Bee Colony Collapse Disorder linked to GMOs? 2 pp http://gmo-journal.com/index.php/2009/09/24/is-bee-colony-collapse-disorder-linked-to-gmos/[4/8/2010 AND http://www.ask-force.org/web/Bees/Gitlin-CCD-GMOS-20090924.PDF

GMO Compass (20121022) Honey Genius Darmstadt Berlin http://www.gmo-compass.org/eng/database/food/238.honey.html AND http://www.ask-force.org/web/Bees/GMO-Compass-Honey-20121022.pdf

GMO Safety (20120505) Following the ECJ Honey Ruling: Will it now be impossible to grow genetically modified plants outdoors? 2 pp http://www.gmo-safety.eu/news/1351.honey-genetically-plants-ecj.html AND http://www.ask-force.org/web/Bees/GMO-Safety-Bees-European-Court-20110915.pdf

Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Petrokofsky, G., Potts, S. G., Raine, N. E., Vanbergen, A. J. and McLean, A. R. (2014) A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators Proceedings of the Royal Society of London B: Biological Sciences 281 1786 http://rspb.royalsocietypublishing.org/royprsb/281/1786/20140558.full.pdf AND 10.1098/rspb.2014.0558 AND http://rspb.royalsocietypublishing.org/content/281/1786/20140558 AND http://www.ask-force.org/web/Bees/Godfray-Restatement-recent-advances-evidence-neonicotinoids-pollinators-20140507.pdf

Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Potts, S. G., Raine, N. E., Vanbergen, A. J. and McLean, A. R. (2015) A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators Proceedings of the Royal Society of London B: Biological Sciences 282 1818 http://rspb.royalsocietypublishing.org/royprsb/282/1818/20151821.full.pdf AND http://www.ask-force.org/web/Bees/Godfray-Restatement-recent-advances-evidence-neonicotinoids-pollinators-20150924.pdf

Gonzalez, S. (2010) Mind, Disrupted, How Toxic Chemicals May Change How We Think and Who We Are, A Biomonitoring Project with Leaders of the

Learning and Developmental Disabilities Community Learning and Developmental Disabilities Initiative (LDDI), whose leadership partners include: the American Association on Intellectual and Developmental Disabilities (AAIDD), the Autism Society (ASA), the Learning Disabilities Association of America (LDA), and the National Association for the Dually Diagnosed (NADD). 36 pp www.minddisrupted.org AND http://www.botanischergarten.ch/Pesticides/Gonzalez-Mind-Disrupted-report-2010.pdf

Goulson, D. (2015) Neonicotinoids impact bumblebee colony fitness in the field; a reanalysis of the UK's Food & Environment Research Agency 2012 experiment Peerj 3 ISBN/2167-8359 <Go to ISI>://WOS:000352297200005 AND http://www.ask-force.org/web/Bees/Goulson-Neonicotinoids-impact-bumblebee-2015.pdf

Gray, A., Peterson, M. and Teale, A. (2010) An update on recent colony losses in Scotland from a sample survey covering 2006-2008 Journal of Apicultural Research 49 1 129-131 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500028

Guttierrez, D. (2009) Honey bee collapse strikes Japan, up to fifty percent of honey bees gone Natural News 28. April 2009 www.naturalnews.com/026151_Japan_honeybees_honey.html

Guzman-Novoa, E., Emsen, B., Unger, P., Espinosa-Montano, L. G. and Petukhova, T. (2012) Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of Varroa destructor mites in selected strains of worker honey bees (Apis mellifera L.) Journal of Invertebrate Pathology 110 3 314-320 pp ISBN/0022-2011 <Go to ISI>://WOS:000305362700007 AND http://www.ask-force.org/web/Bees/Guzman-Novoa-Genotypic-Variability-Varroa-2012.pdf

Haddad, N., Bataeneh, A., Albaba, I., Obeid, D. and Abrulrahman, S. (2009) Status of colony losses in the Middle East. Proceedings of the 41st Apimondia Congress Montpellier, France 36 pp
Haefeker, W. (2008) Co-existenceo f GM-cropsw ith beekeeping- impact of GM-crops on the supply chain for honey and othcr bee products Peter Lang Frankfurt a.M. http://www.ask-force.org/web/Bt/Haefeker-Coexistence-Bees-2008.PDF

Happach-Kasan, C. (2009) Honigvernichtung im vergangenen Jahr war freiwillig - Pollen von gentechnisch veränderten Pflanzen im Honig unproblematisch German Bundestag, Pressemitteilung Berlin 2 pp http://www.ask-force.org/web/Bees/Happach-Kasan-Honigvernichtung1-2009.pdf

Harpur, B. A., Kent, C. and Zayed, A. (2012) Variation in differentiation across the honey bee, Apis mellifera, genome: signs of adaptive selection Genome 55 10 719-719 pp ISBN/0831-2796 <Go to ISI>://WOS:000311829100017 AND http://www.ask-force.org/web/Bees/Harpur-Variation-in-Differentation-Apis-2012.pdf

Harpur, B. A., Minaei, S., Kent, C. F. and Zayed, A. (2012) Management increases genetic diversity of honey bees via admixture Molecular Ecology 21 18 4414-4421 pp ISBN/1365-294X http://dx.doi.org/10.1111/j.1365-294X.2012.05614.x AND http://www.force.org/web/Bees/Harpur-Management-Increases-Genetic-Diversity-2012.pdf

Harpur, B. A., Minaei, S., Kent, C. F. and Zayed, A. (2013) Admixture increases diversity in managed honey bees: Reply to De la Rua etal. (2013) Molecular Ecology 22 12 3211-3215 pp ISBN/0962-1083 <Go to ISI>://WOS:000320396100005 AND http://www.ask-force.org/web/Bees/Harpur-Admixture-Increases-Reply-Rua-2013.pdf

Harpur, B. A., Sobhani, M. and Zayed, A. (2013) A review of the consequences of complementary sex determination and diploid male production on mating failures in the Hymenoptera Entomologia Experimentalis Et Applicata 146 1 156-164 pp ISBN/0013-8703 <Go to ISI>://WOS:000312222600016 AND http://www.ask-force.org/web/Bees/Harpur-Review-Consequences-Complementary-Sex-2013.pdf

Hartfelder, K., Bitondi, M. M. G., Brent, C. S., Guidugli-Lazzarini, K. R., Simoes, Z. L. P., Stabentheiner, A., Tanaka, E. D. and Wang, Y. (2013) Standard methods for physiology and biochemistry research in Apis mellifera Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300006 AND http://www.ask-force.org/web/Bees/Hartfelder-Standard-Physiology-Chemistry-Apis-Mellifera-20130901.pdf

Harz, M., Mueller, F. and Rademacher, E. (2010) Organic acids: Acute toxicity on Apis mellifera and recovery in the haemolymph Journal of Apicultural Research 49 1 95-96 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500014

Hatjina, F., Bouga, M., Karatasou, A., Kontothanasi, A., Charistos, L., Emmanouil, C., Emmanouil, N. and Maistros, A.-D. (2010) Data on honey bee losses in Greece: a preliminary note Journal of Apicultural Research 49 1 116-118 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500023

Hatjina, F., Papaefthimiou, C., Charistos, L., Dogaroglu, T., Bouga, M., Emmanouil, C. and Arnold, G. (2013) Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo Apidologie 44 4 467-480 pp ISBN/0044-8435 <Go to ISI>://WOS:000319878900011 AND http://www.ask-force.org/web/Bees/Hatjina-Sublethal-Doses-Imidacloprid-2013.pdf

Hendrikx, P., Chauzat, M., Debin, M., Neuman, P., Fries, I., Ritter, W., Brown, M., Mutinelli, F., Le Conte, Y. and Gregorc, A. (2009) Bee Mortality and Bee Surveillance in Europe, SCIENTIFIC REPORT submitted to EFSA 217 pp http://www.efsa.europa.eu/cs/BlobServer/External_Rep/027e.pdf?ssbinary=true AND http://www.ask-force.org/web/Bees/Hendrikx-Bee-Mortality-Surveillance-Europe-2009.pdf

Hernandez-Garcia, R., De la Rua, P. and Serrano, J. (2009) Mating frequency in Apis mellifera iberiensis queens Journal of Apicultural Research 48 2 121-125 pp ISBN/0021-8839 <Go to ISI>://WOS:000265703400006

Herzig, R., Liebendorfer, L., Urech, M., Ammann, K., Cuecheva, M. and Landolt, W. (1989) Passive Biomonitoring With Lichens As A Part Of An Integrated Biological Measuring System For Monitoring Air-Pollution In Switzerland International Journal of Environmental Analytical Chemistry 35 1 43-57 pp ISBN/0306-7319 <Go to ISI>://WOS:A1989T753600004 AND http://www.ask-force.org/web/Flechten/Passive-Biomonitoring.pdf

Herzig, R., Urech, M., Liebendörfer, L., Ammann Klaus, Guecheva, M. and Landolt, W. (1990) Lichens as Biological Indicators of Air Pollution in Switzerland: Passive Biomonitoring as a Part of an Integrated Measuring System for Monitoring Air Pollution VCH Weinheim u. New York 448, 317- 332 3-527-28054-5 and 0-89573-962-3/3-527-28054-5 and 0-89573-962-3 http://www.ask-force.org/web/Flechten/Herzig-Lichens-Biol-Ind-1990.PDF

Higes, M., Martin-Hernandez, R., Botias, C., Bailon, E. G., Gonzalez-Porto, A. V., Barrios, L., del Nozal, M. J., Bernal, J. L., Jimenez, J. J., Palencia, P. G. and Meana, A. (2008a) How natural infection by Nosema ceranae causes honeybee colony collapse Environmental Microbiology 10 10 2659-2669 pp ISBN/1462-2912 <Go to ISI>://WOS:000259147900017 AND http://www.ask-force.org/web/Bees/Higes-Natural-Infection-Nosema-2008.pdf

Higes, M., Martin-Hernandez, R., Garrido-Bailon, E., Botias, C., Garcia-Palencia, P. and Meana, A. (2008b) Regurgitated pellets of Merops apiaster as fomites of infective Nosema ceranae (Microsporidia) spores Environmental Microbiology 10 5 1374-1379 pp ISBN/1462-2912 <Go to ISI>://WOS:000254666700024

Hoefer Eberhard and Jany Klaus (2014) Honig-Urteil: Außer Spesen nichts gewesen NOVO Argumente NOVO München 1-6 pp http://novoargumente.com/magazin.php/novo_notizen/artikel/0001520#comments AND http://www.ask-force.org/web/Bees/Hoefer-Jany-Honig-Urteil-Ausser-Spesen-nichts-gewesen-201402.pdf AND English translation: http://www.ask-force.org/web/Bees/Hoefer-Jany-Honey-Judgement-NOVO-201402.pdf

Huang, Z. Y., Hanley, A. V., Pett, W. L., Langenberger, M. and Duan, J. J. (2004) Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees Journal of Economic Entomology 97 5 1517-1523 pp <Go to ISI>://000224653200005 AND http://www.ask-force.org/web/Bees/Huang-Field-Bees-2004.pdf

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Jaffe, R., Jensen, A. B., Koehler, A., Magyar, J. P., Ozkyrym, A., Pirk, C. W. W., Rose, R., Strauss, U., Tanner, G., Tarpy, D. R., van der Steen, J. J. M., Vaudo, A., Vejsnaes, F., Wilde, J., Williams, G. R. and Zheng, H.-Q. (2013) Miscellaneous standard methods for Apis mellifera research Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800008 AND http://www.ask-force.org/web/Bees/Human-Miscellaneous-standard-methods-Apis-mellifera-research-2015.pdf

Ivanova, E. N. and Petrov, P. P. (2010) Regional differences in honey bee winter losses in Bulgaria during the period 2006-9 Journal of Apicultural Research 49 1 102-103 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500017

Jaffe, R., Dietemann, V., Allsopp, M. H., Costa, C., Crewe, R. M., Dall'olio, R., de la Rua, P., El-Niweiri, M. A. A., Fries, I., Kezic, N., Meusel, M. S., Paxton, R. J., Shaibi, T., Stolle, E. and Moritz, R. F. A. (2010) Estimating the Density of Honeybee Colonies across Their Natural Range to Fill the Gap in Pollinator Decline Censuses Conservation Biology 24 2 583-593 pp ISBN/0888-8892 <Go to ISI>://WOS:000275877600028

Jany, K. D. (2012) Auswirkungen und Herausforderungen des EuGH-Urteils zu Pollen im Honig Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety 7 3 175-177 pp ISBN/1661-5751 <Go to ISI>://WOS:000307766100001 AND http://www.ask-force.org/web/Bees/Jany-Auswirkungen-Herausforderungen-Pollen-Honig-2012.pdf

Jany Klaus-Dieter and Eberhard Höfer (2015) The German Beekeepers and Modern Plant Biotechnology Wissenschaftsverlag Dr. Kovac Hamburg Hamburg 192 pp ISBN 978-3-8300-8224-8/ISBN 978-3-8300-8224-8 http://www.verlagdrkovac.de/3-8300-8224-X.htm?lang=english AND http://www.ask-force.org/web/Bees/Beekeeper-Jany-Hoefer-2015a.pdf

Jany Klaus and Hoefer Eberhard (20140130) Die deutsche Imkerschaft und die Grüne Gentechnik. Ein chronologischer Überblick zum Kampf der deutschen Imkerschaft gegen die Grüne Gen- technik zum Schutz von Honig und anderen Bienenprodukten. With Annexes 2-4 pp http://www.ask-force.org/web/Bees/Jany-Hoefer-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-2-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-4-Chronologie-Deutsche-Imker-2014.pdf AND http://www.ask-force.org/web/Bees/Jany-Hoefer-Annex-3-Chronologie-Deutsche-Imker-2014.pdf AND Summary in English http://www.ask-force.org/web/Bees/Jany-Hoefer-Chronology-English-Summary-2014.pdf

Jany Klaus Dieter and Eberhard Höfer (2014) Die deutsche Imkerschaft und Grüne Gentechnik Verlag Dr. Kovac Hamburg 210 pp SBN 978-3-8300-8038-1/SBN 978-3-8300-8038-1 http://www.verlagdrkovac.de/978-3-8300-8038-1.htm AND for personal use only: http://www.ask-force.org/web/Bees/Jany-Hoefer-Deutsche-Imkerschaft-Gentechnik-2014.pdf

Jara, L., Cepero, A., Garrido-Bailon, E., Martin-Hernandez, R., Higes, M. and De la Rua, P. (2012) Linking evolutionary lineage with parasite and pathogen prevalence in the Iberian honey bee Journal of Invertebrate Pathology 110 1 8-13 pp ISBN/0022-2011 <Go to ISI>://WOS:000303639100002

Jensen, A. B., Aronstein, K., Flores, J. M., Vojvodic, S., Palacio, M. A. and Spivak, M. (2013) Standard methods for fungal brood disease research Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300013 AND http://www.ask-force.org/web/Bees/Jensen-Standard-Fungal-Brood-Disease-20130901.pdf

Johnson, R. (2007) Recent Honey Bee Colony Declines CSR Report for Congress, Order Code RL33938 Washington 13 pp CSR Report for Congress, Order Code RL33938/CSR Report for Congress, Order Code RL33938 http://www.ask-force.org/web/Bees/Johnson-CCD-CRS-2007.pdf

Johnson, R. M., Evans, J. D., Robinson, G. E. and Berenbaum, M. R. (2009) Changes in transcript abundance relating to colony collapse disorder in honey bees (Apis mellifera) Proceedings of the National Academy of Sciences 14790–14795 pp http://www.pnas.org/content/early/2009/08/21/0906970106.abstract AND http://www.ask-force.org/web/Bees/Johnson-Changes-Transscript-CCD-2009.pdf

Johnson, R. M., Pollock, H. S. and Berenbaum, M. R. (2009) Synergistic Interactions Between In-Hive Miticides in Apis mellifera Journal of Economic Entomology 102 2 474-479 pp ISBN/0022-0493 <Go to ISI>://WOS:000264899500003 AND http://www.ask-force.org/web/Bees/Johnson-Synergistic-Actions-2009.pdf

Kaatz, H. H. (2004) Auswirkungen von Bt-Maispollen auf die Honigbiene - Methodenentwicklung zu Wirkungsprüfung und Monitoring. Schlussbericht 2004 zu Nr. 3.3 BNest-BMBF 98 (2001 - 2004) Universität Jena, Institut für Ernährungswissenschaften LB Apidologie Bonn, Germany 1-48 pp http://edok01.tib.uni-hannover.de/edoks/e01fb07/541693832.pdf AND http://www.ask-force.org/web/Bees/Kaatz-Abschlussbericht-Verbundprojekt-54169383-2004.pdf

Kaatz, H. H., Hagedorn, H. H. and Engels, W. (1985) Culture of Honeybee Organs - Development of a New Medium and the Importance of Tracheation In Vitro Cellular & Developmental Biology 21 6 347-352 pp <Go to ISI>://A1985ATV0900007 AND http://www.ask-force.org/web/Bees/Kaatz-Culture-Honeybee-organs-development-1985.pdf

Kaatz, H. H., Hildebrandt, H. and Engels, W. (1992) Primer Effect of Queen Pheromone on Juvenile-Hormone Biosynthesis in Adult Worker Honey-Bees Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 162 7 588-592 pp <Go to ISI>://A1992KA03600003 AND http://www.ask-force.org/web/Bees/Kaatz-Primer-effect-queen-pheromione-biosynthesis-1992.pdf

Kaiser, L., Pham-Delegue, M. H. and Ramirez-Romero, R. (2001) Bt corn and insect helpers Biofutur 2001 207 30-33 pp <Go to ISI>://WOS:000166867400010 AND http://www.ask-force.org/web/Bt1/Kaiser-Mais-Bt-Insectes-auxiliaires-2001.pdf

Kauffeld, N. M., Everitt, J. H. and Taylor, E. A. (1976) Honey bee problems in the Rio Grande Valley of Texas American Bee Journal 116 220-222, 232 pp

Kennedy, C. M., Lonsdorf, E., Neel, M. C., Williams, N. M., Ricketts, T. H., Winfree, R., Bommarco, R., Brittain, C., Burley, A. L., Cariveau, D., Carvalheiro, L. G., Chacoff, N. P., Cunningham, S. A., Danforth, B. N., Dudenhoffer, J. H., Elle, E., Gaines, H. R., Garibaldi, L. A., Gratton, C., Holzschuh, A., Isaacs, R., Javorek, S. K., Jha, S., Klein, A. M., Krewenka, K., Mandelik, Y., Mayfield, M. M., Morandin, L., Neame, L. A., Otieno, M., Park, M., Potts, S. G., Rundlof, M., Saez, A., Steffan-Dewenter, I., Taki, H., Viana, B. F., Westphal, C., Wilson, J. K., Greenleaf, S. S. and Kremen, C. (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems Ecology Letters 16 5 584-599 pp ISBN/1461-023X <Go to ISI>://WOS:000318077200003 AND http://www.ask-force.org/web/Bees/Kennedy-Global-Quantitative-Synthesis-Landscape-2013.pdf

Kent, C. F., Minaei, S., Harpur, B. A. and Zayed, A. (2012) Recombination is associated with the evolution of genome structure and worker behavior in honey bees Proceedings of the National Academy of Sciences of the United States of America 109 44 18012-18017 pp ISBN/0027-8424 <Go to ISI>://WOS:000311149900069 AND http://www.ask-force.org/web/Bees/Kent-Recombination-Associated-Evolution-2012.pdf

Kent, C. F., Minaei, S., Harpur, B. A. and Zayed, A. (2013) Reply to Hunt et al.: Worker-biased genes have high guanine-cytosine content and rates of nucleotide diversity in the honey bee Proceedings of the National Academy of Sciences of the United States of America 110 6 E447-E447 pp ISBN/0027-8424 <Go to ISI>://WOS:000315209800002 AND http://www.ask-force.org/web/Bees/Kent-Reply-Hunt-worker-2013.pdf

Kessler, S. C., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C. and Wright, G. A. (2015) Bees prefer foods containing neonicotinoid pesticides Nature 521 7550 74-U145 pp ISBN/0028-0836 <Go to ISI>://WOS:000354040900035 AND http://www.ask-force.org/web/Bees/Kessler-Bees-prefer-foods-containing-neonicotinoid-pesticides-2015.pdf

Kleinschmidt, G. and Kondos, A. (1979) Colony management for low quality pollens The Australasian Beekeeper. 81 5-6 pp

Kovács-Hostyánszki, A., Haenke, S., Batáry, P., Jauker, B., Báldi, A., Tscharntke, T. and Holzschuh, A. (2013) Contrasting effects of mass-flowering crops on bee pollination of hedge plants at different spatial and temporal scales Ecological Applications 23 8 1938-1946 pp ISBN/1051-0761 http://dx.doi.org/10.1890/12-2012.1 AND http://www.ask-force.org/web/Bees/Kovacs-Hosty-Contrasting-Effects-Wild-Bees-Oilseed-2013.pdf

Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G. and Given, K. (2012) Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields PLoS ONE 7 1 e29268 pp http://dx.doi.org/10.1371%2Fjournal.pone.0029268 AND http://www.ask-force.org/web/Bees/Krupke-Multiple-Routes-Bees-2012.pdf

Kulincevic, J., Ball, B. and Mladjan, V. (1990) Viruses in Honey-Bee Colonies Infested with Varroa Jacobsoni - 1st Findings in Yugoslavia Acta Veterinaria-Beograd 40 1 37-42 pp <Go to ISI>://A1990DB33900006

Kulincevic, J. and Mladjan, V. (1988) Preventive Treatment of American Foulbrood (Bacillus-Larvae) with Sugar-Oil Antibiotic Patties Acta Veterinaria-Beograd 38 5-6 267-272 pp <Go to ISI>://A1988T909400008

Kulincevic, J., Stairs, G. R. and Rothenbuhler.Wc (1969) A Disease of Honey Bee Causing Behavioral Changes and Mortality Journal of Invertebrate Pathology 14 1 13-& pp <Go to ISI>://A1969D709100003

Kulincevic, J. M., Rinderer, T. E. and Mladjan, V. J. (1991a) Effects of Fluvalinate and Amitraz on Bee Lice (Braula-Coeca Nitzsch) in Honey-Bee (Apis-Mellifera L) Colonies in Yugoslavia Apidologie 22 1 43-47 pp <Go to ISI>://A1991FG51000006

Kulincevic, J. M., Rinderer, T. E., Mladjan, V. J. and Buco, S. M. (1991b) Control of Varroa-Jacobsoni in Honeybee Colonies in Yugoslavia by Fumigation with Low-Doses of Fluvalinate or Amitraz Apidologie 22 2 147-153 pp <Go to ISI>://A1991FP08900007

Kulincevic, J. M., Rinderer, T. E., Mladjan, V. J. and Buco, S. M. (1992) 5 Years of Bidirectional Genetic Selection for Honey-Bees Resistant and Susceptible to Varroa-Jacobsoni Apidologie 23 5 443-452 pp <Go to ISI>://A1992KF90500006

Kulincevic, J. M., Rinderer, T. E. and Urosevic, D. J. (1988) Seasonality and Colony Variation of Reproducing and Non-Reproducing Varroa-Jacobsoni Females in Western Honey Bee (Apis-Mellifera) Worker Brood Apidologie 19 2 173-179 pp <Go to ISI>://A1988Q546500007

Kulincevic, J. M., Rothenbuehler, W. C. and Stairs, G. R. (1973) Effect of Presence of a Queen Upon Outbreak of a Hairless-Black Syndrome in Honey Bee Journal of Invertebrate Pathology 21 3 241-247 pp <Go to ISI>://A1973P718300004

Kulincevic, J. M. and Rothenbuhler, W. C. (1975) Selection for Resistance and Susceptibility to Hairless-Black Syndrome in Honeybee Journal of Invertebrate Pathology 25 3 289-295 pp <Go to ISI>://A1975AC29400003

Kulincevic, J. M. and Rothenbuhler, W. C. (1982) Selection for Length of Life in the Honeybee (Apis-Mellifera) Apidologie 13 4 347-352 pp <Go to ISI>://A1982QB14100022

Kulincevic, J. M. and Rothenbuhler, W. C. (1989a) The Effects of Artificial Infection with Chronic Bee Paralysis Virus on Queens from Strains of Honeybee Resistant or Susceptible to Hairless-Black Syndrome Journal of Apicultural Research 28 2 79-80 pp <Go to ISI>://A1989DD17500004

Kulincevic, J. M. and Rothenbuhler, W. C. (1989b) Variation in Food Consumed by Caged Worker Bees from Different Colonies Journal of Apicultural Research 28 1 26-29 pp <Go to ISI>://A1989CV53200004

Kulincevic, J. M., Rothenbuhler, W. C. and Rinderer, T. E. (1982) Disappearing Disease .1. - Effects of Certain Protein-Sources Given to Honeybee Colonies in Florida American Bee Journal 122 3 189-191 pp <Go to ISI>://A1982NE81900006 NOT IN NEBIS, NOT IN EZB

Kulincevic, J. M., Rothenbuhler, W. C. and Rinderer, T. E. (1983) Disappearing Disease .2. Effects of Certain Protein-Sources on Brood Rearing and Length of Life in the Honey Bee under Laboratory Conditions American Bee Journal 123 1 50-53 pp <Go to ISI>://A1983QA25300010 NOT IN NEBIS, NOT IN EZB

Kulincevic, J. M., Rothenbuhler, W. C. and Rinderer, T. E. (1984) Disappearing Disease .3. a Comparison of 7 Different Stocks of the Honey Bee (Apis-Mellifera) Ohio Agricultural Research and Development Center Research Bulletin 1160 1-21 pp <Go to ISI>://A1984TD14900001 AND http://www.ask-force.org/web/Bees/Kulincevic-Disappearing-1984.pdf AND https://kb.osu.edu/dspace/handle/1811/24687

Laloi, D., Sandoz, J. C., Picard-Nizou, A. L., Marchesi, A., Pouvreau, A., Tasei, J. N., Poppy, G. and Pham-Delegue, M. H. (1999) Olfactory conditioning of the proboscis extension in bumble bees Entomologia Experimentalis Et Applicata 90 2 123-129 pp <Go to ISI>://000080275700002

Lamp, B., Url, A., Seitz, K., Eichhorn, J., Riedel, C., Sinn, L. J., Indik, S., Köglberger, H. and Rümenapf, T. (2016) Construction and Rescue of a Molecular Clone of Deformed Wing Virus (DWV) PLOS ONE 11 11 e0164639 pp http://dx.doi.org/10.1371%2Fjournal.pone.0164639 AND http://www.ask-force.org/web/Bees/Lamp-Construction-Rescue-Molecular-Clone-Deformed-Wing-Virus.pdf

Laurino, D., Manino, A., Patetta, A. and Porporato, M. (2013) Toxicity of neonicotinoid insecticides on different honey bee genotypes Bulletin of Insectology 66 1 119-126 pp ISBN/1721-8861 <Go to ISI>://WOS:000319310300017 AND http://www.ask-force.org/web/Bees/Laurino-Toxicity-Neonicotinoid-Insecticides-2013.pdf

Le Conte, Y. and Navajas, M. (2008a) Climate change : impact on bee populations and their illnesses Revue Scientifique Et Technique-Office International Des Epizooties 27 2 485-497 pp ISBN/0253-1933 <Go to ISI>://WOS:000259353700016

Le Conte, Y. and Navajas, M. (2008b) Climate change: impact on honey bee populations and diseases Revue Scientifique Et Technique-Office International Des Epizooties 27 2 499-510 pp ISBN/0253-1933 <Go to ISI>://WOS:000259353700017 AND http://www.ask-force.org/web/Bees/Leconte-Climate-Change-Bees-2008.pdf

Lee, K., Reuter, G. and Spivak, M. (2010) Standardized Sampling Plan to Detect Varroa Density in Colonies and Apiaries American Bee Journal 150 12 1151-1155 pp ISBN/0002-7626 <Go to ISI>://WOS:000285403100016 AND http://www.ask-force.org/web/Bees/Lee-Standard-Detect-Varroa-2010.pdf

Lefebvre, B. and Bassand, D. (2001) Bee selectivity of MAVRIK((R)) (tau-fluvalinate) in tank mix with ERIA((R)) (Difenoconazole, Ergosterol Biosynthesis Inhibitor-EBI). Short, medium and long term effects under semi-field conditions Hazards of Pesticides to Bees 98 71-77 pp ISBN/0293-1915 <Go to ISI>://WOS:000170386300003 NO full text available

Liebendorfer, L., Herzig, R., Urech, M. and Ammann, K. (1988) Evaluation und Kalibrierung der Schweizer Flechten-Indikationsmethode mit wichtigen Luftschadstoffen (Evaluation and Calibration of the Swiss Lichen-Indication- Method with Important Air-Pollutants) Staub Reinhaltung Der Luft 48 6 233-238 pp <Go to ISI>://A1988N818300005 and http://www.ask-force.org/web/Flechten/Liebendoerfer-Evaluierung-Staub-1988.pdf

Lindsley, D. L. and Zimm, G. G. (1992) The genome of Drosophila melanogaster. Academic Press San Diego, USA
Locke, B., Le Conte, Y., Crauser, D. and Fries, I. (2012) Host adaptations reduce the reproductive success of Varroa destructor in two distinct European honey bee populations Ecology and Evolution 2 6 1144-1150 pp ISBN/2045-7758 <Go to ISI>://WOS:000312447900005 AND http://www.ask-force.org/web/Bees/Locke-Host-Adaptations-reduce-reproductive-Success-Varroa-2012.pdf

Logrieco, A., Bottalico, A., Mulé, G., Moretti, A. and Perrone, G. (2004) Epidemiology of Toxigenic Fungi and their Associated Mycotoxins for Some Mediterranean Crops European Journal of Plant Pathology 109 7 645-667 pp http://dx.doi.org/10.1023/A:1026033021542 AND http://www.ask-force.org/web/Bt/Pettitt-Association-Fusarium-2004.pdf

Loper, G. M., Wolf, W. W. and Taylor, O. R., Jr. (1992) Honey Bee Drone Flyways and Congregation Areas: Radar Observations Journal of the Kansas Entomological Society 65 3 223-230 pp ISBN/00228567 http://www.jstor.org/stable/25085360 AND http://www.ask-force.org/web/Bees/Loper-Bee-Drone-Flyways-1992.pdf

LU Chensheng, K. M. W., Richard A. CALLAHAN, (2014) Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder Bulletin of Insectology 67 1 125-130 pp http://www.ask-force.org/web/Bees/Lu-Sub-Lethal-Exposure-Neonicotinoids-Bees-2014.pdf

Mahmood, R., Wagchoure, E. S., Raja, S. and Sarwar, G. (2012) Control of Varroa destructor Using Oxalic Acid, Formic Acid and Bayvarol Strip in Apis mellifera (Hymenoptera: Apidae) Colonies Pakistan Journal of Zoology 44 6 1473-1477 pp ISBN/0030-9923 <Go to ISI>://WOS:000313462300002 AND http://www.ask-force.org/web/Bees/Mahmood-Control-Ectoparasitic-Mites-Thymol-Oxalic-2012.pdf

Malone, L. A. and Giacon, H. A. (1996) Effects of Nosema apis Zander on inbred New Zealand honey bees (Apis mellifera ligustica L) Apidologie 27 6 479-486 pp ISBN/0044-8435 <Go to ISI>://WOS:A1996WX22900007 AND http://www.ask-force.org/web/Bees/Malone-Effects-Nosema-1996.pdf

Malone, L. A. and Pham-Delegue, M. H. (2001) Effects of transgene products on honey bees (Apis mellifera) and bumblebees (Bombus sp.) Apidologie 32 4 287-304 pp <Go to ISI>://000170394700001 AND http://www.ask-force.org/web/Bees/Malone-transgene-2001.pdf

Maori, E., Lavi, S., Mozes-Koch, R., Gantman, Y., Peretz, Y., Edelbaum, O., Tanne, E. and Sela, I. (2007) Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination Journal of General Virology 88 11/12 3428-3438 pp <Go to ISI>://WOS:000251619500026 AND http://www.ask-force.org/web/Bees/Maori-Isolation-Israeli-Acute-2007.pdf

Maori, E., Paldi, N., Shafir, S., Kalev, H., Tsur, E., Glick, E. and Sela, I. (2009) IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion Insect Molecular Biology 18 1 55-60 pp ISBN/0962-1075 <Go to ISI>://WOS:000262516400006 AND http://www.ask-force.org/web/Bees/Maori-IAPV-Silenced-2009.pdf

Martin, S. J., Ball, B. V. and Carreck, N. L. (2010) Prevalence and persistence of deformed wing virus (DWV) in untreated or acaricide-treated Varroa destructor infested honey bee (Apis mellifera) colonies Journal of Apicultural Research 49 1 72-79 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500010

Martin, S. J. and Medina, L. M. (2004) Africanized honeybees have unique tolerance to Varroa mites Trends in Parasitology 20 3 112-114 pp ISBN/1471-4922 <Go to ISI>://WOS:000220183900004 AND http://www.ask-force.org/web/Bees/Martin-Africanized-Honeybees-2004.pdf

Matsumoto, T. (2013) Reduction in homing flights in the honey bee Apis mellifera after a sublethal dose of neonicotinoid insecticides Bulletin of Insectology 66 1 1-9 pp ISBN/1721-8861 <Go to ISI>://WOS:000319310300001 AND http://www.ask-force.org/web/Bees/Matsumoto-Reduction-Homing-Flights-Neonicotinoid-2013.pdf

May-Itza, W. D., Quezada-Euan, J. J. G., Ayala, R. and De La Rua, P. (2012) Morphometric and genetic analyses differentiate Mesoamerican populations of the endangered stingless bee Melipona beecheii (Hymenoptera: Meliponidae) and support their conservation as two separate units Journal of Insect Conservation 16 5 723-731 pp ISBN/1366-638X <Go to ISI>://WOS:000308725900006

Mayack, C. and Naug, D. (2009) Energetic stress in the honeybee Apis mellifera from Nosema ceranae infection Journal of Invertebrate Pathology 100 3 185-188 pp ISBN/0022-2011 <Go to ISI>://WOS:000264850500007 AND http://www.ask-force.org/web/Bees/Maycack-Energetic-Stress-Nosema-2009.pdf

McMullan, J. B. and Brown, M. J. F. (2009) A qualitative model of mortality in honey bee (Apis mellifera) colonies infested with tracheal mites (Acarapis woodi) Experimental and Applied Acarology 47 3 225-234 pp ISBN/0168-8162 <Go to ISI>://WOS:000262972100005 AND http://www.ask-force.org/web/Bees/McMullan-Qualitative-Model-Mortality-2009.pdf

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L. P., Chauzat, M. P., Classen, C., Colin, M. E., Dupont, T., Girolami, V., Johnson, R., Le Conte, Y., Luckmann, J., Marzaro, M., Pistorius, J., Porrini, C., Schur, A., Sgolastra, F., Delso, N. S., van der Steen, J. J. M., Wallner, K., Alaux, C., Biron, D. G., Blot, N., Bogo, G., Brunet, J. L., Delbac, F., Diogon, M., El Alaoui, H., Provost, B., Tosi, S. and Vidau, C. (2013) Standard methods for toxicology research in Apis mellifera, (The COLOSS beebook: Toxicology) Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800012 AND http://www.ask-force.org/web/Bees/Medrzycki-Standard-Methods-toxicology-research-Apis-mellifera-2013.pdf

Medrzycki, P., Sgolastra, F., Bortolotti, L., Bogo, G., Tosi, S., Padovani, E., Porrini, C. and Sabatini, A. G. (2010) Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides Journal of Apicultural Research 49 1 52-59 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500007

Mehlo, L., Gahakwa, D., Nghia, P. T., Loc, N. T., Capell, T., Gatehouse, J. A., Gatehouse, A. M. R. and Christou, P. (2005) An alternative strategy for sustainable pest resistance in genetically enhanced crops PNAS 102 22 7812-7816 pp http://www.pnas.org/cgi/content/abstract/102/22/7812 and http://www.ask-force.org/web/Bt/Mehlo-Atern-Bt-PNAS-2005.pdf

Meixner, M. D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E. and Buechler, R. (2010) Conserving diversity and vitality for honey bee breeding Journal of Apicultural Research 49 1 85-92 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500012

Meixner, M. D., Pinto, M. A., Bouga, M., Kryger, P., Ivanova, E. and Fuchs, S. (2013) Standard methods for characterising subspecies and ecotypes of Apis mellifera Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800004 AND http://www.ask-force.org/web/Bees/Meixner-Standard-methods-subspecies-ecotypes-Apis-mellifera-2015.pdf

Mueller-Jung, J. (2008) Vergifteter Honig, Wie die Imker-Lobby die Forschung bloßstellen wollte, FAZ Frankfurter Allgemeine Zeitung Frankfurt a.M. pp http://www.ask-force.org/web/Bees/Mueller-Jung-Vergifteter-Honig-FAZ-2009.pdf

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. and Pettis, J. S. (2010) High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health PLoS ONE 5 3 ISBN/1932-6203 Go to ISI>://WOS:000275809700007 AND http://www.ask-force.org/web/Bees/Mullin-High-Levels-Miticides-Agrochemicals-2010.pdf

Munkvold, G. P. (2004) Epidemiology of Fusarium diseases and their mycotoxins in maize ears European Journal of Plant Pathology 109 7 705-713 pp <Go to ISI>://000185661500006 AND http://www.ask-force.org/web/Mycotoxins/Munkvold-Epidemiology-Fusarium-2004.pdf

Munoz, I., Dall'Olio, R., Lodesani, M. and De la Rua, P. (2009) Population genetic structure of coastal Croatian honeybees (Apis mellifera carnica) Apidologie 40 6 617-626 pp ISBN/0044-8435 <Go to ISI>://WOS:000273050200004

Munoz, I. and De la Rua, P. (2012) TEMPORAL ANALYSIS OF THE GENETIC DIVERSITY IN A HONEY BEE MATING AREA OF AN ISLAND POPULATION (LA PALMA, CANARY ISLANDS, SPAIN) Journal of Apicultural Science 56 1 41-49 pp ISBN/1643-4439 <Go to ISI>://WOS:000305773900005

Munoz, I., Stevanovic, J., Stanimirovic, Z. and De la Rua, P. (2012) GENETIC VARIATION OF Apis mellifera FROM SERBIA INFERRED FROM MITOCHONDRIAL ANALYSIS Journal of Apicultural Science 56 1 59-69 pp ISBN/1643-4439 <Go to ISI>://WOS:000305773900007

Mutinelli, F., Costa, C., Lodesani, M., Baggio, A., Medrzycki, P., Formato, G. and Porrini, C. (2010) Honey bee colony losses in Italy Journal of Apicultural Research 49 1 119-120 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500024

Neuman, P. and Carreck, N. L. (2010) Honey bee colony losses, guest editorial, open source Journal of Apicultural Research 49 1 1-6 pp DOI 10.3896/IBRA.1.49.1.01, open source AND http://www.ask-force.org/web/Bees/Neumann-Honey-Bee-Colony-Losses-2010.pdf

Neumann, P., Evans, J. D., Pettis, J. S., Pirk, C. W. W., Schafer, M. O., Tanner, G. and Ellis, J. D. (2013) Standard methods for small hive beetle research Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800017 AND http://www.ask-force.org/web/Bees/Neumann-Standard-methods-small-hive-beetle-research-2015.pdf

Neumann, P., Yanez, O., Fries, I. and de Miranda, J. R. (2012) Varroa invasion and virus adaptation Trends in Parasitology 28 9 353-354 pp ISBN/1471-4922 <Go to ISI>://WOS:000308777800001 AND http://www.ask-force.org/web/Bees/Neumann-Varroa-Invasion-Virus-Adaption-2012.pdf

Nguyen, B. K., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Tuirionet, B., Verheggen, F. J., Berkvens, D., De Pauw, E. and Haubruge, E. (2009) Does Imidacloprid Seed-Treated Maize Have an Impact on Honey Bee Mortality? Journal of Economic Entomology 102 2 616-623 pp ISBN/0022-0493 <Go to ISI>://WOS:000264899500021 AND http://www.ask-force.org/web/Bees/Nguyen-Imidacloprid-Seed-2009.pdf

Noleppa Steffen and Hahn Thomas (2013) The Value of Neonicotinoid seed treatment in the European Union. A socioeconomic and environmental review. HFFA Working Paper 01 96 pp http://www.hffa.info/files/wp_1_13_1.pdf AND http://www.ask-force.org/web/Bees/Noleppa-Value-Neonicotinoid-Seed-Treatment-2013.pdf

Nuyttens, D., Devarrewaere, W., Verboven, P. and Foque, D. (2013) Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review Pest Management Science 69 5 564-575 pp ISBN/1526-498X <Go to ISI>://WOS:000317621500002 AND http://www.ask-force.org/web/Bees/Nuyttens-Pesticide-Laden-Dust-Emission-Seeds-3013.pdf

Oertel, E. (1965) Many bee colonies dead of an unknown cause American Bee Journal 105 48-49 pp
OPERA, Dr. Anne Alix Ministry of Agriculture France, Dr. Helen Thompson National Bee Unit UK, Dr. Kiki Machera Benaki Phytopathological Institute Greece, Jens Pistorius Julius KühnInstitut Germany, Dr. Konstantinos Kasiotis Benaki Phytopathological Institute Greece, Dr. Ettore Capri OPERA Research Centre, I., Mike Brown National Bee Unit UK and Alexandru Marchis OPERA (2011,) Farming Bee-good, Bee Health Europe, first report OPERA Piacenza, Brussels 52 pp http://www.ask-force.org/web/Bees/OPERA-Alix-Farming-Bee-Good-Bee-Health-Europe-2011.pdf

OPERA, Dr. Ettore Capri, O. R. C., Dr. Mariano Higes, L. o. B. P. o. C. A. J., Spain,, Dr. Konstantinos Kasiotis, B. P. I., Greece,, Dr. Kyriaki Machera Benaki Phytopathological Institute, G., Alexandru Marchis, O. R. C., Dr Stephen J. Martin, S. U., UK,, Jens Pistorius, J. K.-I., Germany,, Thomas Steeger, E. P. A., U.S.A, and Dr. Helen Thompson, N. B. U., UK and Selwyn Wilkins, National Bee Unit, UK, (2013) Bee health in Europe - Facts & figures 2013, Compendium of the latest information on bee health in Europe Universita Cattolica del Sacro Cuore, Piacenza, Italia 64 pp http://www.ask-force.org/web/Bees/OPERA-Capri-Bea-Health-Europe-Facts-Figures-2013.pdf

ORourke, M. K. and Buchmann, S. L. (1991) STANDARDIZED ANALYTICAL TECHNIQUES FOR BEE-COLLECTED POLLEN Environmental Entomology 20 2 507-513 pp ISBN/0046-225X <Go to ISI>://WOS:A1991FH31500017 AND http://www.ask-force.org/web/Bees/ORourke-standardazed-analytical-techniques-bee-collection-1991.pdf

Pajuelo, A. G., Torres, C. and Bermejo, F. J. O. (2008) Colony losses: a double blind trial on the influence of supplementary protein nutrition and preventative treatment with fumagillin against Nosema ceranae Journal of Apicultural Research 47 1 84-86 pp ISBN/0021-8839 <Go to ISI>://WOS:000254014000014 AND http://www.ask-force.org/web/Bees/Pajuelo-Colony-Losses-2008.pdf

Palacios, G., Hui, J., Quan, P. L., Kalkstein, A., Honkavuori, K. S., Bussetti, A. V., Conlan, S., Evans, J., Chen, Y. P., vanEngelsdorp, D., Efrat, H., Pettis, J., Cox-Foster, D., Holmes, E. C., Briese, T. and Lipkin, W. I. (2008) Genetic analysis of Israel acute paralysis virus: Distinct clusters are circulating in the United States Journal of Virology 82 13 6209-6217 pp <Go to ISI>://WOS:000256947300012 AND http://www.ask-force.org/web/Bees/Palacios-Genetic-Analysis-2008.pdf

Palmer, M. J., Moffat, C., Saranzewa, N., Harvey, J., Wright, G. A. and Connolly, C. N. (2013) Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees Nature Communications 4 ISBN/2041-1723 <Go to ISI>://WOS:000318873900087 AND http://www.ask-force.org/web/Bees/Palmer-Cholinergic-Pesticides-Cause-Mushroom-Body-2013.pdf

Panchin, A. Y. and Tuzhikov, A. I. (2016) Published GMO studies find no evidence of harm when corrected for multiple comparisons Critical Reviews in Biotechnology 1-5 pp ISBN/0738-8551 http://dx.doi.org/10.3109/07388551.2015.1130684 AND http://www.ask-force.org/web/Seralini/Panchin-published-GMO-studies-no-evidence-harm-2016.pdf

Papst, C., Utz, H. F., Melchinger, A. E., Eder, J., Magg, T., Klein, D. and Bohn, M. (2005) Mycotoxins produced by Fusarium spp. in isogenic Bt vs. non-Bt maize hybrids under European corn borer pressure Agronomy Journal 97 1 219-224 pp <Go to ISI>://000226783300030 AND http://www.ask-force.org/web/Bt/Papst-Mycotoxin-Fusarium-2005.pdf

Parasharami, V. A., Naik, V. B., von Arnold, S., Nadgauda, R. S. and Clapham, D. H. (2006) Stable transformation of mature zygotic embryos and regeneration of transgenic plants of chir pine (Pinus roxbughii Sarg.) Plant Cell Reports 24 12 708-714 pp <Go to ISI>://WOS:000234454700003

Paxton, R. J. (2010) Does infection by Nosema ceranae cause "Colony Collapse Disorder" in honey bees (Apis mellifera)? Journal of Apicultural Research 49 1 80-84 pp http://www.ibra.org.uk/articles/20091221_22 AND 10.3896/IBRA.1.49.1.11 <http://dx.doi.org/10.3896/IBRA.1.49.1.11> AND open access http://www.ask-force.org/web/Bees/Paxton-Does-Infection-Nosema-2010.pdf

Pettis, J. S. and Delaplane, K. S. (2010) Coordinated responses to honey bee decline in the USA Apidologie 41 3 256-263 pp ISBN/0044-8435 <Go to ISI>://WOS:000279029200004 AND http://www.ask-force.org/web/Bees/Pettis-Coordinated-Responses-Decline-2010.pdf

Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R. and vanEngelsdorp, D. (2013a) Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen <italic>Nosema ceranae</italic> PLoS ONE 8 7 e70182 pp http://dx.doi.org/10.1371%2Fjournal.pone.0070182 AND http://www.ask-force.org/web/Bees/Pettis-Crop-Pollination-Exposes-Pesticides-2013.pdf

Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R. and Vanengelsdorp, D. (2013b) Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae Plos One 8 7 ISBN/1932-6203 <Go to ISI>://WOS:000322167900111 AND http://www.ask-force.org/web/Bees/Pettis-Crop-Pollination-Exposes-Bees-Pesticides-2013.pdf

Pettis, J. S., vanEngelsdorp, D., Johnson, J. and Dively, G. (2012) Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema Naturwissenschaften 99 2 153-158 pp ISBN/0028-1042 <Go to ISI>://WOS:000300682000008 AND http://www.ask-force.org/web/Bees/Pettis-Pesticide-Exposure-Nosema-2012.pdf

Pham-Délègue, M., Picard-Nizou AL, Arnorld G, Grison R, Toppan A, Olsen L, Masson C (1992) Impact of genetically modified rapeseed on insect pollinators (honeybees) Proceedings of the 2nd International Symposium on "the biosafety results of field tests of genetically modified plants and micro-organisms, May 11-14, 1992 Goslar, Germany

Pierce, C. M. F., Solter, L. F. and Weinzierl, R. A. (2001) Interactions between Nosema pyrausta (Microsporidia : Nosematidae) and Bacillus thuringiensis subsp kurstaki in the European corn borer (Lepidoptera : Pyralidae) Journal of Economic Entomology 94 6 1361-1368 pp <Go to ISI>://000172741000007 AND http://www.ask-force.org/web/Bt/Pierce-Interactions-2001.pdf

Pietri, A., Bertuzzi, T., Pallaroni, L. and Piva, G. (2004) Occurrence of mycotoxins and ergosterol in maize harvested over 5 years in Northern Italy Food Additives and Contaminants 21 5 479-487 pp <Go to ISI>://000221241900009 AND http://www.ask-force.org/web/Bt/Pietri-Ocurrence-Mycotoxins-2004.pdf

Pinto, M. A., Henriques, D., Neto, M., Guedes, H., Munoz, I., Azevedo, J. C. and de la Rua, P. (2013) Maternal diversity patterns of Ibero-Atlantic populations reveal further complexity of Iberian honeybees Apidologie 44 4 430-439 pp ISBN/0044-8435 <Go to ISI>://WOS:000319878900007

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. (2010) Global pollinator declines: trends, impacts and drivers Trends in Ecology & Evolution 25 6 345-353 pp ISBN/0169-5347 <Go to ISI>://WOS:000278682500005 AND http://www.ask-force.org/web/Bees/Potts-Global-pollinator-declines-2010.pdf

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., Neumann, P. and Settele, J. (2010) Declines of managed honey bees and beekeepers in Europe Journal of Apicultural Research 49 1 15-22 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500003 AND http://www.ask-force.org/web/Bees/Potts-Declines-managed-honey-bees-Europe-2010.pdf

Ptaszynska, A. A., Borsuk, G., Anusiewicz, M. and Mulenko, W. (2012) Location of Nosema spp. spores within the body of the honey bee Medycyna Weterynaryjna 68 10 618-621 pp ISBN/0025-8628 <Go to ISI>://WOS:000309377600010 AND http://www.ask-force.org/web/Bees/Ptaszynska-Location-Nosema-spores-Bees-2012.pdf

Radloff, S. E., Hepburn, H. R., Hepburn, C. and de la Rua, P. (2001) Morphometric affinities and population structure of honey bees of the Balearic Islands (Spain) Journal of Apicultural Research 40 3-4 97-103 pp ISBN/0021-8839 <Go to ISI>://WOS:000178286500003

Ramirez-Romero, R., Chaufaux, J. and Pham-Delegue, M. H. (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach Apidologie 36 4 601-611 pp <Go to ISI>://000235837400007 AND http://www.ask-force.org/web/Bees/Ramirez-Romero-Effects-2005.pdf

Ramirez-Romero, R., Desneux, N., A., D., Chaffiol, A. and Pham-Delegue, M. H. (2008a) Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? In Press, Corrected Proof http://www.sciencedirect.com/science/article/B6WDM-4RWK072-2/2/123e7dfc2ecddadee7da81bca89cc97e AND http://www.ask-force.org/web/Bt/Ramirez-Romero-Affect-Bees-2008.pdf

Ramirez-Romero, R., Desneux, N., A., D., Chaffiol, A. and Pham-Delegue, M. H. (2008b) Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? Ecotoxicology and Environmental Safety In Press, Corrected Proof http://www.sciencedirect.com/science/article/pii/S0147651307003065 AND http://www.ask-force.org/web/Bt/Ramirez-Romero-Affect-Bees-2008.pdf

Rands, S. A. and Whitney, H. M. (2010) Effects of pollinator density-dependent preferences on field margin visitations in the midst of agricultural monocultures: A modelling approach Ecological Modelling 221 9 1310-1316 pp ISBN/0304-3800 <Go to ISI>://WOS:000276933200008 AND http://www.ask-force.org/web/Bees/Rands-Effects-Pollinator-Density-dependent-Field-Margin-2010.pdf

Rennie, J., White, P. B. and Harvey, E. J. (1921) Isle of Wight Disease in hive bees Transactions of the Royal Society of Edinburgh 52 737-779 pp

Retschnig, G., Neumann, P. and Williams, G. R. (2014) Thiacloprid-Nosema ceranae interactions in honey bees: Host survivorship but not parasite reproduction is dependent on pesticide dose Journal of Invertebrate Pathology 118 18-19 pp ISBN/0022-2011 <Go to ISI>://WOS:000335541000003 AND http://www.ask-force.org/web/Bees/Retschnig-Thiacloprid-Nosema-ceranae-interactions-bees-2014.pdf

Retschnig, G., Williams, G. R., Mehmann, M. M., Yanez, O., de Miranda, J. R. and Neumann, P. (2014) Sex-Specific Differences in Pathogen Susceptibility in Honey Bees (Apis mellifera) Plos One 9 1 ISBN/1932-6203 <Go to ISI>://WOS:000330237000028 AND http://www.ask-force.org/web/Bees/Retschnig-Sex-Specific-Differences-Pathogen-Susceptibility-Bees-2014.pdf

Ribiere, M., Olivier, V., Blanchard, P., Schurr, F., Celle, O., Drajnudel, P., Faucon, J. P., Thiery, R. and Chauzat, M. P. (2008) The collapse of bee colonies: the CCD case ("Colony collapse disorder") and the IAPV virus (Israeli acute paralysis virus) Virologie 12 5 319-322 pp ISBN/1267-8694 <Go to ISI>://WOS:000262852300001 AND http://www.ask-force.org/web/Bees/Ribiere-CCD-Virologie-2008.pdf

Rivolet Patrick, Belval Olivier and Kastler Guy (20130729) Lettre Ouverte a l'attention de Mr. le Ministre Stephane Le Foll: Objet : OGM cachés, demande d'opposition au développement des colzas et tournesols tolérants aux herbicides Collectif « Appel de Poitiers » – Patrick Rivolet, UNAF – Olivier Belval, Confederation Paysanne – Guy Kastler, 2 pp http://www.confederationpaysanne.fr/rp_article.php?id=1822 AND http://www.ask-force.org/web/Bees/OGM-Caches-Appel-Poitiers-VTH-Abeilles-201111.pdf

Roberts, S. P. M. and Potts, S. G. (2010) The status of European non-Apis bees Journal of Apicultural Research 49 1 137-138 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500031

Rosenkranz, P., Aumeier, P. and Ziegelmann, B. (2010) Biology and control of Varroa destructor Journal of Invertebrate Pathology 103 S96-S119 pp ISBN/0022-2011 <Go to ISI>://WOS:000273993100011 AND http://www.ask-force.org/web/Bees/Rosenkranz-Biology-Control-Varroa-2010.pdf

Rossi, C. D., Roat, T. C., Tavares, D. A., Cintra-Socolowski, P. and Malaspina, O. (2013) Effects of sublethal doses of imidacloprid in malpighian tubules of africanized Apis mellifera (Hymenoptera, Apidae) Microscopy Research and Technique 76 5 552-558 pp ISBN/1059-910X <Go to ISI>://WOS:000317299500015 AND http://www.ask-force.org/web/Bees/Rossi-Effect-Sublethal-Doses-Imidacloprid-2013.pdf

Roudel, M., Aufauvre, J., Corbara, B., Delbac, F. and Blot, N. (2013) New insights on the genetic diversity of the honeybee parasite Nosema ceranae based on multilocus sequence analysis Parasitology 140 11 1346-1356 pp ISBN/0031-1820 <Go to ISI>://WOS:000323383200004 AND http://www.ask-force.org/web/Bees/Roudel-genetic-diversity-Nosema-ceranae-2013.pdf

Roulston, T. a. H. and Goodell, K. (2011) The Role of Resources and Risks in Regulating Wild Bee Populations 293-312 978-0-8243-0156-9/978-0-8243-0156-9 <Go to ISI>://WOS:000286841900016 AND http://www.ask-force.org/web/Bees/Roulston-Role-Resources-Risks-Regulating-Wild-Bee-2011.pdf

Ruiz, C., May-Itza, W. D., Quezada-Euan, J. J. G. and De la Rua, P. (2013) Presence of nuclear copies of mitochondrial origin (NUMTs) in two related species of stingless bee genus Melipona (Hymenoptera: Meliponini) Journal of Zoological Systematics and Evolutionary Research 51 2 107-113 pp ISBN/0947-5745 <Go to ISI>://WOS:000318045300003

Runckel, C., Flenniken, M. L., Engel, J. C., Ruby, J. G., Ganem, D., Andino, R. and DeRisi, J. L. (2011) Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, Nosema, and Crithidia Plos One 6 6 ISBN/1932-6203 <Go to ISI>://WOS:000291607000017 AND http://www.ask-force.org/web/Bees/Runckel-Temporal-_Analysis-Viruses-201106.pdf

Sammataro, D., de Guzman, L., George, S., Ochoa, R. and Otis, G. (2013) Standard methods for tracheal mite research Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800018 AND http://www.ask-force.org/web/Bees/Sammataro-Standard-methods-tracheal-mite-research-2015.pdf

Sandrock, C., Tanadini, L. G., Pettis, J. S., Biesmeijer, J. C., Potts, S. G. and Neumann, P. (2014) Sublethal neonicotinoid insecticide exposure reduces solitary bee reproductive success Agricultural and Forest Entomology 16 2 119-128 pp ISBN/1461-9555 <Go to ISI>://WOS:000334030300002 AND http://www.ask-force.org/web/Bees/Sandrock-Sublethal-neonic-exposure-2013.pdf

Sanford, M. T. (1987) Diseases and Pests of the Honey Bee Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida CIR766 16 pp http://edis.ifas.ufl.edu/BODY_AA090#FOOTNOTE_1 AND http://www.ask-force.org/web/Bees/Sanford-Diseases-1987.pdf

Sanford, M. T. (2007) Colony Collapse Disorder (CCD), an update Bee Culture May 17-19 pp http://www.beeculture.com/storycms/index.cfm?cat=Story&recordID=541 AND http://www.ask-force.org/web/Bees/Sanford-Insecticides-CCD-2007.pdf

Santrac, V., Granato, A. and Mutinelli, F. (2010) Detection of Nosema ceranae in Apis mellifera from Bosnia and Herzegovina Journal of Apicultural Research 49 1 100-101 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500016

Savoy, A. C., Lupan, D. M., Manalo, P. B., Roberts, J. S., Schlageter, A. M., Weinhold, L. C. and Kozel, T. R. (1997) Acute lethal toxicity following passive immunization for treatment of murine cryptococcosis Infection and Immunity 65 5 1800-1807 pp ISBN/0019-9567 <Go to ISI>://WOS:A1997WW39800033

Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., Grunewald, B., Hahshold, S., Karrer, M., Koeniger, G., Koeniger, N., Menzel, R., Mujagic, S., Radspieler, G., Schmickl, T., Schneider, C., Siegel, A. J., Szopek, M. and Thenius, R. (2013) Standard methods for behavioural studies of Apis mellifera Journal of Apicultural Research 52 4 ISBN/0021-8839 <Go to ISI>://WOS:000323845800003 AND http://www.ask-force.org/web/Bees/Scheiner-Standard-methods-behavioural-studies-Apis-2013.pdf

Schindle Shannon and Zienkiewicz, M. (20140930) EU Feeling Impact of Neonics Ban. European growers and industry are feeling the sting of the two-year restriction on the use of neonicotinoids eruopeanseed 1 1 5 pp http://european-seed.com/eu-feeling-impact-neonics-ban/ AND http://www.ask-force.org/web/Bees/Shannon-Zinkiewicz-EU-feeling-impact-on-Neonics-ban-20140930.pdf

Schneider, C. W., Tautz, J., Grunewald, B. and Fuchs, S. (2012) RFID Tracking of Sublethal Effects of Two Neonicotinoid Insecticides on the Foraging Behavior of Apis mellifera Plos One 7 1 ISBN/1932-6203 <Go to ISI>://WOS:000301355700076

Schoening, C., Gisder, S., Geiselhardt, S., Kretschmann, I., Bienefeld, K., Hilker, M. and Genersch, E. (2012) Evidence for damage-dependent hygienic behaviour towards Varroa destructor-parasitised brood in the western honey bee, Apis mellifera Journal of Experimental Biology 215 2 264-271 pp ISBN/0022-0949 <Go to ISI>://WOS:000298404800013 AND http://www.ask-force.org/web/Bees/Schoening-Evidence-Damage-Dependent-Behaviour-Varroa-2012.pdf

Science20 (20151023) Bees Addicted To Neonics: A Failure of Science Journalism Science20 Science Blogging http://www.science20.com/news_articles/bees_addicted_to_neonics_a_failure_of_science_journalism-158154 AND http://www.ask-force.org/web/Bees/Science-2-0-Bees-Addicted-to-Neonics-Failure-Science-Journalism-20151023.pdf

Scienceindustries (20130618) Bienengesundheit, Fact Sheet ScienceIndustries 1 pp http://www.scienceindustries.ch/_file/12424/20130618-fact-sheet-biene-mediendossier.pdf AND http://www.ask-force.org/web/Bees/ScienceIndustries-Factsheet-Bene-Mediendossier-20130618.pdf

Seeley, T. D. and Tarpy, D. R. (2007) Queen promiscuity lowers disease within honeybee colonies Proceedings of the Royal Society B-Biological Sciences 274 1606 67-72 pp ISBN/0962-8452 <Go to ISI>://WOS:000242765300009 AND http://www.ask-force.org/web/Bees/Seeley-Queen-Promiscuity-Lowers-Disease-2007.pdf

Seufert, V., Ramankutty, N. and Foley, J. A. (2012) Comparing the yields of organic and conventional agriculture Nature advance online publication ISBN/1476-4687 http://dx.doi.org/10.1038/nature11069 AND http://www.nature.com/nature/journal/vaop/ncurrent/abs/nature11069.html#supplementary-information AND http://www.ask-force.org/web/Organic/Seufert-Comparing-Yields-2012.pdf AND http://www.ask-force.org/web/Organic/Seufert-Supplement-Excluded-2012.xlsb AND http://www.ask-force.org/web/Organic/Seufert-Supplementary-PDF.pdf AND http://www.ask-force.org/web/Organic/Seufert-Excell-Adressen-2012.xlsb

Shaibi, T., Munoz, I., Dall'Olio, R., Lodesani, M., De la Rua, P. and Moritz, R. F. A. (2009) Apis mellifera evolutionary lineages in Northern Africa: Libya, where orient meets occident Insectes Sociaux 56 3 293-300 pp ISBN/0020-1812 <Go to ISI>://WOS:000268102600010

Sharma, V. P. and Kumar, N. R. (2009) Changes in honeybee behaviour and biology under the influence of cellphone radiations Current Science 98 10 1376-1378 pp ISBN/0022-3042 <Go to ISI>://WOS:000268550400621 AND http://www.ask-force.org/web/Bees/Sharma-Changes-Honeybee-Cellphone-2010.pdf AND an alarmist newspaper flash http://www.ask-force.org/web/Bees/Lean-CCD-Cellphones-The-Independent-20070415.PDF

Sheets, L. P., Li, A. A., Minnema, D. J., Collier, R. H., Creek, M. R. and Peffer, R. C. (2016) A critical review of neonicotinoid insecticides for developmental neurotoxicity Critical Reviews in Toxicology 46 2 153-190 pp ISBN/1040-8444 <Go to ISI>://WOS:000368449400003 AND http://www.ask-force.org/web/Bees/Sheens-Critical-review-neonicotinoid-developmental-neurotoxicity-2016.pdf

Sherman, P. W., Seeley, T. D. and Reeve, H. K. (1998) Parasites, pathogens, and polyandry in honey bees American Naturalist 151 4 392-396 pp ISBN/0003-0147 <Go to ISI>://WOS:000072752400008

Shi, X. B., Jiang, L. L., Wang, H. Y., Qiao, K., Wang, D. and Wang, K. Y. (2011) Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, Aphis gossypii Pest Management Science 67 12 1528-1533 pp ISBN/1526-498X <Go to ISI>://WOS:000297511700006

Simon, B., Cenis, J. L. and De La Rua, P. (2007) Distribution patterns of the Q and B biotypes of Bemisia tabaci in the Mediterranean Basin based on microsatellite variation Entomologia Experimentalis Et Applicata 124 3 327-336 pp ISBN/0013-8703 <Go to ISI>://WOS:000248964600013

Snart, C. J. (2011) Transcriptome analysis of honey bee larvae following neonicotinoid exposure University of Nottingham Dissertation submitted to the University of Nottingham in partial fulfilment of the requirements for the degree of Masters of Research with in Advanced Genomics and Proteomics Nottingham pp http://etheses.nottingham.ac.uk/2333/2/Transcriptome__Analysis_of_Honey_Bee_Larvae_following_Neonicotinoid_Exposure.pdf AND http://www.ask-force.org/web/Bees/Snart-Transcriptom-Analysis-Bee-Neonicotinoid-2011.pdf

Soroker, V., Hetzroni, A., Yacobson, B., Voet, H. and Slabezki, S. (2009) Colony losses in Israel: incidence of viral infection and beehive populations. Proceedings of the 41st Apimondia Congress Montpellier, France 38 pp

Spleen, A. M., Lengerich, E. J., Rennich, K., Caron, D., Rose, R., Pettis, J. S., Henson, M., Wilkes, J. T., Wilson, M., Stitzinger, J., Lee, K., Andree, M., Snyder, R., vanEngelsdorp, D. and Bee Informed, P. (2013) A national survey of managed honey bee 2011-12 winter colony losses in the United States: results from the Bee Informed Partnership Journal of Apicultural Research 52 2 ISBN/0021-8839 <Go to ISI>://WOS:000317041800007 AND http://www.ask-force.org/web/Bees/Spleen-National-Survey-Managed-Honey-Bees-2013.pdf

Stokstad, E. (2007a) ENTOMOLOGY: The Case of the Empty Hives Science 316 5827 970-972 pp http://www.sciencemag.org/cgi/content/summary/316/5827/970 AND http://www.ask-force.org/web/Bees/Stockstad-Empty-2007.pdf

Stokstad, E. (2007b) GENOMICS: Puzzling Decline of U.S. Bees Linked to Virus From Australia Science %R 10.1126/science.317.5843.1304 317 5843 1304-1305 pp http://www.sciencemag.org AND http://www.ask-force.org/web/Bees/Stockstad-IAPV-Culprit-2007.pdf

Stoner, K. A. and Eitzer, B. D. (2012) Movement of Soil-Applied Imidacloprid and Thiamethoxam into Nectar and Pollen of Squash (Cucurbita pepo) Plos One 7 6 ISBN/1932-6203 <Go to ISI>://WOS:000305825800019 AND http://www.ask-force.org/web/Bees/Stoner-Movement-Soil-Applied-Imidacloprid-Squash-2012.pdf

Straub, L., Williams, G. R., Pettis, J., Fries, I. and Neumann, P. (2015) Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors Current Opinion in Insect Science 12 109-112 pp ISBN/2214-5745 <Go to ISI>://WOS:000369017900016 AND http://www.ask-force.org/web/Bees/Straub-Superorganism-resilience-2015.pdf

Tapparo, A., Giorio, C., Solda, L., Bogialli, S., Marton, D., Marzaro, M. and Girolami, V. (2013) UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations Analytical and Bioanalytical Chemistry 405 2-3 1007-1014 pp ISBN/1618-2642 <Go to ISI>://WOS:000313650800048 AND http://www.ask-force.org/web/Bees/Tapparo-UHPLC-DAD-Method-Determination-Neonicotinoid-2013.pdf

Tapparo, A., Marton, D., Giorio, C., Zanella, A., Solda, L., Marzaro, M., Vivan, L. and Girolami, V. (2012) Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds Environmental Science & Technology 46 5 2592-2599 pp ISBN/0013-936X <Go to ISI>://WOS:000301023700018 AND http://www.ask-force.org/web/Bees/Tapparo-Assessment-Environmental-Exposure-Honeybees-2012.pdf

Teixeira, E. W., Chen, Y. P., Message, D., Pettis, J. and Evans, J. D. (2008) Virus infections in Brazilian honey bees Journal of Invertebrate Pathology 99 1 117-119 pp ISBN/0022-2011 <Go to ISI>://WOS:000259131400018 AND http://www.ask-force.org/web/Bees/Weinstein-Teixeira-Virus-Brazil-2008.pdf

Tenczar, E. G. and Krischik, V. A. (2007) Comparison of standard (granular and drench) and novel (tablet, stick soak, and root dip) imidacloprid treatments for cottonwood leaf beetle (Coleoptera : Chrysomelidae) management on hybrid poplar Journal of Economic Entomology 100 5 1611-1621 pp ISBN/0022-0493 <Go to ISI>://WOS:000250154700016 AND http://www.ask-force.org/web/Bees/Tenczar-Comparison-Standard-Novel-Imidacloprid-Treatments-2007.pdf

Tew, J. E. (2002) Bee Culture's Beeyard Dr. James E. Tew, State Specialist, Beekeeping, The Ohio State University, Wooster, OH 44691 http://www.orsba.org/htdocs/download/Dtew.htm AND http://www.ask-force.org/web/Bees/Tew-Bee-Cultures-Beeyard-2002.PDF

Tofilski, A. (2008) Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies Apidologie 39 5 558-U32 pp ISBN/0044-8435 <Go to ISI>://WOS:000260532100010 AND http://www.ask-force.org/web/Bees/Tofilski-Using-geometric-morphometrics-standard-Apis-subspecies-2008.pdf

Tokarz, R., Firth, C., Street, C., Cox-Foster, D. L. and Lipkin, W. I. (2011) Lack of Evidence for an Association between Iridovirus and Colony Collapse Disorder Plos One 6 6 ISBN/1932-6203 <Go to ISI>://WOS:000292291800049 AND http://www.ask-force.org/web/Bees/Tokarz-Lack-Evidence-Iridovirus-CCD-2011.pdf

Topolska, G., Gajda, A., Pohorecka, K., Bober, A., Kasprzak, S., Skubida, M. and Semkiw, P. (2010) Winter colony losses in Poland Journal of Apicultural Research 49 1 126-128 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500027

Traynor, K. S., Pettis, J. S., Tarpy, D. R., Mullin, C. A., Frazier, J. L., Frazier, M. and vanEngelsdorp, D. (2016) In-hive Pesticide Exposome: Assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States Scientific Reports 6 33207 pp http://dx.doi.org/10.1038/srep33207 AND 10.1038/srep33207 AND http://www.nature.com/articles/srep33207#supplementary-information AND http://www.ask-force.org/web/Bees/Traynor-In-Hive-Pesticide-Eyposome-US-2016.pdf

Tuck, S. L., Winqvist, C., Mota, F., Ahnström, J., Turnbull, L. A. and Bengtsson, J. (2014) Land-use intensity and the effects of organic farming on biodiversity: a hierarchical meta-analysis Journal of Applied Ecology n/a-n/a pp ISBN/1365-2664 http://dx.doi.org/10.1111/1365-2664.12219 AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-Land-use-Intensity-Effects-Organic-Farming-2014.pdf AND http:www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0001-AppendixS1-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0002-AppendixS2-20140230.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0003-TableS1-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0004-TableS2-20140203.pdf AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0005-TableS3-20140203.csv AND http://www.ask-force.org/web/Biotech-Biodiv/Tuck-jpe12219-sup-0005-TableS3-20140203.xla

Upton John (20130827) Syngenta to take a continent to court to upend pesticide ban Grist, a beacon in the smog 9 pp http://grist.org/news/syngenta-to-take-a-continent-to-court-to-upend-pesticide-ban/ AND http://www.ask-force.org/web/Bees/Upton-Syngenta-Takes-Continent-Court-20130827.pdf

van der Zee, R. (2010) Colony losses in the Netherlands Journal of Apicultural Research 49 1 121-123 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500025

van der Zee, R., Pisa, L., Andonov, S., Brodschneider, R., Charriere, J.-D., Chlebo, R., Coffey, M. F., Crailsheim, K., Dahle, B., Gajda, A., Gray, A., Drazic, M. M., Higes, M., Kauko, L., Kence, A., Kence, M., Kezic, N., Kiprijanovska, H., Kralj, J., Kristiansen, P., Martin Hernandez, R., Mutinelli, F., Bach Kim, N., Otten, C., Ozkirim, A., Pernal, S. F., Peterson, M., Ramsay, G., Santrac, V., Soroker, V., Topolska, G., Uzunov, A., Vejsnaes, F., Wei, S. and Wilkins, S. (2012) Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10 Journal of Apicultural Research 51 1 91-114 pp ISBN/0021-8839 <Go to ISI>://WOS:000299995700012 AND http://www.ask-force.org/web/Bees/vanderZee-Managed-Honey-Bee-Losses-2012.pdf

Van Dijk, T. C., Van Staalduinen, M. A. and Van der Sluijs, J. P. (2013) Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid Plos One 8 5 ISBN/1932-6203 <Go to ISI>://WOS:000319167000043 AND http://www.ask-force.org/web/Bees/Van-Dijk-Macro-Invertebrate-Decline-Water-Imidacloprid-2013.pdf

Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Brown, M., Bryden, J., Budge, G. E., Bull, J. C., Carvel, C., Challinor, A. J., Connolly, C. N., Evans, D. J., Feil, E. J., Garratt, M. P., Greco, M. K., Heard, M. S., Jansen, V. A. A., Keeling, M. J., Kunis, W. E., Marris, G. C., Memmott, J., Murray, J. T., Nicolson, S. W., Osborne, J. L., Paxton, R. J., Pirk, C. W. W., Polce, C., Potts, S. G., Priest, N. K., Raine, N. E., Roberts, S., Ryabov, E. V., Shafir, S., Shirley, M. D. F., Simpson, S. J., Stevenson, P. C., Stone, G. N., Termansen, M., Wright, G. A. and Insect Pollinators, I. (2013) Threats to an ecosystem service: pressures on pollinators Frontiers in Ecology and the Environment 11 5 251-259 pp ISBN/1540-9295 <Go to ISI>://WOS:000319857700017 AND http://www.ask-force.org/web/Bees/Vanbergen-Threats-to-an-ecosystem-service-Polinators-2013.pdf

vanEngelsdorp, D., Caron, D., Hayes, J., Underwood, R., Henson, M., Rennich, K., Spleen, A., Andree, M., Snyder, R., Lee, K., Roccasecca, K., Wilson, M., Wilkes, J., Lengerich, E., Pettis, J. and Bee Informed, P. (2012) A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership Journal of Apicultural Research 51 1 115-124 pp ISBN/0021-8839 <Go to ISI>://WOS:000299995700013 AND http://www.ask-force.org/web/Bees/vanEngelsdorp-National-Survey-US-2012.pdf

VanEngelsdorp, D., Evans, J. D., Donovall, L., Mullin, C., Frazier, M., Frazier, J., Tarpy, D. R., Hayes, J. and Pettis, J. S. (2009a) "Entombed Pollen": A new condition in honey bee colonies associated with increased risk of colony mortality Journal of Invertebrate Pathology 101 2 147-149 pp ISBN/0022-2011 <Go to ISI>://WOS:000267382800012 AND http://www.ask-force.org/web/Bees/VanEngelsdorp-Entombed-Pollen-2009.pdf

vanEngelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D. R. and Pettis, J. S. (2009b) Colony Collapse Disorder: A Descriptive Study PLoS ONE 4 7 Article No.: e6481 pp ISBN/1932-6203 <Go to ISI>://BIOSIS:PREV200900520575 AND http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0006481 AND http://www.ask-force.org/web/Bees/VanEngelsdorp-Descriptive-PLoSone-2009.pdf

vanEngelsdorp, D., Hayes, J., Jr., Underwood, R. M. and Pettis, J. (2008) A Survey of Honey Bee Colony Losses in the US, Fall 2007 to Spring 2008 PLoS ONE 3 12 Article No.: e4071 pp ISBN/1932-6203 <Go to ISI>://BIOSIS:PREV200900336218 AND http://www.ask-force.org/web/Bees/vanEngelsdorp-Survey-Colony-Losses-2008.pdf

vanEngelsdorp, D., Hayes, J., Underwood, R. M. and Pettis, J. S. (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009 Journal of Apicultural Research 49 1 7-14 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500002

vanEngelsdorp, D., Lengerich, E., Spleen, A., Dainat, B., Cresswell, J., Baylis, K., Nguyen, B. K., Soroker, V., Underwood, R., Human, H., Le Conte, Y. and Saegerman, C. (2013) Standard epidemiological methods to understand and improve Apis mellifera health Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300008 AND http://www.ask-force.org/web/Bees/vanEngelsdorp-Standard-Epidemiological-Methods-corr-20130901.pdf

vanEngelsdorp, D. and Meixner, M. D. (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them, open source Journal of Invertebrate Pathology 103 Supplement 1 S80-S95 pp ISBN/0022-2011 http://www.sciencedirect.com/science/article/B6WJV-4XNMC3B-4/2/243a511760d9697483f95c9d96a9794e AND http://www.ask-force.org/web/Bees/vanEngelsdorp-Historical-Review-Managed-2010.pdf

Vazquez-Padron, R. I., de la Riva, G., Aguero, G., Silva, Y., Pham, S. M., Soberon, M., Bravo, A. and Aitouche, A. (2004) Cryptic endotoxic nature of Bacillus thuringiensis Cry1Ab insecticidal crystal protein Febs Letters 570 1-3 30-36 pp <Go to ISI>://WOS:000222885400006 AND http://www.ask-force.org/web/Bt1/Vazquez-Cryptic-endotoxic-nature-Bt-2004.pdf

Vejsnaes, F., Nielsen, S. L. and Kryger, P. (2010) Factors involved in the recent increase in colony losses in Denmark Journal of Apicultural Research 49 1 109-110 pp ISBN/0021-8839 <Go to ISI>://WOS:000276090500020

Villa, J. D., Bourgeois, A. L. and Danka, R. G. (2013) Negative evidence for effects of genetic origin of bees on Nosema ceranae, positive evidence for effects of Nosema ceranae on bees Apidologie 44 5 511-518 pp ISBN/0044-8435 <Go to ISI>://WOS:000323246700003 AND http://www.ask-force.org/web/Bees/Villa-Negative-Evidence-Origin-Positive-Effects-Bees-2013.pdf

Vojvodic, S., Boomsma, J. J., Eilenberg, J. and Jensen, A. B. (2012) Virulence of mixed fungal infections in honey bee brood Frontiers in Zoology 9 ISBN/1742-9994 <Go to ISI>://WOS:000305830800001 AND http://www.ask-force.org/web/Bees/Vojvodic-Virulence-Mixed-Fungal-Infections-2012.pdf

Wagner Sheldon L. (1983) Clinical Toxicology of Agricultural Chemicals Noyes Pubns; First edition (January 1983) Park Ridge, New Jersey USA 306 pp ISBN-10: 0815509308 AND ISBN-13: 978-0815509301/ISBN-10: 0815509308 AND ISBN-13: 978-0815509301 http://www.amazon.com/Clinical-Toxicology-Agricultural-Chemicals-Sheldon/dp/0815509308/ref=sr_1_1?ie=UTF8&qid=1424248872&sr=8-1&keywords=Wagner+Clinical+Toxicology Bibl. KA AND selection http://www.ask-force.org/web/Bees/Wagner-Clinical-Toxicology-Ag-Chemicals-1983.pdf

Wang, R. M., Wang, Z. H., Yang, H., Wang, Y. Z. and Deng, A. P. (2012) Highly sensitive and specific detection of neonicotinoid insecticide imidacloprid in environmental and food samples by a polyclonal antibody-based enzyme-linked immunosorbent assay Journal of the Science of Food and Agriculture 92 6 1253-1260 pp ISBN/0022-5142 <Go to ISI>://WOS:000301645900013 AND http://www.ask-force.org/web/Bees/Wang-Highly-Sensitive-Detection-Neonicotinoid-2012.pdf

Wanner, H., Ammann, K., Berlincourt, P., Filliger, P., Herzig, R., Liebendörfer, L., Rickli, R. and Urech, M. (1986) Urban Meteorology and Air Pollution in Biel - Bienne, Switzerland. Workshop on Urban Meteorology International Symposium on Climatology 2. 1986 Freiburger Geogr.Hefte 3 pp http://www.ask-force.org/web/Flechten/Wanner-Urban-Meteorol-Biel-1986.PDF

Watanabe, M. E. (2008) Colony collapse disorder: Many suspects, no smoking gun Bioscience 58 5 384-388 pp <Go to ISI>://WOS:000255971600005 AND http://www.ask-force.org/web/Bees/Watanabe-CCD-Many-Suspects-2008.pdf

Williams, G. R., Alaux, C., Costa, C., Csaki, T., Doublet, V., Eisenhardt, D., Fries, I., Kuhn, R., McMahon, D. P., Medrzycki, P., Murray, T. E., Natsopoulou, M. E., Neumann, P., Oliver, R., Paxton, R. J., Pernal, S. F., Shutler, D., Tanner, G., van der Steen, J. J. M. and Brodschneider, R. (2013) Standard methods for maintaining adult Apis mellifera in cages under in vitro laboratory conditions Journal of Apicultural Research 52 1 ISBN/0021-8839 <Go to ISI>://WOS:000315730300004 AND http://www.ask-force.org/web/Bees/Williams-Standard-Maintaining-Adult-Apis-Cages-20130901.pdf

Williams, G. R., Dietemann, V., Ellis, J. D. and Neumann, P. (2012) An update on the COLOSS network and the "BEEBOOK: standard methodologies for Apis mellifera research" Journal of Apicultural Research 51 2 151-153 pp ISBN/0021-8839 <Go to ISI>://WOS:000302295500001 AND http://www.ask-force.org/web/Bees/Williams-Update-COLOSS-BEEBOOK-Network-2012.pdf

Williams, J. L. and Kauffeld, N. M. (1974) Winter conditions in commercial colonies in Louisiana 114 219-221 pp
Williamson, S. M., Baker, D. D. and Wright, G. A. (2013) Acute exposure to a sublethal dose of imidacloprid and coumaphos enhances olfactory learning and memory in the honeybee Apis mellifera Invertebrate Neuroscience 13 1 63-70 pp ISBN/1354-2516 <Go to ISI>://WOS:000320000900008 AND http://www.ask-force.org/web/Bees/Williamson-Acute-Exposure-Sublethal-Dose-Imidacloprid-2013.pdf

Williamson, S. M. and Wright, G. A. (2013) Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees Journal of Experimental Biology 216 10 1799-1807 pp ISBN/0022-0949 <Go to ISI>://WOS:000318483600013 AND http://www.ask-force.org/web/Bees/Williamson-Exposure-Multiple-Cholinergic-Pesticides-2013.pdf

Wilson, W. T. and Menapace, D. M. (1979) Disappearing disease of honey bees: A survey of the United States American Bee Journal 119, Part I and Part II 118-119 and 184-186, 217 pp
Winter, T. (2007) Gießen: Bio-Honig kontra Hi-Tech-Getreide, Frankfurter Allgemeine Zeitung FAZ Frankfurt a.M. pp http://www.faz.net/s/Rub9FAE69CECEA948EAAFE2806B54BF78AA/Doc~EB6CB684869E54C368AE66524EDBF48DD~ATpl~Ecommon~Scontent.html AND http://www.ask-force.org/web/Bees/Winter-Bio-Honig-Contra-Hi-Tech-Getreise-FAZ-2007.pdf

Wittstock, S., Kaatz, H. H. and Menzel, R. (1993) Inhibition of Brain Protein-Synthesis by Cycloheximide Does Not Affect Formation of Long-Term-Memory in Honeybees after Olfactory Conditioning Journal of Neuroscience 13 4 1379-1386 pp <Go to ISI>://A1993KV71100006 AND http://www.ask-force.org/web/Bees/Wittstock-Inhibition-Brain-Protein-Synthesis-Cycloheximide-Not-Affect-1993.pdf

Wu-Smart, J. and Spivak, M. (2016) Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development Scientific Reports 6 32108 pp http://dx.doi.org/10.1038/srep32108 AND http://www.ask-force.org/web/Bees/Wu-Smart-Sublethal-effects-dietary-neonic-exposure-bee-fecundity-2016.pdf

Wu, F. (2007) Bt corn and impact on mycotoxins CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 2 060 1-8 pp http://www.cababstractsplus.org/cabreviews AND http://www.ask-force.org/web/Bt/Wu-Corn-Impact-CAB-2007.pdf

Yanez, K. P., Bernal, J. L., Nozal, M. J., Martin, M. T. and Bernal, J. (2013) Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column Journal of Chromatography A 1285 110-117 pp ISBN/0021-9673 <Go to ISI>://WOS:000317167400013 AND http://www.ask-force.org/web/Bees/Yanez-Determination-Seven-Neonicotinoid-Bee-Wax-2013.pdf

Yang, E. C., Chuang, Y. C., Chen, Y. L. and Chang, L. H. (2008) Abnormal Foraging Behavior Induced by Sublethal Dosage of Imidacloprid in the Honey Bee (Hymenoptera: Apidae) Journal of Economic Entomology 101 6 1743-1748 pp ISBN/0022-0493 <Go to ISI>://WOS:000261430700004 AND http://www.ask-force.org/web/Bees/Yang-Abnormal-Foraging-Behavior-2008.pdf