GM crops not the reason for the bee colony collapse disorder (CCD)

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1. The Issue:

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”? and the German Contribution talks in the headline about “Aids in the Bee Hive”. And instead of making the allegation that GM crops are the real culprit themselves, they interview a well known beekeeper and opponent of GM crops.

Latsch, G. (2007)
Aids im Bienenstock, Der Spiegel 12 pp 58-59 (Der Spiegel Article)

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

As a well known lobbyist for beekeepers Mr. Haefeker has numerous times spoken out against GM crops, in the Spiegel interview he does not make an exception:

“Walter Haefeker is a man who is used to painting grim scenarios. He sits on the board of directors of the German Beekeepers Association (DBIB) and is vice president of the European Professional Beekeepers Association. And because griping is part of a lobbyist’s trade, it is practically his professional duty to warn that “the very existence of beekeeping is at stake.” The problem, says Haefeker, has a number of causes, one being the varroa mite, introduced from Asia, and another is the widespread practice in agriculture of spraying wildflowers with herbicides and practicing monoculture. Another possible cause, according to Haefeker, is the controversial and growing use of genetic engineering in agriculture.” (Latsch, 2007a, b)

The Spiegel text is notoriously cited, here an example of a pseudoscientific article from the GMO-journal, discussing the CCD and trying to link it to GM crops without a shred of evidence, but nice citations of texts only publishing assumptions:

http://www.botanischergarten.ch/Bees/Gitlin-CCD-GMOS-20090924.PDF

1.2. 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, 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. Another pseudo-problem is presented by the German PM Christel Happach-Kasan, speaker of the Liberal Party on agriculture and biotechnology and member of the parliamentary expert group on genetic engineering. She makes 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 intend to destroy this kind of contaminated honey although the legislators and regulators do not require this. More about this debate between German organic farmers and GM crop promoters under a new website Bienen & Agrogentechnik 1. The controversy whether organic honey contaminated by Bt maize pollen is now in the local courts with some contradicting sentences. (Winter, 2007).

1.3. More propaganda of opponents of GM crops, without scientific evidence, but lots of assumptions and questionable principles

(Donovan, 2009)

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 & Cummings, 2007)

“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?

1 Bienen und Agrogentechnik: http://www.bienen-gentechnik.de/
The “number one psychic” of the US got a message from the Great Spirit: it must be the GM crops causing the CCD:
“\textit{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 honey bee 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}.”

2. 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 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 can be labeled as anti-GM-crop scare propaganda. The possible causes – still not really nailed down as the main factor, are infections of parasites, viral infection, as a sum multiple stress factors as we will see below. The recent report for EFSA confirms this summary (Hendrikx et al., 2009). See the extended summary of this most comprehensive report published in December 2009 under 3.1.

3. General comments

3.1. Recent scientific reviews on CCD
The best and most recent summary of the CCD situation has just been published by the Journal of Apicultural Research, here fully cited as an open source publication:

\textbf{Neuman, P. and N. L. Carreck (2010).} “Honey bee colony losses, guest editorial, open source.” \textit{Journal of Apicultural Research} \textbf{49}(1): 1-6. DOI 10.3896/IBRA.1.49.1.01, open source AND \url{http://www.botanischergarten.ch/Bees/Neumann-Honey-Bee- Colony-Losses-2010.pdf} including all citations (as far as already available on WOS)

\textit{Honey bee colony losses, guest editorial, (from (Neuman & 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 honey bee (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 honey bee 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 honey bee, 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 & 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 honey bee 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 & Ball, 1991)

Fig. 1 The Varroa destructor equator of global colony losses. So far, elevated colony losses have recently been reported from Europe (Crailsheim et al., 2009), the USA ((vanEngelsdorp et al., 2009a) and 2010), the Middle East (Haddad et al., 2009; Soroker et al., 2009) and Japan (Guttierrez, 2009) but not from South America, Africa and Australia. Colonies of African honey bees and Africanized honey bees 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 & 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 & Ball, 1991). Other scientists soon made suggestions. By 1912, (Fantham & 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 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 & 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.
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 (Van Engelsdorp et al., 2009; vanEngelsdorp et al., 2009a; VanEngelsdorp et al., 2009b) and not colony losses per se. Indeed, honey bee colonies can die in many ways, and CCD is just one of them (vanEngelsdorp & Meixner, 2010). Finally, since both honey bee 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 honey bees survive without treatment for V. destructor (Martin & 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 honey bee pathogens are now also almost globally distributed, for example Nosema spp. and several viruses (Allen & Ball, 1996; Ellis &
Munn, 2005; Fries; Maori et al., 2007). Multiple infections with pathogens and also interactions between pathogens and other suspected drivers of honey bee 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.

Assigning 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 standardisation 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 honey bee Colony LOSSes http://www.coloss.org/) has therefore been created to coordinate efforts to explain and prevent large scale losses of honey bee 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 & 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. 4 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 honey bee 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 honey bee 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 & 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 honey bee colony losses: viruses (Berthoud et al., 2010; Carreck et al., 2010a,b; Martin et al., 2010); open access: Nosema ceranae (Paxton, 2010); Santrac et al., 2010); Varroa destructor (Carreck et al., 2010b; Dahle, 2010; Martin et al., 2010); pesticides (Chauzat et al., 2010b; Medrzycki et al., 2010); the effects of acaricides (Harz et al., 2010); the loss of genetic diversity (Meixner et al., 2010; and loss of habitats (Potts 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 (Brodscnieder and Crailsheim, 2010; Brodschneider et al., 2010); Bosnia and Herzegovia (Santrac et al., 2010); Bulgaria (Ivanova and Petrov, 2010); Canada (Currie et al., 2010);
Croatia (Gajger et al., 2010); Denmark (Vejsnæs and Kryger, 2010); England (Aston, 2010); France (Chauzat et al., 2010a,c); Greece (Hatjina et al., 2010); Italy (Mutinelli et al., 2010); the Netherlands (Van der Zee, 2010); Norway (Dahle, 2010); Poland (Topolska et al., 2010); Scotland (Gray et al., 2010); Switzerland (Charrière and Neumann, 2010); and the USA (Ellis et al., 2010; vanEnglesdorp et al., 2010). Finally, two further papers consider the general status of both managed honey bees (Potts et al., 2010) and non-Apis bees (Roberts and Potts, 2010) in Europe. (all citations 2010 stem from the special issue of Journal of Apicultural Research, see http://www.ibra.org.uk/articles/specialissue2010, they will be cited after having appeared on Web of Science.

Another account on the CCD has been released last year by a committee of specialists in this field (Hendrikx 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 apiculturalist reports. It also includes the screening of ca. 100 specialized websites on apiculture. The results of this study deserve to be summarized extensively:


“The bee surveillance project sought information on both the prevalence of honey bee 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 et al., 2009)
Some useful illustrations from this open source report:

![Graph showing national percentages of colonies lost during winter from 2000 to 2009 in Denmark, Finland, Germany, Sweden, England and Wales.](image)

**Fig. 5 National percentages of colonies lost during winter from 2000 to 2009 in Denmark, Finland, Germany, Sweden, England and Wales.** From (Hendrikx et al., 2009)

“The figure 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 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 et al., 2009)
Fig. 7 Types of factors occurring in the studied literature from (Hendrikx 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 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 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 electromagnetic radiations, another consensus arises: the role of either of these two factors on colony losses is absent (Table 15).”

Other recent and comprehensive summaries of the present day knowledge about the CCD has just been published, a confirmation for a multitude of causes of CCD.


Abstract: Honey bees 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 honey bee populations have been increasing, the rate of increase is not keeping pace with demand. Further, honey bee populations have not been increasing in all parts of the world, and have declined in many nations in Europe and in North America. Managed honey bee 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 honey bees, followed by a detailed description of some of the historical and present day factors that influence honey bee populations, with particular emphasis on colony populations in Europe and the United States. (vanEngelsdorp & Meixner, 2010)


“Background: Over the last two winters, there have been large-scale, unexplained losses of managed honey bee (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 honey bee resistance to pesticides, are highlighted.” (vanEngelsdorp et al., 2009a)

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


“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 Honey Bees.

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 Wiki:

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

More: An EU project has been started in December 2008, and soon one can expect results, 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 the the overall stress of bees:

3.2. GM crops ruled out as a possible cause of CCD with excellent scientific and historic arguments.

The most comprehensive study of (Hendrikx et al., 2009) with an extensive literature study and a survey based on questionnaires rules out completely the possibility, that magnetic waves of genetic engineering could be a direct cause of CCD.

As a proof 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:

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

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

“INTRODUCTION
Disappearing disease” of the honey bee is a mysterious phenomenon. 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, 1874). Other examples have come from Australia (Beuhne, 1910, 1916) from Louisiana and Texas in 1963-64 (Oertel, 1965; Williams & Kauffeld, 1974), from California in 1964-65 (Foote, 1966), from Mexico in 1977 (Mraz, personal communication), from the Rio Grande Valley in Texas in 1974 (Kauffeld et al., 1976), and from various additional locations in the United States (24). Judging from these reports, there can be no doubt that many beekeepers have suffered devastating losses of bees. An extensive review of literature or1 disappearing disease (DD) is given by Wilson and Menapace (Wilson & 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 & Menapace, 1979) have occurred in the spring. A prominent hypothesis over the last few years has involved some sort of stock detel-oration (Wilson & 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 successfully 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 et al., 1984).

see also other papers of the same authors group: (Kulincevic et al., 1990; Kulincevic & Mladjan, 1988; Kulincevic et al., 1969; Kulincevic et al., 1991a; Kulincevic et al., 1991b, 1992; Kulincevic et al., 1988; Kulincevic et al., 1973; Kulincevic & Rothenbuhler, 1975, 1982, 1989a, b; Kulincevic et al., 1982, 1983).

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, b, c, d, e, f, g, h, i, 2006a, b, 2008a, b, c, d, 2009a, b) 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 et al., 2008):


“Background: Honey bees (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 honey bee populations. Methodology/Principal Findings: We conducted a meta-analysis of 25 studies that independently assessed potential effects of Bt Cry proteins on honey bee 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 honey bee larvae or adults in laboratory settings. Conclusions/Significance: Although the additional stresses that honey bees 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 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 honey bee development and that the use of transgenic canola does not pose any threat to honey bees.
Another feeding study 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.

However, when you read about the details of the paper, you discover that in the feeding experiments with realistic amounts of Bt toxin (3 ppb - parts per billion), the honey bees 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 honey bees 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).”


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/B6WD8-4RA6172M/2/2/123e7dfe2e6dadee7da81bca89cc97e

“Genetically modified Bt crops are increasingly used worldwide but side effects and especially sublethal effects on beneficial insects remain poorly studied. Honey bees 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 honey bees. Following a complementary bioassay, our experiments evaluated effects of the Cry1Ab on three major life traits of young adult honey bees: (a) survival of honey bees during sub-chronic exposure to Cry1Ab, (b) feeding behavior, and (c) learning performance at the time that honey bees 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 honey bees. However, honey bee feeding behavior was affected when exposed to the highest concentration of Cry1Ab protein, with honey bees taking longer to imbibe the contaminated syrup. Moreover, honey bees exposed to 5000 ppb of Cry1Ab had disturbed learning performances. Honey bees 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 honey bee development and that the use of transgenic canola dose not pose any threat to honey bees. (Huang et al., 2004)
foraging efficiency. The implications of these results are discussed in terms of risks of transgenic Bt crops for honey bees. “(Ramirez-Romero et al., 2008).

4. More recent scientific information on possible causes of CCD

4.1. Recent Honey Bee Colony Declines, Summary in Science and CSR Report for the US Congress

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.

ENTOMOLOGY: The Case of the Empty Hives. Science %R 10.1126/science.316.5827.970, 316, 5827, pp 970-972

“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 honey bees. 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 honey bees, 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 honey bees, there is evidence that the metabolites (break down products) of this material are even more toxic than the parent substance.” (Stokstad, 2007a, b)


“This report examines the recent sharp decline in U.S. honey bee 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. Honey bees 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 honey bees as commercial pollinators in the United States is estimated at about $15 billion annually.
Honey bee 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 honey bee 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 et al., 2009b)


(van Engelsdorp et al., 2006)


A Survey of Honey Bee Colony Losses in the US, Fall 2007 to Spring 2008. PLoS ONE, 3, 12, pp Article No.: e4071

<Go to ISI>://BIOSIS:PREV200900336218 AND


Background: Honey bees 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 honey bee 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 honey bee 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. (van Engelsdorp et al., 2006; van Engelsdorp 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 & Vanengelsdorp, 2009; VanEngelsdorp et al., 2009b):

VanEngelsdorp, D., Hayes, J., & Pettis, J. (2009b)
Electronic Source: Preliminary Results: A Survey of Honey Bee Colonies Losses in the U.S. Between September 2008 and April 2009
http://maarec.cas.psu.edu/pdfs/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
(Cox-Foster & 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

4.2. Possible shortage of bee colonies for pollination security in agriculture: no scientific evidence
The most recent two publications sum up the concerns, whether we will have an acute shortage of pollinators with the vanishing bee populations. According to (Aizen et al., 2008; Aizen & 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.


"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 et al., 2008).


"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 honey-bee 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 honey-bee 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 & Harder, 2009)

4.3. Possible protein deficiency as a follow up of a picorna–like viral infection

(Johnson et al., 2009a) 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 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.


“Colony collapse disorder (CCD) is a mysterious disappearance of honey bees 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 honey bee 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.”

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 polyadenylated 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 et al., 2009a)

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:


“The varroa mite, Varroa destructor Anderson & Trueman, is a devastating pest of honey bees, 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 honey bees, 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 coumaphos 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 honey bee 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 et al., 2009b)
4.4. **Imidacloprid or other pesticides as possible cause of CCD?**

In France, the use of Imidacloprid was blamed for the cause of CCD and was therefore banned. [http://en.wikipedia.org/wiki/Imidacloprid_effects_on_bee_population](http://en.wikipedia.org/wiki/Imidacloprid_effects_on_bee_population)

It’s of course difficult to be sure if this particular insecticide is the cause of the problem, it was banned in France, but many different pesticides could cause this problem. The FAO website gives a list of relative toxicity of pesticides. [http://www.fao.org/docrep/X0083E/X0083E09.htm](http://www.fao.org/docrep/X0083E/X0083E09.htm)

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

Does Imidacloprid Seed-Treated Maize Have an Impact on Honey Bee Mortality? Journal of Economic Entomology, 102, 2, pp 616-623


“Beekeepers suspected maize. Zea mays L., treated with imidacloprid to result in substantial loss of honey bee (Hymenoptera: Apidae) colonies in Belgium. The objective of this study was to investigate the potential impact of maize grown from imidacloprid-treated seeds on honey bee 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 honey bees. (Nguyen et al., 2009)

4.5. **A novel way of intoxication of honey bees:**

**Translocation through guttation drops**

**Experiments with imidacloprid from coated corn seedlings.**

A recent paper claims detrimental impact of pesticides through guttation: (Girolami 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.

The death of honeybees, Apis mellifera 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. 9 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 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 (log_{10} 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 (log_{10} 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 \((\log_{10} 2)\) 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 \((\text{LC}_{50})\), for which poisoning solutions are kept available for longer time. Results are in agreement with (Yang et al., 2008) who reported that the imidacloprid concentration \(3 \text{ mg/liter}\) in a sugar solution is the threshold preventing bees to return to foraging. This value is close to the one \((6 \text{ mg/liter})\) 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.**

4.6. **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:


"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." (Van Engelsdorp et al., 2009)

4.7. **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:


"Parasites are dependent on their hosts for energy to reproduce and can exert a significant nutritional stress on them. Energetic demand placed on 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 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 & Naug, 2009)

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


“THE REPORT “A METAGENOMIC SURVEY OF microbes in honey bee 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 et al., 2008; Cox-Foster et al., 2007a; Cox-Foster et al., 2007b)


“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 et al., 2007; Maori et al., 2009)


“Israel acute paralysis virus (IAPV) is associated with colony collapse disorder of honey bees. 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 et al., 2008)
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 et al., 2008)


“This work describes the first molecular-genetic evidence for viruses in Brazilian honey bee 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 et al., 2008)

4.9. Natural infection by Nosema ceranae or other infections the cause of CCD?


“In recent years, honeybees (Apis mellifera) have 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 et al., 2008a; Higes et al., 2008b)

Serious losses of honey bee 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 ceranae. 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 & Kondos, 1979* candel in the USA, (Sanford, 1987, 2007; Savoy 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.int/main <http://www.eea.eu.eu.int/main>). (Pajuelo et al., 2008).


“The tracheal mite has been associated with colony deaths worldwide since the mite was first discovered in 1919. Yet controversy about its role in honey bee 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 honey bees. 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 honey bees, 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 & Brown, 2009)

**4.10. Could climate change contribute to the colony collapse of honey bees?**


“The European honey bee, *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 honey bee’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 honey bees 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 honey bee development. In this article, the authors examine the potential impact of climate change on honey bee behaviour, physiology and distribution, as well as on the evolution of the honey bee’s interaction with diseases. Conservation measures will be needed to prevent the loss of this rich genetic diversity of honey bees and to preserve ecotypes that are so valuable for world biodiversity.” (Le Conte & Navajas, 2008a, b)
### 4.11. Could Electrosmog be the cause of the Colony Collapse Disorder?

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


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 & Kumar, 2009).

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

### 4.12. Summary: Colony Collapse Disorder: Many suspects, no smoking gun


“From the text:

*The evidence to date Honey bees (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 et al., 2008; Cox-Foster 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 honey bees 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 honey bees 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).
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