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Varroa mites and honey bee health: can *Varroa* explain part of the colony losses?*

Yves LE CONTE¹, Marion ELLIS², Wolfgang RITTER³

¹ INRA, UMR 406 Abeilles et Environnement, Laboratoire de Biologie et Protection de l'abeille, 84914 Avignon Cedex 9, France

² University of Nebraska, Department of Entomology, Lincoln, NE 68583, USA

³ CVUA, Fachgebiet Bienen, 79108 Freiburg, Germany

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Abstract – Since 2006, disastrous colony losses have been reported in Europe and North America. The causes of the losses were not readily apparent and have been attributed to overwintering mortalities and to a new phenomenon called Colony Collapse Disorder. Most scientists agree that there is no single explanation for the extensive colony losses but that interactions between different stresses are involved. As the presence of *Varroa* in each colony places an important pressure on bee health, we here address the question of how *Varroa* contributes to the recent surge in honey bee colony losses.

Varroa destructor / *Apis mellifera* / colony collapse disorder / winter losses / honey bee stressors

The *Varroa* mite (*Varroa destructor*) switched from its natural host (*Apis cerana*) to *Apis mellifera*, the European honey bee (HB), when HBs were moved into areas where *Apis cerana* is endemic. In *Apis mellifera* the mite found a far less resistant host, and subsequently spread nearly worldwide (Peng et al., 1987; Oldroyd, 1999). Because *Varroa* and HBs have not co-evolved for a long period of time, they do not exhibit an adapted host-parasite relationship, resulting in *Varroa* often killing its host. During the first years after its introduction in Europe and North America the mite could be easily controlled by one to two chemical treatments per year. Colony losses have continued to increase since the turn of the millennium despite the development of more intensive control measures. Since 2006, disastrous colony losses have been reported in Europe and North America. The causes of the losses were not readily apparent and have

been attributed to overwintering mortalities and to a new phenomenon called Colony Collapse Disorder (CCD). Most scientists agree that there is no single explanation for the extensive colony losses but that interactions between different stresses are likely involved. As the presence of *Varroa* in every colony places an important pressure on bee health, we here address the question of how *Varroa* contributes to the recent surge in HB colony losses.

There are undoubtedly various causes for recent colony losses. However, CCD and wintering mortalities have been cited as the most frequent reasons. CCD was first reported in HB colonies in the USA. One interesting observation is that at the time of collapse, *Varroa* mite populations were not at levels known to cause economic injury or population decline (vanEngelsdorp et al., 2009). Three different descriptive case analyses have been conducted on colonies having CCD symptoms. The first used a metagenomic approach to look at candidate pathogens associated with CCD

Corresponding author: Y. Le Conte,
leconte@avignon.inra.fr

* Manuscript editor: Marla Spivak

and found only one organism, Israeli acute paralysis virus (IAPV), to be strongly correlated with CCD (Cox-Foster et al., 2007). Another study looked at changes in transcript abundance of bees related to CCD. Sixty-five transcripts were identified as potential markers for CCD, but elevated expression of pesticide response genes was not observed, and genes involved in immune response showed no clear trend in expression pattern despite the increased prevalence of viruses and other pathogens in CCD colonies (Johnson et al., 2009). A third epizootic study characterized CCD and the risk factors associated with populations exhibiting CCD (vanEngelsdorp et al., 2009). Bees in CCD colonies had higher pathogen loads and were co-infected with a greater number of pathogens compared to control populations, suggesting an interaction between pathogens and other stress factors in CCD and a possible legacy effect of mite parasitism (vanEngelsdorp et al., 2009). An important point is that descriptive case studies only showed the state of the bees when they were collected. *Varroa* populations could have differed prior to sampling, causing an immunosuppression response possibly leading to subsequent pathogen and virus development. Like all descriptive studies, definitive statements cannot be made concerning factors causing CCD, and there is no clear evidence to date to suggest that *Varroa* is or is not involved. The hypothesis that CCD is due to the invasive *Varroa* mite and its capability to suppress immune responses cannot be excluded, and in fact is supported by the study of vanEngelsdorp et al. (2009).

Wintering mortalities are well known to beekeepers, and some losses are inevitable. Twenty years ago, it was acceptable to have 5 to 10% winter colony losses. Today, the losses are often up to 20% or more in many areas (see the special issue on colony losses in *Journal of Apicultural Research*, 2010). Different causes can explain winter mortality: queen losses, lack of adequate food reserves, low fall populations, poor foraging conditions, diseases and parasites, including failure of treatments to adequately control *Varroa*. For an analysis of the causes of winter losses it is very important to consider how and when the losses occur and

the symptoms. A large number of dead bees at the hive bottom or in front of the entrance point to food scarcity or a disease like *Nosema*. Empty nests with brood and food suggest that *Varroa* may be the cause (Ritter, 1988). Possibly in contrast to CCD, in those colonies the existing bees and brood generally exhibited a high level of *Varroa* infestation (Ritter et al., 1984). Bees as well as brood parasitized by *Varroa* are nearly 100% infested by Deformed Wing Virus (DWV) (Genersch, 2005), and often also by Acute Bee Paralysis Virus (ABPV) (Ball, 1985).

Many physical and physiological detrimental effects of the *Varroa* mite have been described at the individual bee and colony levels. Repeated *Varroa* feeding on adult bee and brood hemolymph injures the bees physically, reduces their protein content and wet and dry body weights, and interferes with organ development (Schneider and Drescher, 1987; Bowen-Walker and Gunn, 2001). The parasitic mite and the viruses they vector contribute to morphological deformities (small body size, shortened abdomen, deformed wings), which reduce vigor and longevity, and they also influence flight duration and the homing ability of foragers (Schneider and Drescher, 1987; Koch and Ritter, 1991; Romero-Vera and Otero-Colina, 2002; Garedew et al., 2004; Kralj and Fuchs, 2006). The mite weakens the bee's immune system, suppressing the expression of immune-related genes and increasing DWV viral titers, both of which reduce worker survivorship and colony fitness (Yang and Cox-Foster, 2005, 2007). A number of viruses including DWV, ABPV, Chronic Bee Paralysis Virus (CBPV), Slow Bee Paralysis Virus (SPV), Black Queen Cell Virus (BQCV), Kashmir Bee Virus (KBV), Cloudy Wing Virus (CWV), and Sacbrood Virus (SBV) have been shown to be associated to varying degrees with *V. destructor* infestation (Ball and Allen, 1988; Allen and Ball, 1996; Martin, 1998, 2001; Tentcheva et al., 2004; Carreck et al., 2010; Martin et al., 2010). However, recent findings did not find evidence of CWV transmission by the mite (Carreck et al., 2010).

The role of *Varroa* mites as a vector in transmitting viruses from infected individuals

to healthy bees has been demonstrated. *Varroa* feeds on brood and adult bees and moves quickly from one bee to another (Le Conte and Arnold, 1987). The *Varroa* mite can facilitate the horizontal transmission of DWV, SBV, APV, IAPV and KBV viruses from nurse bees to larvae through larval food and via brood to adults (Ball, 1985; Chen et al., 2004). It can also be transmitted vertically by drones via semen and by queens via virus infected eggs (Yue et al., 2006, 2007). In addition, there is evidence for horizontal mite-to-mite transmission of viruses (Bowen-Walker et al., 1999; Chen et al., 2004, 2005). A few of those virus can replicate in the *Varroa* mite and are present in mite saliva, which suggests that *Varroa* is likely an active biological vector for bee viruses (Ongus et al., 2004; Shen et al., 2005; Chen et al., 2006). Clinical morphological symptoms like deformed wings and shortened abdomens only develop when *Varroa* is associated with DWV, which is lethal to the bees (Ball and Allen, 1988; Martin, 1998; Bowen-Walker et al., 1999; Martin, 2001; Tentcheva et al., 2006). ABPV and DWV are highly pathogenic, vectored by *V. destructor*, adding to the pathology of mite feeding injury (Chen et al., 2006). Both viruses were recently reported to be correlated with HB winter losses (Highfield et al., 2009; Berthoud et al., 2010). DWV could potentially act independently of *Varroa* mites to bring about colony losses (Highfield et al., 2009). Finally, the co-infections *V. destructor* with viruses has been shown to play a major role in *Varroa*-induced HB colony collapse (Martin, 1998, 2001).

At the colony level, untreated *Varroa*-infested colonies usually die within six months to two years of mite infestation. The length of the time span does not only depend on the mites' potential to multiply in bee brood but also depends on the *Varroa* invasion pressure from nearby colonies. A high bee density combined with severe *Varroa* infestation accelerates bee death (Ritter et al., 1984). When adult bees are infected prior to overwintering, they survive a shorter time compared to mite-free workers and they do not transition from summer to winter bees. As a consequence, they do not live as long as winter bees and are less able

to contribute to the build-up of colony strength in the early spring. When high mite levels are present, parasitized colonies collapse during the winter (Kovac and Crailsheim, 1988; Boecking and Genersch, 2008).

Therefore, *V. destructor* continues to be a very serious threat of the HB and the invasive parasitic mite still decimates HB populations throughout the world. It has been the case in the USA where it reached disastrous proportions especially during winters of 1995 to 1996 and 2000 and 2001 with colony deaths reaching 50 to 100% in many beekeeping operations (Kaplan, 2008; Pettis and Delaplane, 2010, this issue). The situation is similar in Europe (Moritz et al., 2010, this issue). In central Europe, a high number of colony losses occurred in the winter of 2002 to 2003 (Hendriks et al., 2009), and in southern Europe especially during the year of 2005 (Higes, 2005). The history of *Varroa* in the USA and Europe make it logical to ask what role *Varroa* parasitism plays in the recent HB losses worldwide.

The *Varroa* mite has been present for many years in most of the countries that are reporting an increase in colony losses at the moment. While mite populations can be controlled, one can still speculate that the losses can be due, at least in part, to *Varroa* infestations in the colonies. If so, what has changed regarding *Varroa* biology or the environment that can explain losses compared to 20 years ago? Potential reasons include changes in *Varroa* and host bee biology and population dynamics changes, *Varroa* control with acaricides, indirect effects of *Varroa* treatments and synergistic effects of multiple factors.

VARROA AND BEE HOST BIOLOGY

V. destructor genetic variability could explain differential colony losses if different haplotypes or strains of mites have different degrees of virulence to the bees, leading to differences in host-parasite equilibrium. It has been shown that two major mite haplotypes have spread over the world, the Japanese and the Korean haplotypes (Anderson, 2000), which might have different virulence depending on the haplotype (Correa-Marques et al., 2003; Vandame and Palacio, 2010).

Each haplotype has a clonal genetic structure (Solignac et al., 2005). While it is unlikely that different *Varroa* haplotypes could explain the recent colony losses, two new haplotypes of *Varroa destructor* were recently described in *A. mellifera* colonies in Southeast Asia (Navajas et al., 2009). Nothing is known about their virulence on European bees outside Asia, but those haplotypes might be a new threat to consider for *A. mellifera* bees outside Asia. Importation of bees from Asia should be considered carefully, since the impact of introducing Asian haplotypes to *A. mellifera* populations outside of Asia is unknown. The recent discovery of the Japanese haplotype in Spain makes it evident that HB market exchanges can disperse pathogens and parasites (Munoz et al., 2008).

V. destructor is a pseudo-haplo-diploid parasite species (Martin et al., 1997; Harris and Harbo, 1999) reproducing mainly through brother-sister matings, a system which largely favors the fixation of new mutations (Cornuet et al., 2006). Co-evolution of the host and the parasite is driven by mutations of both the mite and the bee, which can lead to a more or less stable equilibrium. Heritable behavioral and physiological traits can be involved in *Varroa* tolerance (see Büchler et al., 2010; Rinderer et al., 2010, this issue), but the mite may counter-select those traits to increase its fitness. Mite reproduction is an important trait in *Varroa* population dynamics and differential reproduction rates had been observed since the first infestations on HB were detected (Anderson, 2000). Changes in the host-parasite chemical ecology can influence *Varroa* populations and mite reproduction as the mite can be differentially attracted depending on inhibitory and stimulatory factors coming from different types of brood (Nazzi and Milani, 1996; Trouiller and Milani, 1999).

Population dynamics

At the time of the first introduction of *Varroa* in Germany, studies often found 7 000 to 11 000 mites in a colony 4 years after the initial infestation (Ritter and Penschil, 1982; Fries et al., 1994). At that time, the economic threshold was determined to be 200 fallen mites per

day in July (Ritter et al., 1984). It is now unusual to find such high *Varroa* infestation levels. Today, German beekeepers are required to start treatment if the natural mite drop exceeds 10 mites per day, a level that indicates the colony is close to collapse. In Germany, a colony mite load above 3 000 mites indicates the colony is close to collapse (Boecking and Genersch, 2008). Interactions between viruses and *Varroa* mite infestation are key to understanding colony collapse as it has been shown that the bees can tolerate a higher *Varroa* load if they are not infested by viruses (Martin, 2001). Sumpter and Martin (2004) worked on a model illustrating why different mite levels associated with colony collapse exist. As many viruses naturally infest HB colonies (Gauthier et al., 2007), to date we cannot exclude mite-virus interactions (even the recently identified IAPV virus) as a cause of variation in the mite load threshold required to cause colony losses.

VARROA CONTROL WITH ACARICIDES

Is acaricide control still a feasible way to manage *Varroa* populations? When the mite arrived in Europe in the 1970s, efficient control was rapidly deployed using bromopropylate, fluvalinate, amitraz and coumaphos in easy-to-use formulations. In 1995, the first occurrence of mite resistance to fluvalinate, a pyrethroid, was observed in Southern Europe, making the chemical compound unusable for *Varroa* control. The mites have developed resistance to fluvalinate and to other pyrethroids like flumethrin (Milani, 1995; Hillesheim et al., 1996), thus rendering pyrethroids ineffective as a class of miticides. In spite of alternating among chemical controls, the mite became resistant to other miticides like coumaphos and amitraz as observed especially in the USA (Milani, 1999; Elzen et al., 2000).

There is no chemical treatment with 100% effectiveness. Treatments that kill susceptible individuals leave the more resistant mites to produce the next generation, and over time, the mite population becomes increasingly resistant. Natural substances such as oxalic acid and thymol have not yet resulted in resistant

mite populations, but while they reduce mite populations, they are not consistently highly effective in all situations. The lack of effective miticides to control *Varroa* lets the mite populations grow to injurious levels triggering colony collapse directly by the number of mites per bee or indirectly by decreasing bee immunity and favoring virus multiplication. Moreover, *Varroa* resistance to acaricides favors the escalation of chemical applications leading to increased doses and residues of miticides in the hive. The amount and number of fat soluble residues from mite controls accumulating in the hives and in bee products, especially in the wax comb, are especially frightening (Wallner, 1999; Bogdanov, 2006; Martel et al., 2007). A recent study on the synergistic effects of fluvalinate and coumaphos showed a large increase in the toxicity of fluvalinate to young bees that had been treated previously with coumaphos, suggesting that HB mortality may occur with the application of sublethal doses of miticide when tau-fluvalinate and coumaphos are simultaneously present in the hive (Johnson et al., 2009). Other alternative controls including more natural substances like the essential oil, thymol, can result in build up of residues in wax over the years of treatments (Floris et al., 2004) and become toxic to the bees. The wax in those colonies can be melted to make foundation wax, but many miticides are stable in beeswax and can be reintroduced through contaminated foundation, favoring *Varroa* resistance to the miticides. Getting rid of miticide residues in beeswax is a widespread problem in beekeeping. Miticide residues can become more toxic when combined with agricultural crop pesticide residues when contaminated pollen loads are brought back to the hive by the foragers, sometimes in significant concentrations (Chauzat et al., 2006). The residues make the pesticide stress factor much more important for the bee as lethal synergetic interactions between pesticides and miticides can occur (Colin and Belzunces, 1992; Johnson et al., 2009). Miticides and pesticides accumulate in the colony matrices as a function of time, and the residues are more important now compared to 20 years ago. This chemical stress is being studied, as it could at least partly

explain colony losses ((Johnson et al., 2010), this issue). On the other hand, some chemical substances, especially the organic acids and essential oils, exhibit a disinfectant effect. When they are used for *Varroa* control, the pathogenic but also beneficial fungi and bacteria present in a healthy colony may be destroyed (Vasquez et al., 2009). A healthy colony's microflora appears to be an important part of the natural defense against diseases in a bee colony as demonstrated by their inhibitory effect, reducing susceptibility to *Ascosphaera apis* (Gilliam, 1997). Colony microflora may act in a similar way for other pathogens.

INDIRECT EFFECTS OF VARROA TREATMENT AND BEEKEEPING MANAGEMENT PRACTICES

The quality of *Varroa* control by beekeepers can explain some losses; a lack of treatment, and poor timing of treatments have been reported to be important in HB colony losses (Delaplane and Hood, 1997; Currie and Gatien, 2006). This is especially valid when nectar or honeydew is only harvested at the end of a bee season. To avoid residues in honey a chemical treatment can be done only after the harvest. At this time, the mite population has often already reached injurious levels.

Recent reports of HB colonies surviving *Varroa* mite infestation without treatment present a possible way to understand *Varroa* and HB biology and co-evolution (Ritter, 1993; Kefuss et al., 2004; Fries et al., 2006; Le Conte et al., 2007; Seeley, 2007), but this information needs to be considered carefully as *Varroa*-resistant HBs may not exhibit the same resistance if moved to other areas. For example the number of mites may increase when moving bees from one foraging crop to another one, disrupting the equilibrium between the parasite and the host in a way that it unfavorable to the bees. Also, bees that survive mite infestation may not have characteristics suitable for beekeeping such as honey production or they may be overly aggressive (for more details, see Büchler et al., 2010; and Rinderer et al., 2010, this issue). Moreover, the apparent lack of resistance of bees in many areas

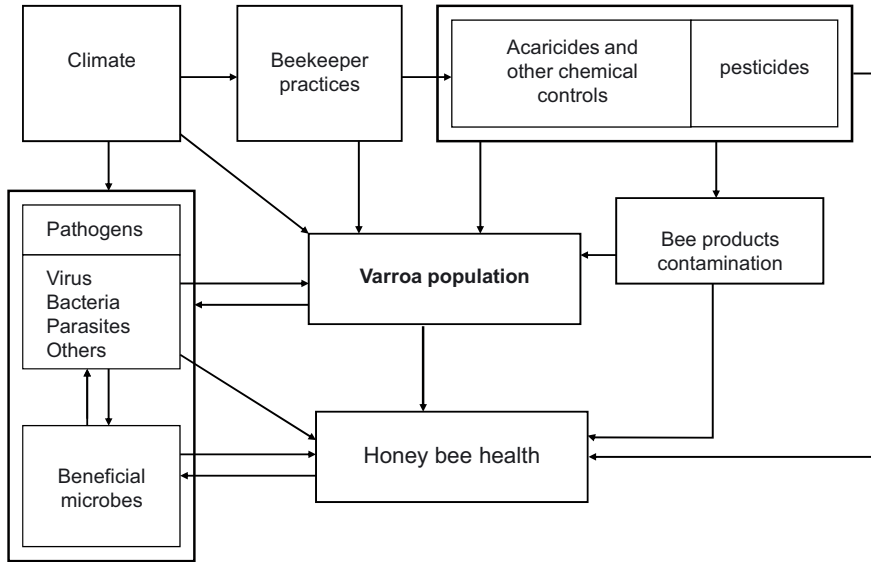


Figure 1. Potential synergistic effects of different stressors on varroa populations and HB health.

is not just a lack of co-evolution, but may be due to the management practice of keeping large numbers of colonies in close proximity where mites can spread even after they kill a colony. The practice of keeping colonies in high-density apiaries makes it easier for *Varroa* transmitted viruses to spread.

Varroa environment

Factors such as weather conditions, longer brood rearing periods and large amounts of drone brood can dramatically increase the mite population size (Calis et al., 1999; Martin, 2001). Global warming can induce longer spring and fall periods resulting in longer brood rearing periods and more drone brood, both of which lead to significantly larger mite population at the end of the year (Le Conte and Navajas, 2008). But longer summers also means longer foraging time which could increase the mite threshold levels (Sumpter and Martin, 2004).

SYNERGISTIC FACTORS

Colonies that died from CCD symptoms had capped brood with low levels of parasitic

mite indicating that the losses could not be attributed to a recent infestation of mites. Instead, vanEngelsdorp et al. (2009) suggested that CCD might be due to an interaction between pathogens and other stress factors, or a possible legacy effect of mite parasitism. Downey et al. (2000) demonstrated that the two parasites, *V. destructor* and *Acarapis woodi*, have biologically synergistic interactions at the individual and colony level that are detrimental to their host colonies (Downey et al., 2000; Downey and Winston, 2001). What about interactions between *Varroa* and other stressors? Many interactions could be involved in colony losses, such as pathogens, pesticides and the environment (climate change) (see Fig. 1). Except for very early data showing that *Varroa* mites kill bee colonies (Ritter et al., 1984), many studies of CCD or winter mortalities have not looked at the origin of the stress that led to losses, but rather at the results. In contrast, recent monitoring in Canada using colonies with different levels of *Varroa* infestation concluded that the *Varroa* mite was the main culprit causing reduced populations and death of overwintered HB colonies (Guzman-Novoa et al., 2010). In addition, a causal approach demonstrated that *V. destructor* is a key factor in winter losses in

Norway (Dahle, 2010). Control strategies for the mite must be developed that do not involve any application of acaricides to avoid side effects. The invasive *Varroa* mite has detrimental physical and physiological effects and is a potential immune-suppressing stress on bees allowing pathogens to multiply unchecked. Thus, interactions between the mite and other pathogens, especially viruses and newly identified pathogens like *Nosema ceranae*, should be studied to quantify their role in HB losses. *Varroa* interactions with miticides and other pesticides are also good candidates to study, as a detrimental synergetic interaction between *Nosema* and a neonicotinoid was recently demonstrated (Alaux et al., 2009). It is also well known that, similar to *Nosema*, the fat body is reduced in bees infested by *Varroa* making them more susceptible to pesticides. The reduction in fat body due to *Nosema* infection seems to be the essential factor, an effect which may also be caused by *Varroa* infestation of brood (Drescher and Schneider, 1987). It is logical that brood reared in a contaminated environment, such as in contaminated wax cells, may be more sensitive to effects of pesticides compared to adult bees, resulting in weakening and other health problems. Other combinations of parasites, pathogens and pesticides need to be studied, which will require a lot of work and coordination between scientists.

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Varroa et santé des abeilles : la présence de cet acarien peut-il expliquer en partie les pertes observées dans les colonies ?

Varroa destructor / Apis mellifera / syndrome d'effondrement des colonies / pertes hivernales / facteurs de stress

Zusammenfassung – Varroa-Milben und Honigbienenengesundheit: Kann Varroa einen Teil der

Völkerverluste erklären? Seit 2006 werden in Europa und Nordamerika katastrophale Völkerverluste gemeldet. Die Ursachen dieser Verluste waren nicht leicht zu erklären, sie wurden als überwinterungsbedingte Mortalitäten bewertet und unter dem Begriff Colony Collapse Disorder (CCD) zusammengefasst. Die meisten Wissenschaftler stimmen dahingehend überein, dass diesen Völkerverlusten keine Einzelursache zugrunde liegt, sondern, dass vermutlich verschiedene Stressfaktoren zusammenwirken. Da der *Varroa*-Befall einen wichtigen Druck auf die Gesundheit der Bienen in den Völkern darstellt, gingen wir der Frage nach, inwiefern *Varroa* an den seit kurzem beobachteten Völkerverlusten beteiligt sein kann.

Verschiedene physikalische und physiologische Faktoren mit negativer Wirkung der *Varroa*-Milbe auf die Gesundheit der einzelnen Biene und den Volkszusammenhang sind bereits bekannt. So führt das wiederholte Saugen von Hämolymphe zu Verletzungen der Bienen, zu erniedrigten Werten im Proteingehalt, sowie dem Lebend- und Trockengewicht und zur Behinderungen in der Organentwicklung. Die parasitische Milbe und die von ihr übertragenen Viren führen zu morphologischen Fehlentwicklungen, reduzierter Widerstandskraft und Lebenserwartung und zu negativen Effekten auf die Flugdauer und Heimfindungsfähigkeit der Sammlerinnen. Die Milbe schwächt das Immunsystem der Bienen, indem die Expression von Genen der Immunantwort reduziert wird. Zusammen mit erhöhten DWV-Viren-Titern reduziert sie damit die Lebensfähigkeit der Arbeiterinnen und die Koloniefitness. Die Rolle der *Varroa*-Milbe als Vektor in der horizontalen und vertikalen Übertragung von Viren ist hingehend bekannt, und Ko-Infektionen von *V. destructor* mit verschiedenen Viren wurden bereits als wichtige Faktoren im *Varroa*-bedingten Zusammenbruch von Völkern beschrieben. *V. destructor* muss deshalb weiterhin als eine ernsthafte Bedrohung der Honigbiene gelten, und weiterhin werden Honigbienenpopulationen weltweit durch diese parasitische Milbe dezimiert.

Die Hypothese, dass CCD durch eindringende *Varroa*-Milben und ihre immunsuppressiven Fähigkeiten hervorgerufen wird, ist damit nicht auszuschliessen und wird durch die Befunde von vanEngelsdorp et al. (2009) gestärkt. Die *Varroa*-Milbe ist seit Jahren in den meisten Ländern vertreten, aus denen auch Völkerverluste gemeldet werden. Obwohl die Milbenpopulationen kontrolliert werden können, kann trotzdem spekulativ postuliert werden, dass die Verluste zumindest teilweise durch einen *Varroa*-Befall der Völker bedingt sein können. Welche Veränderungen in der *Varroa*-Biologie können also die jetzigen Verluste im Vergleich zu denen vor 20 Jahren erklären? Wir diskutieren potentielle Ursachen, einschliesslich Veränderungen in der Biologie und Populationsdynamik von *Varroa* und ihrem Wirt, der Kontrolle von *Varroa* durch Akarizide, indirekte Effekten der

Varroa-Behandlungen, sowie synergistische Effekte multipler Faktoren.

***Varroa destructor* / *Apis mellifera* / Colony Collapse Disorder / Winterverluste / Honigbienen Stressfaktoren**

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