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Original article

The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies*

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Abstract – The Western honey bee, *Apis mellifera*, is the most important animal pollinator in agriculture worldwide providing more than 90% of the commercial pollination services. Due to the development in agriculture the demands for honey bee pollination are steadily increasing stressing the pollination capacity of the global managed honey bee population. Hence, the long-term decline of managed honey bee hives in Europe and North-America is of great concern and stimulated intensive research into the possible factors presumably causing honey bee colony collapse. We here present a four-year study involving more than 1200 bee colonies from about 120 apiaries which were monitored for the entire study period. Bee samples were collected twice a year to analyze various pathogenic factors including the ectoparasitic mite *Varroa destructor*, fungi (*Nosema* spec., *Ascosphaera apis*), the bacterium *Paenibacillus larvae*, and several viruses. Data on environmental factors, beekeeping management practice, and pesticides were also collected. All data were statistically analyzed in respect to the overwintering mortality of the colonies. We can demonstrate for several factors that they are significantly related to the observed winter losses of the monitored honey bee colonies: (i) high varroa infestation level, (ii) infection with deformed wing virus (DWV) and acute bee paralysis virus (ABPV) in autumn, (iii) queen age, and (iv) weakness of the colonies in autumn. No effects could be observed for *Nosema* spec. or pesticides. The implications of these findings will be discussed.

colony losses / *Varroa* / DWV / ABPV / *Nosema* / pesticides

1. INTRODUCTION

The western honey bee *Apis mellifera* L. is among the most important productive livestock due to the role of managed honey bee

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colonies in pollination of many crops, particularly of specialty crops such as nuts, berries, fruits and vegetables. Therefore, the economic value of honey production plays only a minor role compared to the economic value of honey bees as pollinators in agriculture (Morse and Calderone, 2000). For European crops it was estimated that 84% of crop species depend at least to some extent upon animal pollination, with honey bees being the most important animal pollinator (Williams, 1994). However, this figure is misleading since it does not take into account the importance of the crop to consumers. As the majority of the world's staple foods are wind- or passively self-pollinated (wheat, corn, rice), or are vegetatively propagated (potatoes), their production does not depend on and increase with animal pollinators (insects, birds, and bats). These crops account for 65% of global food production, leaving as much as 35% depending on pollinating animals (Klein et al., 2007). 90% of commercial pollination services are provided by managed honey bees, making honey bees the most important commercial pollinator in Europe and worldwide. The demands for agricultural pollination are increasing (Aizen et al., 2008) stressing the pollination capacity of the global managed honey bee population. Hence, it is not surprising that although the global population of managed honey bee hives has increased ~45% during the last half century (Aizen and Harder, 2009) the long-term declines of managed honey bee hives in the USA and some European countries became an issue of widespread interest and concern (Pettis and Delaplane, 2010; Moritz et al., 2010). As a consequence of these concerns research into the many factors presumably afflicting honey bees has been intensified in the recent past. The main focus lies on elucidating the role of pathogens and environmental factors, mainly pesticides, in decreased honey bee vitality and increased colony losses.

Concerning the role of pathogens, there is no question that the global health of honey bees is at risk, threatened by parasitic mites (*Varroa destructor*, *Acarapis woodi*, *Tropilaelaps* spec.), fungi (*Nosema* spec., *Ascosphaera apis*), bacteria (*Paenibacillus larvae*, *Melissococcus plutonius*), viruses, and vermin (small hive beetle). The most recent examples of catastrophic colony losses linked to – but not fully explained by – pathogens have been (i) the as yet mysterious Colony Collapse Disorder (CCD), which resulted in huge honey bee losses in the USA and elsewhere (Cox-Foster et al., 2007; Oldroyd, 2007; vanEngelsdorp et al., 2007), as well as (ii) massive colony losses in Spain since 2006 attributed to *Nosema ceranae* (Higes et al., 2008; Higes et al., 2006; Higes, 2010). In addition, honey bees are negatively affected by many pesticides and fungicides used in agriculture and the chronic exposure to acaricides needed to combat *Varroa destructor* in apiculture (Barnett et al., 2007; Desneux et al., 2007; Karise, 2007; Moncharmont et al., 2003; vanEngelsdorp et al., 2009a; Johnson et al., 2010).

In the winter 2002/2003, the beekeepers in Germany experienced unusual high winter losses with about 30% of the German honey bee population reported dead in spring 2003. Losses were not equally distributed among the beekeepers; instead, the mean of 30% was the result of many beekeepers that lost 80–100% of their hives on one hand and many that observed normal winter losses on the other hand. Similar to CCD, no easy explanation could be found for this phenomenon but has been reported from other European countries since then (Potts et al., 2010).

In response to these 2002/2003 winter losses the German Bee Monitoring Project was initiated in autumn 2004. The aim of this project was to unravel factors which are responsible for increased colony winter losses. The overall idea was to collect in advance colony data and samples of bees and hive products from a great number of colonies in order to use them later for a retrospective explanation of colony mortality. In order to best achieve this aim, more than 1200 bee colonies from about 120 apiaries (10 colonies per apiary) were monitored from autumn 2004 until now. Lost colonies were replaced with colonies originating from the same apiary, preferably with nuclei made from the lost colony in the previous year. Data on the presence of viral, bacterial and fungal pathogens, on varroa infestation level, on the health status

and strength of the colonies at different times of the year, on mite control regimes, on exposure to certain crops, on pesticide residues in rape pollen, and on beekeeping practice were collected by professional bee inspectors and the beekeepers themselves. Rape pollen was chosen for pesticide analyses because in Germany oil seed rape is the most important nectar and pollen source for honey bees in late spring (Horn, 2009) and a risk for the contamination of bee products due to the common and indispensable application of pesticides (Meixner et al., 2009). Colony mortality was recorded and correlated in a statistical analysis using more than 4000 data sets from 2004–2008. The results of this monitoring project will be discussed in the context of the ongoing colony losses in Europe and North-America.

2. MATERIAL AND METHODS

2.1. Structure and organization of the project

In reaction to the unusually high colony losses in Germany in the winter of 2002/2003 the German Bee Monitoring Project was established in autumn 2004 after several round table discussions involving bee scientists, experts of the German Ministry of Agriculture, beekeepers, farmers organizations, and representatives of agrochemical companies. The project was headed by a project board consisting of these partners. Nine scientific bee institutes of different Federal States in Germany were responsible for the coordination of the field work, the data collection, and the supervision of the beekeepers involved in the project. Only those data and samples which originated from selected beekeepers and their monitored colonies (see below) were included in the study and subsequent statistical analyses. Each bee institute supervised 6 to 24 beekeepers and farmers organisations and the bee inspectors and/or scientists were obliged to visit them at least at two of the three sampling dates each year (autumn survey before wintering in October, spring survey after wintering in March/ April or summer survey). During these visits the inspectors/scientists (i) collected detailed information about the previous period, (ii) took samples of bees and hive products from each of the 10 monitoring colonies, and (iii) estimated the population size of the colonies. A single monitoring period lasted from September to August of the following year.

2.2. Description of the apiaries and monitoring of the honey bee colonies

At the beginning of the monitoring project, selected beekeepers in Germany were asked to participate with 10 colonies designated for participation in the German bee monitoring project. Selection of the beekeepers – and, hence, the colonies – aimed at establishing a cohort representing 'all beekeeping' in Germany especially in respect to (i) geographical distribution (Fig. 1), (ii) the number of managed colonies (between 10 and several hundred colonies, Fig. 2), (iii) scale of beekeeping (hobbyist, semi-professional and professional beekeepers, Fig. 2), and (iv) the main nectar flow plants in the proximity of the apiary (i.e., the main source of nectar and pollen) with special focus on intensive crops like oil seed rape, sunflowers, and corn which have been suspected of causing a negative impact on the health of honey bee colonies (Tab. I). The project started in autumn 2004 with 112 beekeepers. In the 2005/2006 season already 123 beekeepers provided data, with 120 beekeepers continuing participation in 2006/2007 and 117 in 2007/2008.

Each participating beekeeper randomly selected ten colonies from his apiary to serve as 'monitoring colonies'. If such a colony collapsed in the course of the study it was replaced with another colony of the same apiary, preferably with a nucleus made from the collapsed colony in the previous year. The ten selected colonies were managed by the beekeeper like the other colonies in the apiary and according to his/her usual beekeeping practice including migration to specific honey crops, production of nuclei, requeening, and varroa treatment. This was to ensure that the colonies involved in the project reflected the entire range of variation in types of hives and management techniques common in and typical for Germany.

2.3. Factors analyzed in individual honey bee colonies

2.3.1. Questionnaires

Prior to the start of the project, participating beekeepers answered a basic questionnaire concerning

Table I. Main nectar flow plants in the vicinity of the apiaries as provided by the monitoring beekeepers at the start of the project.

		[%]			
Main nectar flow plants	No	Low	Middle	High	Not defined
Oil seed rape (*Brassica napus*)	36	11	16	37	
Sunflower (*Helianthus annuus)*	90	3	3	4	
Corn (*Zea mais*)	44	18	16	22	
Honey dew	32	25	16	20	7

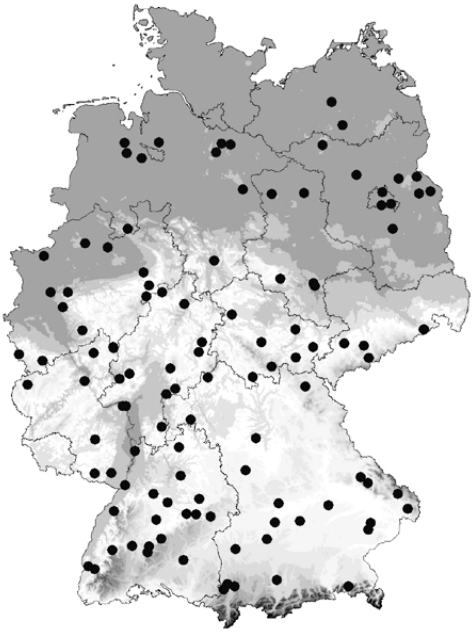

Figure 1. Geographical distribution of the apiaries of the German monitoring project during the year 2005.

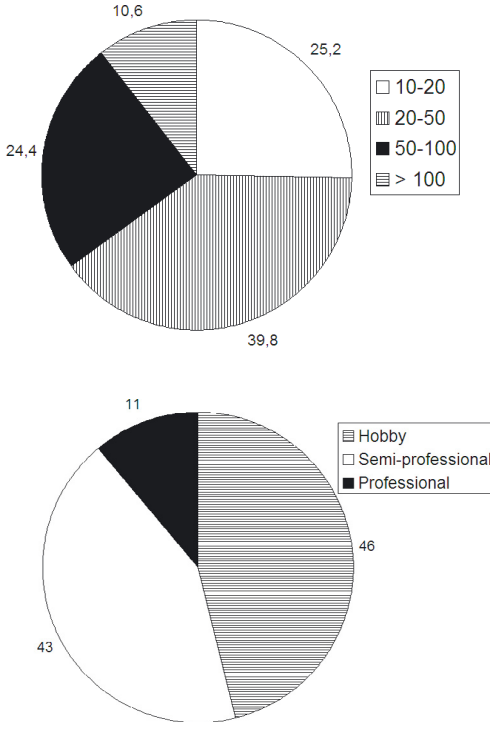

Figure 2. Size of the beekeeping business (number of colonies, upper diagram) and percentages of hobby and professional beekeepers among the participants in the monitoring project (lower diagram).

the total number of colonies, exact location of the apiary and the colonies (if several sites were used), details of the management system (type of hive, migratory beekeeping, and mode of colony multiplication). With additional yearly questionnaires the beekeepers provided information about their honey yields, migrations, production of nuclei, requeening of colonies, varroa treatment(s), abnormal population dynamics, and visible symptoms of diseases. This information was evaluated and verified as far as possible during the regular visits of the bee inspectors/scientists.

2.3.2. Record of colony winter losses

During the spring survey the beekeepers provided the number of colonies which collapsed over winter. A colony was considered dead if (i) no bees were present any more or (ii) the colony was too weak to have a chance to recover during spring (approximately less than three bee frames occupied by bees after winter). Colonies which collapsed between April and September were to be recorded but

no such losses were reported during the course of the study.

2.3.3. *Estimation of the population dynamics of the monitoring colonies*

The population size of each monitoring colony was estimated before (October) and after (March / April) wintering, preferably in colonies with little or no brood, i.e. after (autumn) and before (spring) massive brood rearing. For this purpose, the number of frames covered by bees was counted. The definition of "one frame covered by bees" was determined in training courses of the supervisors in order to standardize this measure as far as possible. In general the following procedure was used: all hives were opened and from two-story hives the upper magazines were tilt forwards. By doing so, all spaces between the combs could be inspected and the numbers of combs covered by bees were recorded. Depending on the climatic region, the date for the spring estimate varied between the apiaries. To avoid overestimating the population size of the overwintered colony the population estimation had to be performed prior to the emergence of the first spring brood. Therefore, the last accepted period for measuring the starting population was the 15th week of the year.

The quotient of the population size before and after the wintering of the colonies were calculated as "overwintering quotient" and represented a measure of the weakening of the colonies over winter and was used to analyze the effect of oilseed rape and the amount of pesticides in bee bread on the wintering of honey bee colonies.

2.3.4. *Sampling and analysis of adult honey bees*

Samples of about 150 adult bees were taken in October, spring and summer. For sampling, a comb with bees was taken from the periphery of the brood nest and the winter cluster, respectively, and the bees were shaken on a piece of plastic wrap and then put into labelled plastic vials. Samples were immediately stored at –20 °C until analysis.

The bees were analyzed for the following pathogens:

***Varroa destructor*:** from the autumn (October) samples, all bees were individually analyzed for varroa mites and the infestation level was calculated as 'number of mites per 100 bees' and given as 'percentage of infestation'. From the analyzed samples, a total of 3589 colony-years with full data sets could be used for the statistical analysis.

***Nosema* spec.:** from all spring samples about 20 bees were homogenized and after the addition of 2 mL water analyzed microscopically (400X). According to the number of *Nosema* spec. spores within the visual field positive samples were classified into weakly (<20 spores), medium (20–100 spores) and strongly (>100 spores) infected. In 1868 colonies from 2005 to 2007 autumn samples were also analyzed for *Nosema* infection and then used for statistical analysis in relation to winter losses.

Honey bee viruses: to limit the costs for the analysis, only one third of the autumn samples were analyzed for five honey bee viruses which were considered relevant in respect to colony losses: Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), sacbrood virus (SBV), deformed wing virus (DWV), and Israeli acute paralysis virus (IAPV). From each bee sample to be analyzed ten bees were taken and decapitated and subsequently total RNA was extracted from the heads for detection of SBV, ABPV, DWV, and IAPV (Siede et al., 2008; Yue and Genersch, 2005). For detection of KBV and also for IAPV, total RNA was extracted from the abdomens. KBV detection was only performed in the first three years of the project and then stopped since very few KBV positive bees were detected only in 2006. Instead, bee samples from 2007 were analyzed for the newly detected IAPV implicated in colony losses in the US (Cox-Foster et al., 2007; Maori et al., 2007). RNA extraction was performed using standard methods (RNeasy Kit, Qiagen) as described previously (Genersch, 2005; Yue et al., 2006). The rather moderate sampling size and the pooling of the bees will not allow the detection of the odd infected bee in the colony but will detect an infection level above 20% (Fries et al., 1984) which can be considered biologically relevant. In addition, a recent study demonstrated that analyzing individual bees has no advantage over analyzing pooled bees and that a pool of 20 bees is sufficient to reliably quantify virus levels in colonies (Highfield et al., 2009). To detect viral RNA, one-step RT-PCR was performed according to standard protocols (One-step RT.PCR kit; Qiagen) and as previously described (Genersch, 2005; Yue et al., 2006). The following temperature scheme was used: 30 min at 50 °C, 15 min at

Table II. Primer sequences used for virus detection.

Virus	Primer sequence	Length of amplicon	Annealing temperature	Reference
KBV	5'GATGAACGTCGACCTATTGA 3' 5'TGTGGGTTGGCTATGAGTCA 3'	414 bp	50.5	(Stoltz et al., 1995)
ABPV	5'CATATTGGCGAGCCACTATG 3' 5'CCACTTCCACACAACTATCG 3'	398 bp	49.5	(Bakonyi et al., 2002)
DWV	5'CCTGCTAATCAACAAGGACCTGG 3' 5'CAGAACCAATGTCTAACGCTAACCC 3'	355 bp	52.0	(Genersch, 2005)
SBV	5'GTGGCAGTGTCAGATAATCC 3' 5'GTCAGAGAATGCGTAGTTCC 3'	816 bp	52.0	(Yue et al., 2006)
IAPV	5'GAGCGTCGATCCCCCGTATGG 3' 5'TCCATTACCACTGCTCCGACAC 3'	524 bp	55.0	(Maori et al., 2007)

95 °C followed by 35 cycles with 30 s at 94 °C, 30 s at the appropriate annealing temperature (see Tab. II), 30 s at 72 °C followed by a final elongation step for 10 min at 72 °C. PCR products (5 µL per reaction) were analyzed on a 1.0% agarose gel. The agarose gel was stained with ethidium bromide and visualized by UV light. Correlation of the electrophoretic mobility of the amplicons with the expected size (Tab. II) was interpreted as specific detection. Specificity of the amplicons was further verified by sequencing (Medigenomix) random amplicons.

During the first two project years, also American foulbrood (*Paenibacillus larvae*) and tracheal mites (*Acarapis woodi*) were analyzed following the OIE guidelines and protocols given by the German National Reference Laboratory for Bee Diseases (Freiburg). However, tracheal mites were never observed and AFB only rarely detected. Therefore, these analyses were not continued in order to save resources.

2.4. Residue analysis

Samples of bee bread (appr. 10 × 10 cm) collected after the blooming period of oilseed rape (*Brassica napus*) were used for residue analysis. Oilseed rape provides a huge amount of nectar and pollen for honey bees in Germany but also represents a source for contamination with pesticides through seed dressing and spray application during the blooming period. Because most pesticides are lipophilic, pollen is considered the best matrix for measuring the exposure of a honey bee colony to pesticides. In the years 2005 and 2006, fifty apiaries were selected for residue analysis based on the microscopic pollen analysis of the honey. Only those which revealed a high input of rape nectar were considered suitable due to a potential exposure to pesticides. In 2007, bee bread samples of nearly all apiaries (n = 110) were analyzed.

All samples were split in two parts, one for the pollen analysis and one for the residue analysis. For the chemical analyses a multi-method (LC-MS/MS, GC-MS) was adapted which allowed the detection and quantification of 258 active ingredients. 5 g beebread were extracted with acetonitrile. After removal of fat and remaining proteins by cooling to –20 °C overnight, solvent was cleaned-up using gel-permeation-chromatography (GPC). The extract was further cleaned by SPE cartridges containing C18, aminopropyl and graphitized carbon black. The final extract was analyzed by GC-MS and LC-MS/MS for 258 pesticides and pesticide metabolites. The limits of quantification were between 3 and 10 µg/kg, in a few cases 15 µg/kg. For all neonicotinoids the limits of detection were at the level of 1 µg/kg.

2.5. Effect of locations with access to oilseed rape

In 2006, we analyzed a possible effect of oilseed rape on the wintering of honey bee colonies. The amount of rape pollen was determined in bulk samples of honeys from the first harvest from apiaries with different access to oilseed rape (n = 142). The overwintering quotient from October 2006 to March/April 2007 was calculated for the sampled colonies and correlated with the amount of rape pollen as determined by microscopic honey analysis.

Table III. Level of *Varroa destructor* infestation – as determined from adult bee samples collected in autumn – in all colonies and given separately for those colonies which survived or collapsed in the subsequent winter.

	Infestation level in % ± sd					
	2004	2005	2006	2007	Σ n	*P*-value[1]
All colonies	3.1 ± 5.8	4.7 ± 8.9	4.4 ± 8.6	5.1 ± 8.5		
(N)	(315)	(1065)	(1092)	(1117)	(3589)	
Surviving colonies	3.1 ± 5.9	3.2 ± 5.9	3.5 ± 6.7	3.6 ± 6.4		
(N)	(311)	(927)	(1013)	(966)	(3217)	
Collapsed colonies	1.7 ± 2.0	14.6 ± 17.0	16.5 ± 18.0	14.8 ± 13.5		< 0.000001
(N)	(4)	(138)	(79)	(151)	(372)	

[1] Varroa infestation in October was compared using Mann-Whitney U-test.

2.6. Data evaluation and statistical analysis

All data were fed into a central database programmed especially for this project. Each data set represents the complete parameters for one colony in one year (i.e. colony parameters of the autumn, spring and summer survey including colony size, all beekeeping practices such as type, date and number of Varroa treatments, migratory beekeeping, honey yield, colony management, the data from the questionnaire (see Sect. 2.3.1), and all laboratory data including honey, pollen and pathogen analysis). For statistical analysis, only complete data sets were considered. In addition, these complete data sets had to pass a plausibility check. Finally, of 5198 colony-level data sets, only 4313 could be used for the statistical analysis.

Some parameters like *Nosema* spec. in autumn and honey bee viruses were not analyzed in all colonies in each year and, therefore, for these parameters only a reduced number of data sets could be analyzed. The exact number of data sets used for each parameter is given in the results (Tabs. III–VI).

We compared surviving and collapsed colonies employing nonparametric tests because the basal assumptions of parametric tests (i.e. normality and constant variance) were not satisfied. All statistical analyses were carried out using Statistica 6.0. Kruskal-Wallis- and median-test proved that there were no significant differences in the distribution of colony losses between the years ($P > 0.05$). Therefore, the data sets of the four years were analyzed together. For each parameter given in Tables III–VI non parametric Mann-Whitney U-tests (varroa infestation rates) and chi^2 tests (honey bee viruses, Nosema infection, beekeeping management) were performed by comparing the survival rates in infested colonies with those in non-infested colonies. A P-value < 0.05 was considered significant.

3. RESULTS

3.1. Colony winter losses

It has to be mentioned that the monitoring beekeepers in general reported a satisfactory development of their colonies during the bee seasons of the study period. This is supported by average honey yields per colony of 39.5 kg in 2005, 49.0 kg in 2006 and 46.3 kg in 2007. Of the 4393 colonies included in the analysis over the four year period, 504 colonies died over winter but most did not show the described symptoms of colony collapse disorder (CCD) (vanEngelsdorp et al., 2007). In 80 of the 504 dead colonies the reason for the collapse could clearly be explained by queen loss (50), food shortage (17), crime (12) and AFB (1). These colonies were not considered in the following statistical analysis. Therefore, to unravel the reasons for inexplicable or not easily explainable winter losses 3889 surviving and 424 dead colonies were used for further analysis.

The average percentage of winter losses ranged from 3.8% (2004/05) to 15.2% in 2005/06 (Fig. 3). However, the losses were not distributed equally among the participating beekeepers. An analysis of all data sets of the four years showed that the majority of the beekeepers had no or only moderate

Table IV. Incidence of *Nosema* spec. and honey bee viruses in the adult bee samples from autumn.

		2004	2005	2006	2007	Σ n
Nosema	n		164	688	1072	1924
	positive %		31.1	21.4	13.8	
	strong infection %		1.2	0.7	2.4	
ABPV	n	182	276	296	350	1104
	positive %	8.8	5.8	6.4	11.7	
SBV	n	182	276	296	350	1104
	positive %	15.4	9.8	5.4	7.4	
DWV	n	182	276	296	350	1104
	positive %	4.4	11.2	20.6	33.4	
KBV	n	182	218	196	-	596
	positive %	0.0	0.0	1.0	-	
IAPV	n	-	-	-	341	341
	positive %	-	-	-	0.0	

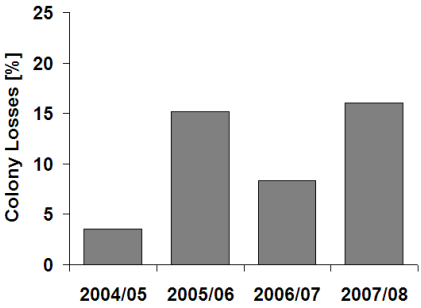

Figure 3. Proportion of lost colonies among the monitored colonies of the beekeepers participating in the project during the four years of the monitoring phase. For each year, the total number of lost colonies was calculated as proportion of all colonies participating in the project.

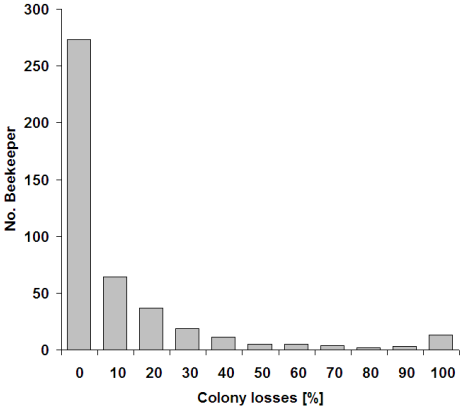

Figure 4. Average distribution of the colony losses during the winters 2004/ 05 – 2007/ 08 (n = 436 beekeeper). One data set represents one beekeeper with 10 colonies.

colony losses during the project period and only in 14.2% of the analyzed cases the losses were higher than 20% (Fig. 4). This distribution – many beekeepers with no or few colony losses and few beekeepers with high losses – were similar for all four winters as confirmed by Kruskal-Wallis- and median-test ($P > 0.05$). In addition to annual variations in winter losses regional variations were also observed, however, these regional differences were not consistent over the four years period. In addition, higher colony losses were not consistently related to certain beekeepers and apiaries.

3.2. Effect of pathogens and parasites on colony winter losses

The varroa infestation rates in October are given in Table III. The infestation rate for *Varroa destructor* ranged from 3.1 in 2004 to 5.1 in 2007. A statistically highly significant ($P < 0.000001$) difference between the varroa infestation rate of surviving colonies and of colonies which collapsed over winter could be demonstrated. Summarizing all data sets, the average varroa infestation in surviving colonies (av. ± s.e.: 3.4 ± 0.1) was significantly lower than in lost colonies (av. ± s.e.: 15.1 ±

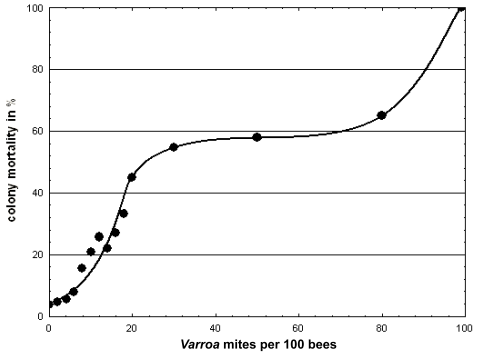

Figure 5. Relation between colony mortality in winter and varroa infestation level calculated as number of mites per 100 bees.

0.7). Even if one considers the huge standard deviation of the individual annual values the damage rate of some colonies might have exceeded the damage threshold level of more than 10 mites per 100 bees in autumn (Liebig, 2001) (Fig. 5). Plotting colony mortality against 'varroa mites per 100 bees' revealed that with as little as 10 mites per 100 bees around 20% of the colonies were prone to collapse over winter and an average of 50% mortality could be expected if more than 20 mites per 100 bees were present in a colony in autumn (Fig. 5). However, some of the colonies with high infestation rates of more than 30% in October were able to survive the winter (Fig. 5). Colony mortality and varroa infestation levels were significantly correlated (Spearman rank order correlation r = 0.996, $P < 0.00001$).

The prevalence of *Nosema* infection in autumn varied between ~31% Nosema positive colonies in 2005 (with a relatively low number of colonies) and less than 14% in 2007 (Tab. IV). Most of the *Nosema* infected colonies did only reveal weak infection levels; as few as 0.7% to 2.4% of the colonies were considered strongly infected.

Bee samples were qualitatively analyzed for the presence of DWV, ABPV, SBV KBV, and IAPV (Tab. IV). While KBV and IAPV could rarely (1.0%) or never be detected in any of the samples, respectively, DWV, ABPV, and SBV were more prevalent. The incidence of these three viruses varied independently from year to year. The incidence of ABPV varied between 5.8% in autumn 2005 and 11.7% in autumn 2007. The proportion of SBV positive samples differed between 5.4% in 2006 and 15.4% in 2004, while autumn samples testing positive for DWV varied between 4.4% in 2004 and 33.4% in 2007. In general, the season 2007/2008 showed the highest level of viral infections in the sampled and analyzed colonies.

In addition to the significant relationship between varroa infestation level and colony winter losses, the occurrence of DWV and ABPV was significantly higher in lost than in surviving colonies (Tab. V). No effect could be proven for SBV and *Nosema* spec. (Tab. V). Analysis of the viral infection status of surviving and collapsed colonies with chi^2-tests revealed that the presence of DWV in autumn was related with a surprisingly high significance ($P = 0.00001$) with winter losses. In other words, colonies which contained clinically infected bees (DWV viral RNA in total head RNA) in autumn had a lower chance to survive winter than colonies which tested negative for DWV. A similar relationship could be demonstrated for ABPV. Again, a significant relation between ABPV infection detected in autumn and colony collapse over the following winter could be established ($P = 0.0039$).

3.3. Effect of beekeeping management on colony winter losses

No effect could be confirmed for the material of the hive (wood vs. Styrofoam) and the "starting condition", respectively (Tab. VI). The latter refers to the common practice among German beekeepers to establish new colonies at the end of the season in order to have stronger and/or healthier colonies for overwintering. These new colonies could be established as nuclei (n) or could be composed of nuclei and "old" colonies (o) in different combinations (Tab. VI). A comparison of all possible colony types (n, o, n+n, o+o, n+o) did not reveal any statistical significance (Chi^2, df = 4; 1.75; $P = 0.78$).

A clear significant effect, however, was proven for the age of the queen: colonies which survived the winter had on average

Table V. Effects of pathogen infection and parasite infestation in October on winter losses of honey bees.

Factor	Total No. of colonies analyzed	No. of survived colonies			No. of collapsed colonies			*P*-value (chi^2)
		total	pathogen positive	pathogen negative	total	pathogen positive	pathogen negative	
DWV	1104	995	173	822	109	44	65	0.00001
ABPV	1104	995	75	920	109	17	92	0.0039
KBV	596	543	2	541	53	0	53	0.658
SBV	1104	995	99	896	109	6	103	0.202
Nosema spec.	1924	1744	317	1427	180	29	151	0.492

Table VI. Effects of beekeeping management on winter losses of honeybee colonies.

Factor	Total No. of analyzed colonies	Total No. of surviving colonies	Total No. of collapsed colonies	*P*-value
Type of beehive	4313	3889 wood / styrofoam (2594) / (1295)	424 wood / styrofoam (282) / (142)	0.94 (chi^2)
Starting condition[1]	4293	3876 O N C (2731) (724) (421)	417 O N C (317) (65) (35)	0.78 (chi^2)
Queen age years (n)	4021	3639 0 1 2 3 4 (2002) (1238) (192) (5) (2)	382 0 1 2 3 4 (156) (181) (45) (0) (0)	0.0052 (chi^2)
Colony strength in October (frames with bees ± sd)	4313	3889 12.3 ± 5.1	424 10.0 ± 5.4	< 0.000001 (t-test)

[1] Old colony from previous year (O), newly formed colony during summer season (N), or combined colonies (C) either o + o, o + n, n + n.

significantly younger queens compared to the colonies which collapsed during winter (chi^2, $P < 0.000001$, Tab. VI). In other words: young queens lowered the risk for a colony to collapse during the winter independently from the above mentioned starting conditions. A clear effect on the overwintering success was also confirmed for the colony strength (= number of bees) in October: the 3889 surviving colonies during the four year period occupied on average 12.3 ± 5.1 bee spaces compared to only 10.0 ± 5.4 in the dead colonies. These differences were highly significant (chi^2, $P < 0.000001$, Tab. VI).

3.4. Effect of oilseed rape on the overwintering quotient

The correlation analysis reveals a positive but not significant correlation between the amount of rape pollen in the honeys harvested in summer and the overwintering coefficient (Fig. 6). Therefore, the hypothesis could not be verified that intensive contact of honey bee colonies to oilseed rape has a negative influence on overwintering.

3.5. Residue analysis of pesticides in bee bread

42 active ingredients have been detected in the 105 analyzed samples of 2005 and 2006 (Tab. VII). In many positive samples more than one substance could be found. Only 25 samples did not reveal any measurable contamination (below limit of detection). The most abundant active substances were coumaphos (46, varroa treatment), boscalid (35, fungicide) and terbuthylazine (32, herbicide). Some samples showed quite high residue amounts

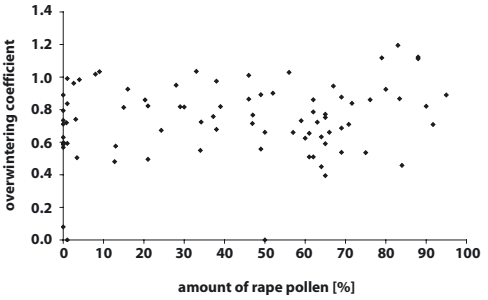

Figure 6. Relation between amounts of rape pollen in honey harvested in summer 2006 and the overwintering coefficient of the colonies in the subsequent winter 2006/2007. Statistical analysis of these data according to Pearson revealed no correlation (r = 0.173, $P = 0.12$).

(the herbizide azoxystrobin, and the fungizide tolylfluanid, for instance). However, these high residue amounts did not correlate with poor colony development. There was no significant difference in overwintering quotient between apiaries with no pesticide residues in the bee bread and those with higher amounts of residues (more than 10 μg/kg of at least one substance; $chi^2 P = 0.999$; F-Test $P = 0.938$; n = 40, bee bread 2006, overwintering 2006–2007). The most prevalent insecticide was thiacloprid (9, max. 199 μg/kg). Other detected insecticides were dimethoate (3 samples), azetamiprid (2), pirimicarb (2), tau-fluvalinate (2), and Lambda-cyhalothrin (1). The amounts of the active substances in these cases were below 10 μg/kg, except for dimethoate (20 μg/kg).

The results of the 110 bee bread samples from 2007 did not reveal striking differences to the results of the samples 2005 and 2006 in terms of the percentage of positive samples and the amounts of active ingredients. 42 active substances have been detected between 1 and 67 times in the 110 samples (Tab. VII). The main substances were again coumaphos (33 times), boscalid (67 times), thiacloprid (62 times), and terbuthylazine (48 times).

Due to their high toxicity to bees the neonicotinoids were of particular interest. However, clothianidin was not detected and imidacloprid was detected only once (3 μg/kg) in the 215 samples collected from 2005–2007.

4. DISCUSSION

For several reasons the German bee monitoring project represents a worldwide unique approach for the analysis of unusually high winter losses:

1. The project was established as a close cooperation between beekeepers and bee scientists enabling the monitoring of colonies managed by 'normal' beekeepers who continued to practice their usual beekeeping routine.
2. To reflect the beekeeping situation in all of Germany in respect to regional peculiarities, the participating apiaries (about 120) with more than 1200 colonies were distributed nationwide.
3. A long-term ongoing study was implemented to observe annual variations.
4. Data on bee pathology, on residue analysis of bee bread and information on the environmental conditions at the site of the apiary and beekeeping management practices were recorded to be analyzed in relation to colony winter losses.

Such a project requires an enormous effort for general coordination, for the supervision of the participating beekeepers, and for the maintenance of the central data base. However, the cooperation with the beekeepers worked out quite well and without major conflicts which is confirmed by the remarkable low fluctuation of less than 5% of the participants over the years. This was and is a prerequisite for the planned long term continuation of the monitoring project. The overall aims of this project were (i) to analyze the occurrence of periodically high winter losses on the basis of verifiable data and (ii) to correlate such losses with the factors measured within the project.

4.1. Colony losses

Periodic colony winter losses of 30% and more have been reported in Germany already for more than 50 years (Gnädinger, 1984) and recently from other countries (Ellis et al., 2010; Giray et al., 2010; vanEngelsdorp et al., 2008). However, during the four winters from

Table VII. : Number of pollen samples of the years 2005 and 2006 (total n = 105) and the year 2007 (total n = 110) which tested positive for the analyzed pesticides.

	Active ingredient	2005 and 2006	2007
Insecticides / Acaricides	Acetamipride	2	0
	Bromopropylate	8	4
	Clofentezine	1	0
	Coumaphos	46	33
	Dimethoate	3	3
	Fenpyroximate	0	2
	Flufenoxurone	0	1
	Imidacloprid	0	1
	Indoxacarbe	0	1
	Lambda-cyhalothrine	1	2
	Methiocarbe	0	22
	Methoxyfenozide	0	5
	Pirimicarb-desmethyl	0	7
	Pirimicarbe	2	3
	Tau-fluvalinate	2	4
	Tebufenozide	2	4
	Tebufenpyrad	0	1
	Thiacloprid	9	62
Fungicides	Azoxystrobin	10	12
	Bitertanol	2	0
	Boscalid	35	67
	Carbendazime	6	7
	Cymoxanile	3	0
	Cyproconazole	4	0
	Cyprodinile	11	0
	Difenconazol	1	3
	Dimethomorph	3	0
	Diphenylamine	0	2
	Epoxiconazole	0	1
	Fenpropimorph	1	7
	Fludioxonil	8	13
	Flusilazole	2	3
	Iprodione	2	1
	Iprovalicarb	1	1
	Kresoxim-methyl	0	4
	Metalaxyl	4	0
	Myclobutanil	5	3
	Penconazol	0	1
	Pyraclostrobine	2	10
	Pyrimethanil	0	6
	Tebuconazole	12	3
	Tolyfluanid	4	2
	Triadimenol	1	0
	Trifloxystrobine	3	0
	Vinclozolin	0	1

Table VII. Continued.

	Active ingredient	2005 and 2006	2007
Herbicides	Chloridazone	5	3
	Ethofumesate	3	3
	Isoproturone	6	25
	Metamitrone	1	4
	Metobromuron	1	0
	Metolachlore	7	15
	Metoxurone	2	0
	Metribuzine	1	0
	Pendimethaline	1	0
	Prosulfocarb	2	18
	Terbuthylazine	32	44

2004/ 05 until 2007/ 08 the mean winter losses of all monitoring beekeepers ranged only between 4% and 15%, but regionally higher losses were reported. An advantage of the cooperation with experienced beekeepers is that fundamental mistakes in the management practice should be rare and, therefore, unexpected colony losses may not be the result of beekeeping mismanagement. This assumption is supported by the fact that only 12 of 4393 died from starvation during winter.

Colony winter losses were not equally distributed among the participating beekeepers. Combining the datasets of all four years, only in 14.2% of the cases the loss rates were higher than 20%. This also means that the majority of the beekeepers had little or only moderate losses. However, as higher colony losses were not consistently related to certain beekeepers and apiaries, respectively, we can state that the colony losses during our monitoring project cannot exclusively be explained by the factor 'beekeeper'.

4.2. Reasons for colony losses

The German Bee monitoring project provided statistical evidence that certain factors are involved in causing winter losses of bee colonies. The identified factors were (i) high mite infestation levels, (ii) clinically relevant DWV infections in autumn, (iii) ABPV infections in autumn, (iv) old queens, and (v) relative colony weakness before overwintering. The main cause of overwintering problems was undoubtedly infestation with the ectoparasitic mite *Varroa destructor* followed by viral infections. Bee colonies exhibiting one or more of the above mentioned factors might have a rather small chance to survive winter according to the results of the project.

The infestation with *Varroa destructor* in fall clearly revealed the highest relation with winter losses of honey bee colonies. As a measure for the infestation rate we used "varroa mites per 100 bees" in October. At that time honey bee colonies in Germany already have produced their winter bee population and usually have little or no brood. It is believed that varroa damages at the colony level already are engendered by the infestation during late summer, when the host population declines, the relative varroa parasitization increases and consequently the production of healthy long-living winter bees is negatively impacted (Amdam et al., 2004; Fries et al., 1994). However, the infestation rate of the "October bees" obviously represents an excellent measure to predict the risk of colony winter losses. For infestation rates between 0 and 20% a nearly exponential increase of the winter losses can be observed. Figure 5 indicates a threshold of 6% for the infestation rate to keep the average colony losses below 10%, which is considered an acceptable colony loss rate for winter. This confirms recent field studies (Liebig, 2001) showing that infestation rates of the winter bees of more than 7% were critical for the winter survival of colonies under German conditions. In the

USA (Delaplane and Hood, 1999) and Canada (Currie and Gatien, 2006) infestation rates of 10% and even more were suggested as thresholds for economic damages. Our results indicate that these thresholds may be too high, at least for German conditions. Our surviving colonies had average infestation rates of approximately 3% whereas the infestation of collapsed colonies was 5 fold higher, on average, with a huge variation (Tab. III). Furthermore, it is likely that more highly infested colonies which did not collapse exhibit sublethal damages which may affect the spring development after overwintering.

Surprisingly, winter mortality only increased from ~55% to ~65% for colonies with infestation rates ranging from 30% to 80% (Fig. 5). This contradicts field experiments where non-treated colonies with infestation rates of more than 30% during summer do not have a chance to survive the following winter (Fries et al., 2003; Rosenkranz et al., 2006) on the one hand, but, on the other hand, figures similar to those obtained in the current study have also been observed in managed colonies in cold climate (Strange and Sheppard, 2001). The surviving monitoring colonies with high varroa infestation may, therefore, be victims of reinvasion after the production of winter bees (Goodwin et al., 2006; Greatti et al., 1992; Renz and Rosenkranz, 2001) or may have been free of secondary infection.

Additionally, we have to consider that nearly all of these colonies had been treated at minimum once against varroa during summer, usually with formic acid. Our results also clearly demonstrate that the varroa treatments performed so far are not sufficiently effective to ensure a regionwide reduction of the varroa infestation and, therefore, minimize the risk of reinvasion.

We can, therefore, state that *Varroa destructor* still represents the major threat for the winter survival of honey bee colonies in Germany. Varroa as a main factor for winter losses have also been proven in recent surveys from Europe (Topolska et al., 2008) and the USA (vanEngelsdorp et al., 2008). The infestation of the "October bees" could be used in extension work to predict the chance for survival of colonies before winter. Varroa treatments should be performed in a way to achieve a bee infestation rate in autumn of less than 5%.

In the literature several studies determining the viral infection status of honey bee colonies can be found (Baker and Schroeder, 2008; Berenyi et al., 2006; Tentcheva et al., 2004). The general picture emerging from these studies is that DWV is the most prevalent virus in Europe with more than 90% of the analyzed colonies being infected. The same seems to be true for Germany (Yue and Genersch, 2005) although no epidemiological study has been performed so far. Unfortunately, DWV incidence of 90–100% in all colonies regardless of whether they are strong, weak or collapsing does not allow correlating DWV infection with colony losses since the mere presence of DWV in otherwise healthy bees is obviously of no clinical relevance (de Miranda and Fries, 2008; Yue et al., 2007). Recently it was shown that the detection of DWV RNA in total RNA extracted from bee heads correlated with clinical symptoms like crippled wings (Yue and Genersch, 2005). However, a small proportion of seemingly healthy bees also show DWV infection in the head (Yue et al., 2007) which is interesting in the context of a recent study involving experimental DWV infection of adult bees. Injecting DWV into the hemolymph caused learning deficits pointing to neurological symptoms being associated with DWV infections (Iqbal and Müller, 2007). Such learning deficits might affect the fitness of individual bees and, hence, colony performance. We therefore chose to diagnose DWV infections by using extracts from bee heads rather than from entire bees. Not surprisingly, we found a rather low rate of DWV positive colonies compared with previous studies (Baker and Schroeder, 2008; Berenyi et al., 2006; Tentcheva et al., 2004) ranging between 4.4% in autumn 2004 and 33.4% in autumn 2007. Statistically relating these data with the observed winter losses revealed a highly significant ($P = 0.00001$) negative effect of DWV infection (characterized by viral detection in 'head') in autumn on winter survival. Most of the collapsed colonies also had high varroa infestation levels confirming the strong association of clinical DWV infections with *V. destructor* infestation (Ball, 1983, 1989; Ball

and Allen, 1988; Bowen-Walker et al., 1999; Gisder et al., 2009; Martin, 2001; Shen et al., 2005; Yang and Cox-Foster, 2005; Yue and Genersch, 2005). However, a few collapsed colonies with DWV showed no or low mite infestation levels (data not shown). This suggests that DWV can contribute to colony collapse even in the absence of *V. destructor* as also implicated by a recent study (Highfield et al., 2009). Another possibility is that a clinical DWV infection of the colony initiated by high mite infestation rates during spring and summer can cause colony collapse in winter even though *V. destructor* had been successfully eliminated during late summer and autumn treatments.

ABPV infection in autumn was also significantly related ($P = 0.0039$) to colony collapse in the following winter confirming the results of a recent study from a small region in Germany (Siede et al., 2008). In this study virus detection in extracts from entire bees was compared with detection in head extracts. It was shown that both methods were equally meaningful although ABPV detection in total RNA from head produced slightly more significant results (Siede et al., 2008). Again, although most of the collapsed colonies with ABPV showed high mite infestation levels, a few of these colonies had low infestation levels. The same explanations as already outlined for DWV might also hold true for some ABPV associated colony collapses: Either ABPV can sometimes cause colony collapse even in the absence of *V. destructor* or eliminating *V. destructor* once a fatal ABPV infection has already been initiated in the colony does no longer change the fate of the colony.

No negative effect of SBV or KBV on winter survival could be demonstrated in our study. The rates of infection were consistent with other studies (Baker and Schroeder, 2008; Berenyi et al., 2006; Tentcheva et al., 2004). For detection of SBV again only RNA extracted from bees' heads has been used as opposed to whole bee extracts commonly used in the literature. SBV is a brood pathogen but persistence in the hypopharyngeal glands of adult bees drives virus transmission in the colony (Bailey and Ball, 1991). Due to this tissue tropism of SBV, using head extracts of adult bees for SBV diagnosis was the method of choice for increasing detection sensitivity. KBV was only detected in a few colonies. This is in accordance with the worldwide distribution of KBV which is most prevalent in North America and New Zealand but less prevalent in Europe (de Miranda et al., 2010) where ABPV is most prevalent (de Miranda et al., 2010). In addition, KBV was also the least prevalent virus found in other European studies on the incidence of bee viruses in diseased and healthy colonies (Baker and Schroeder, 2008; Berenyi et al., 2006; Tentcheva et al., 2004). For both SBV and KBV the missing correlation with colony collapse is in accordance with previous studies revealing a rather low incidence of these two viruses and even a higher prevalence in healthy colonies when compared to weak or collapsing colonies (Berenyi et al., 2006; Tentcheva et al., 2004).

In summary, a clear relation between winter losses and viral infection in autumn could only be established for DWV and ABPV. ABPV can be considered a member of the highly virulent ABPV-KBV-IPAV virus complex (de Miranda et al., 2010) with all members being extremely virulent when injected into pupae or adults (Bailey and Ball, 1991; Bailey et al., 1963). It is therefore not surprising that ABPV as well as IAPV are implicated in colony losses (Cox-Foster et al., 2007; Siede et al., 2008) and that ABPV was involved in winter losses of monitoring colonies of the study at hand. So far, DWV has been implicated in colony losses in only one study (Highfield et al., 2009). Other studies might have missed this relation because if 90–100% of the colonies are diagnosed as DWV-positive but only 10–30% of the colonies collapse statistical tests will not reveal a relation between DWV infection and colony collapse. Therefore, it is important to differentiate between clinically irrelevant and clinically relevant infections of colonies and to detect only those colonies which carry a clinically relevant infection. This can be achieved by quantifying DWV loads in asymptomatic bees and colonies since DWV loads exceeding 1×10^8 copies per asymptomatic worker bee in winter seem to be fatal for the colony even in the absence of high mite infestation levels

(Highfield et al., 2009) or by considering the differences in DWV tissue tropism between overtly and covertly infected bees (de Miranda and Genersch, 2010; Gisder et al., 2009; Yue and Genersch, 2005) and restricting DWV diagnosis to total RNA extracted from head as done in the study at hand.

Recently, infections with *Nosema ceranae* leading to an usual form of nosemosis have been implicated in severe colony losses in Spain and it was suggested that this unusual form of nosemosis is the main cause of inexplicable colony losses and CCD-like phenomena in Europe if not worldwide (Higes et al., 2006–2009; Martin-Hernandez et al., 2007) due to the high virulence of *Nosema ceranae* and its exceptional biotic potential even at higher temperatures (Martin-Hernandez et al., 2009). These assumptions are in contrast to several other studies identifying IAPV as reliable marker for CCD (Cox-Foster et al., 2007) or showing that CCD symptoms can be reduced by anti-viral treatment (Maori et al., 2009) or ruling out *Nosema* spec. as cause for colony losses (Chauzat et al., 2007; Johnson et al., 2009; vanEngelsdorp et al., 2009b; Chen and Huang, 2010). Likewise, the results obtained with the German bee monitoring project did not reveal any relation between infection with *Nosema* spec. and winter losses although both *Nosema* species are prevalent in Germany (Klee et al., 2007). Since no losses occurred during summer although colonies were infected by *Nosema* spec. it can also be ruled out that infection with *Nosema* spec. killed colonies between spring and autumn as described in the Spanish studies (Higes et al., 2008; Martin-Hernandez et al., 2007). A weak point of the study at hand is that the differentiation between the *Nosema* species has been performed only sporadically and, therefore, could not be included in the statistical analyses. Nevertheless, colony losses caused by *Nosema ceranae* would not have been masked by this approach and, therefore, the interpretation that *Nosema* spec. did not cause colony losses in Germany during the study period is valid.

Another factor which could be significantly related to winter losses was the age of the queen heading the monitored colony. For the first time we could demonstrate that colonies headed by young queens have a significantly higher chance to survive the winter compared to colonies with older queens. A possible reason for this queen-age-effect could be a significantly higher brood and bee production in colonies with young queens accompanied by a lower infestation with varroa mites (Akyol et al., 2007). However, the detailed reasons for the higher vitality of colonies headed by younger queens remain elusive.

The analysis of pesticide residues in pollen (bee bread) as performed in the course of the German bee monitoring project was the first such screening in Germany. As expected, the results show that pollen is contaminated with a plethora of chemical substances originating from the agricultural practice of using pesticides but also from the apicultural necessity of using acaricides. During bloom of oilseed rape many pesticides are used and, hence, they can be detected in many pollen samples. Likewise, pollen samples from apiaries in regions with intensive cultivation of rape showed a higher contamination level. It is generally assumed that although individual substances might not have a negative effect on individual bees and colonies (i.e. non-toxic for bees), the simultaneous contamination of pollen with several agrochemical substances will have a negative effect on larvae or nurse bees consuming such multi-contaminated pollen. These presumed sublethal effects than negatively influence colony development eventually leading to colony collapse. The contaminations identified in bee bread in the course of the German bee monitoring project were indeed mainly substances which are considered non-toxic for bees. In addition, the observed amounts of the residues were quite low, i.e. three orders of magnitude lower than the respective LD_{50} (http://sitem.herts.ac.uk/aeru/footprint/en/index.htm). Accordingly, no relation between contamination of pollen and colony development or winter losses could be demonstrated in the course of the project although special emphasis was put into this aspect. Still, further investigations and controlled experiments with improved methodology (Pham-Delègue et al., 2002) are undoubtedly necessary because several studies

did prove negative effects of pesticides on honey bees (Decourtye et al., 2003, 2004; Moncharmont et al., 2003; Johnson et al., 2010).

4.3. Conclusion

A panel of factors have been analyzed for their role in winter losses of honey bee colonies in Germany. Among all these factors, infestation with *Varroa destructor* turned out to play the key role. Based on the results presented it is safe to state that *Varroa destructor* is the dominant killer of honey bee colonies during winter. In addition to high varroa infestation levels, DWV and ABPV infections in autumn significantly lower the winter survival of honey bee colonies as do old queens heading overwintering colonies. That a weak colony has not the best chance to survive the winter is rather trivial but the fact that we observed such winter losses due to colony weakness shows that beekeepers still winter weak colonies. It is safe to assume that these identified factors are not specific for winter losses in Germany but that these results have wider implications. *Varroa destructor*, viral infections, old queens, colony weakness for sure are also responsible for winter losses in many other European regions and may be even in parts of North-America. This does not rule out that from year to year other, additional factors also play a role in colony losses and that the reasons for the periodically occurring, unusually high winter losses of more than 30% are different from what we observed during the last five years with rather normal winter losses. The continuation of the project is important to allow the generation of a database that can provide an explanation for winter losses using statistical evidence.

A negative effect of pesticide residues in bee bread from spring on the survival of the bee colonies in the subsequent winter could not be proven, however, our approach was not proposed to record sublethal and chronic effects of multiply contaminated pollen. For such issues, more extensive sampling procedures and enhanced methods are required.

From the results of this study we can deduce a general recommendation for beekeepers who want to successfully bring their colonies through the winter season: an effective treatment against *Varroa destructor* is the best life insurance for honey bee colonies. In addition, wintering strong colonies headed by young queens will improve the chances of the colonies to stay alive over winter. Following these recommendations will not generate eternal honey bee colonies but will definitely reduce colony winter mortality.

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Le programme de surveillance de l'abeille en Allemagne : une étude à long terme pour comprendre les pertes hivernales importantes constatées périodiquement dans les colonies d'abeilles.

perte des colonies / *Varroa* / DWV / virus des ailes déformées / APV/ virus de la paralysie aiguë / *Nosema* / pesticides

Zusammenfassung – Das Deutsche Bienenmonitoring: Eine Langzeitstudie zum Verständnis periodisch auftretender, hoher Winterverluste bei Honigbienenvölkern. Die Honigbiene *Apis mellifera* ist weltweit der wichtigste Bestäuber in der Landwirtschaft und nach aktuellen Schätzungen wird der globale Bedarf an kommerzieller Bestäubung weiter steigen. Dadurch stellt der seit Jahren zu beobachtende stetige Rückgang der Bienenvölker in Nord-Amerika und Europa ein ernsthaftes Problem für die Landwirtschaft dar. Für die Abnahme der Bienenvölker werden neben wirtschaftlichen Faktoren vor allem periodisch auftretende Völkerverluste verantwortlich gemacht, für die aber

eine eindeutige Ursachenanalyse bisher fehlt.
Zur Ursachenaufklärung von Winterverlusten führten wir von 2004 bis 2009 ein Monitoringprojekt durch, in dem mehr als 1200 Bienenvölker auf 125 über ganz Deutschland verteilten Bienenständen (Abb. 1) kontinuierlich beprobt und kontrolliert wurden. Die beteiligten „Monitoringimker" stellten hierfür 10 ihrer Völker zur Verfügung und lieferten Daten zu Honigerträgen, Wanderungen und Ablegerbildung. Mitarbeiter der Bieneninstitute nahmen zweimal im Jahr Bienenproben für Krankheitsuntersuchungen (*Nosema spec, Varroa destructor*, 4 verschiedene Bienenviren) sowie Bienenbrotproben für Rückstandsuntersuchungen. Die Stärke der Bienenvölker wurde bei der Ein- und Auswinterung bestimmt; als „Überwinterungsverlust" wurden Völker definiert, die tot waren bzw. nicht genug Bienen für eine erfolgreiche Frühjahrsentwicklung aufwiesen.
Die Winterverluste schwankten zwischen 3,5 % und 15,2 % (Abb. 3) mit ungleicher Verteilung innerhalb der beteiligten Imker (Abb. 4). Für die Ursachenanalyse wurden die überlebenden mit den zusammengebrochenen Völkern verglichen. Dabei zeigten sich die größten und hochsignifikanten ($P <$ 0,000001, U-Test) Unterschiede beim Varroabefall der Bienen im Oktober (Tab. III, Abb. 5). Ebenfalls hochsignifikante Unterschiede ergaben sich für die Bienenviren DWV ($P <$ 0,00001) und APBV ($P <$ 0,0039), nicht jedoch für KBV, SBV und den Nosemabefall (Tab. V). Erstaunlicherweise waren Völker mit jungen Königinnen signifikant seltener von Winterverlusten betroffen als mit älteren Königinnen (Tab. VI), während z. B. Beutenmaterial oder Rähmchenmaß keine Rolle spielten.
Bei den insgesamt in drei Jahren auf Pestizidrückstände untersuchten 215 Bienenbrotproben wurden insgesamt über 50 Wirkstoffe (von 256) nachgewiesen, die meisten im Spurenbereich. Häufig wurden mehrere Wirkstoffe gefunden und nur etwas mehr als 20 % der Proben waren frei von messbaren Rückständen (Tab. VII). Neonikotinoide wurden nur in einer einzigen Probe nachgewiesen. Es konnte keine Korrelation von Rückstandswerten mit Winterverlusten festgestellt werden. Es gab auch keinen Zusammenhang zwischen der Überwinterung von Bienenvölkern und dem Umfang des zuvor eingetragenen Rapshonigs (Abb. 6).
Unser Projekt zeigt, dass der Varroabefall im Herbst (zusammen mit den assoziierten Sekundärinfektionen) eine Hauptursache für Überwinterungsverluste darstellt. Eine konsequente Varroabehandlung und starke Bienenvölker mit jungen Königinnen sind daher die wichtigste Empfehlung, um Winterverlusten vorzubeugen. Ein zusätzlicher Einfluss der übrigen Faktoren kann nicht ausgeschlossen werden, hierfür sind aber modifizierte Versuchsansätze notwendig.

Völkerverluste / Varroa / DWV / ABPV / Nosema / Pestizide

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