incident schemes honeybee incidents are likely to go undetected or the cause unproven. The decrease in the numbers of incidents reported supports the ascertain that such scheme positively contribute to the regulatory process and can provide confidence in the risk assessment approaches.

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Periodical honey bee colony losses in Germany: preliminary results from a four years monitoring project

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Abstract

Within the framework of the German Bee Monitoring Project winter losses of bee colonies were evaluated from the database of 120 beekeepers and 1200 bee colonies by assessing the following parameters: data on the apiary (site, nuclei, movement of colonies, *Varroa* treatment), strength of the colonies in autumn and spring, honey yields, residues in bee bread (stored pollen), bee disease analysis.

During the last four years the winter losses of the monitoring beekeepers were between 8 and 16% and showed regional differences. The loss rates were clearly lower than those of non-monitoring beekeepers.

In 215 bee bread samples analysed with a sensitive multi-method, more than 55 active ingredients were found. Most active ingredients were found in traces but often in combinations. Primarily fungicides, varroacides and herbicides were found. Clothianidin was not found in any sample. Imidacloprid was found in one sample at the limit of detection.

4400 data sets were statistically analysed for the identification of triggers with negative influence on overwintering. The winter losses were significantly correlated with *Varroa* infestations and virus infections in autumn. It was concluded that no acute effects on honey bees have to be expected on the basis of the evaluated residue data. For testing potential sublethal or long term effects a useful test design has to be developed. The project will be continued in 2009.

Keywords: German Bee Monitoring Project, colony losses, *Apis mellifera, overwintering*

Introduction

The German Bee Monitoring Project was established in 2004 with the aim of finding explanations for periodical colony losses. It was considered advisable to involve all people dealing with bees and apiaries for this long term and large project with scientific approach and standing in the focus of the press and public political discussions. The founded project council consisted of national beekeeper associations, farmer association, authorities, German apicultural institutes and chemical industry. Financial support was given by the chemical industry on a level of nearly 50%.

The project cooperation partners planned the project. For collecting data of bee colonies it was decided to work on a large basis. A unique structure was established for assessing the health status of colonies effectively and scientifically. This is the first and only long-term monitoring project in the world providing verified data.

Data assessments

Data on the development of 1200 bee colonies in 120 apiaries spread all over Germany were assessed over four years by standardized methods. More than 100.000 data were assessed and about 5200 statistically analysable records of colonies were created. The participating apiaries represent the whole\ German spectrum in size, beekeeping management and use of honey flow. The beekeepers provided general data about their apiaries (all colonies, which means about 7000 colonies in total). Data on the apiary, site of the apiary (climate, honey flow, plant protection measures), colony losses, honey yields, beekeeping practice (movement of colonies, *Varroa* treatment, nuclei) were assessed per season.

The beekeepers, with a supervisor of the responsible bee institute, focussed on details of the 10 monitored colonies such as the population dynamics (strength and the development of the colonies before and after overwintering) and samplings (bee samples for diseases) three times per season, honey samples two times per season, one bee bread sample.

The collected bee samples were analysed in the laboratory for *Varroa* infestations, virus infections (ABPV - Acute Bee Paralysis Virus, DWV - Deformed Wing Virus, KBV - Kashmere Bee Virus, SBV - Sacbrood Bee Virus, IAPV - Israel Acute Paralysis Virus since 2007), *Nosema* sp. infection, *Malpighamoeba mellificae* infection and *Acarapis woodi* infestation. The collected honey samples were analysed for their botanical origin. The bee bread samples were analysed for residues with a multi-method detecting 258 active ingredients by an independent laboratory (LUFA institute, Speyer). All data were saved in a central database.

Results and conclusions

The most important results were summed up by the German apiculture research institutes in annual interim reports. The following results are part of the interim report 2004 to 2008[1].

Colony losses

The average winter losses were lower than those of the disaster year 2002/2003 with a loss rate of 28.9%. Noticeable are the annual and regional differences. Among them quite high losses occurred (table 1a). In single cases high losses occurred up to total losses (table 1b). Over 10% of the monitoring beekeepers had no losses over the four project years. The loss rates of the monitoring beekeepers were about 50% lower than those of non-monitoring beekeepers as it appeared from surveys over the project years. Maybe that the

monitoring beekeepers represent a "positive selection" and were better supervised. It was concluded that good management has a big influence on the bee health.

Table 1a Overwintering losses (in %) of monitored colonies. Over 7000 colonies were monitored by beekeepers, supervised by the bee institutes

Supervising bee institute	**2004/2005**	**2005/2006**	**2006/2007**	**2007/2008**
Number of colonies before overwintering (n)	**7240**	**7168**	**7013**	**7187**
Celle	2,7	4,0	18,5	7,6
Freiburg	12,0	14,0	15,9	18,5
Halle	11,6	13,6	7,2	36,5
Hohenheim	6,3	2,2	1,4	1,8
Hohen-Neuendorf	9,0	24,8	3,1	17,8
Kirchhain	7,1	13,9	12,0	15,1
Mayen	5,2	12,1	6,1	16,9
Münster	5,7	14,1	0,4	14,0
Veitshöchheim	11,5	16,2	15,0	14,6
Total	**7,9** [a)]	**12,8** [a)]	**8,8** [a)]	**15,9** [a)]
	6,6 [b)]	**13,1** [b)]	**11,0** [b)]	**12,8** [b)]

[a)] Average percentage of losses; [b)] Losses calculated over the total number of colonies

Table 1b Winter loss levels of participating apiaries. Of all apiaries participating during the four project years nearly 1/3 had no losses while about 15% had losses over 20%.

Level of losses [%]	**Apiaries [number]**	**Apiaries [%]**
0	156	32,8
0-10	157	33,0
10-20	89	18,7
20-30	31	6,5
30-40	15	3,2
40-50	13	2,7
50-60	3	0,6
60-70	1	0,2
70-80	5	1,1
80-90	2	0,4
90-100	4	0,8

Honey yields

The reported project years were good up to very good years of honey yields with almost more than one honey flow during the seasons (table 2).

Table 2 Average honey yield per participating colony in kg/colony

Supervising bee institute	2004/2005	2005/2006	2006/2007	2007/2008
Celle	41,7	41,0	40,3	40,2
Freiburg	28,6	66,3	87,5	-
Halle	38,9	49,5	49,5	37,5
Hohenheim	32,8	57,3	34,3	21,3
Hohen-Neuendorf	37,9	55,8	50,9	51,8
Kirchhain	44,6	44,3	40,2	39,9
Mayen	43,5	38,3	41,0	37,8
Münster	49,4	45,6	38,7	16,4
Veitshöchheim	37,9	42,7	34,7	24,5
Total	**39,5**	**49,0**	**46,3**	**33,7**

Bee diseases

Varroa

As one of the most important criteria the *Varroa* infestation level was assessed at the start of winter (after late summer treatment).

The *Varroa* infestation before winter varied between the supervising institutes and years of monitoring. During the first project years the average was under 5%. In 2007, the year of high *Varroa* infestations, the average of 6% was clearly higher (table 3). As the high *Varroa* infestation was widespread, all bee institutes warned in good time. Probably the warnings for a consequent treatment were better put into effect by the participating beekeepers than by others.

Table 3 Average level of *Varroa* infestation (in %).in adult bees of the participating bee colonies in October at the start of winter

Supervising bee institute	2005/2006	2006/2007	2007/2008
Celle	2,6	4,2	3,3
Freiburg	2,0	6,4	11,0
Halle	9,1	5,5	11,7
Hohenheim	2,4	3,6	2,5
Hohen-Neuendorf	7,1	3,3	4,5
Kirchhain	8,7	5,9	4,8
Mayen	3,2	2,9	4,0
Münster	Bottom board diagnosis	Bottom board diagnosis	7,8
Veitshöchheim	3,6	5,5	4,3
Total	**4,8**	**4,7**	**6,0**

The damages caused by *Varroa* were limited. An infestation level of 6% means an average of 600 mites per colony if the strength of the colony is 10.000 bees at the start of winter. The absolute damage threshold is at 10%. The infestation levels found in the monitored colonies show that some of the colonies exceeded this threshold. Some high losses with individual participating beekeepers could be related to delayed or insufficient *Varroa* treatment.

Bee viruses

Depending on the project year and the virus type the percentage of positive samples was between 6% and 33% (table 4). Noticeable is the high level of positive DWV samples in autumn 2007. The high *Varroa* infestation levels found in 2007 are probably relevant. Surprisingly, the occurrence of viruses was very different between the German regions. The KBV was only found in two samples during the whole monitoring period. It is worth mentioning that only bee heads were analysed. This leads to less positive results than by analysing whole bees

Table 4 Average viruses infection levels (in % of analysed samples) in autumn bee samples. Not all samples were analysed because of high costs. The number of analysed samples is given in the table.

	Acute Paralyse Virus (ABPV) in %				**Sacbrood Virus (SBV) in %**			
Institute	**2004/05**	**2005/06**	**2006/07**	**2007/08**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Celle	4,2	33,3	19,5	2,6	0	13,9	17,1	5,1
Freiburg	20,0	7,4	0,0	0,0	0,0	22,2	14,8	18,5
Halle	18,2	40,0	13,3	16,7	13,6	0,0	0,0	5,6
Hohenheim	0,0	0,0	2,2	22,2	33,3	37,8	-	28,9
Hoh-Neuendorf	2,9	8,5	0,0	3,0	1,4	0,0	0,0	1,5
Kirchhain	16,9	21,2	22,2	30,3	16,9	6,1	11,1	6,1
Mayen	0,0	30,3	8,3	22,2	20,0	9,1	8,3	8,3
Münster	0,0	0,0	0,0	23,3	0,0	0,0	0,0	6,7
Veitshöchheim	0,0	0,0	0,0	0,0	12,1	1,4	8,1	0,0
Total	**6,4**	**12,1**	**6,1**	**11,1**	**12,4**	**9,1**	**7,6**	**7,9**

	Deformed Wing Virus (DWV) in %				**Number of analysed samples n**			
Institute	**2004/05**	**2005/06**	**2006/07**	**2007/08**	**2004/05**	**2005/06**	**2006/07**	**2007/08**
Celle	8,3	38,9	56,1	33,3	24	36	41	39
Freiburg	0,0	22,2	14,8	37,0	15	27	27	27
Halle	18,2	60,0	26,7	44,4	22	15	15	18
Hohenheim	2,4	0,0	2,2	66,7	42	45	45	45
Hoh-Neuendorf	21,7	14,0	23,2	25,8	69	94	69	66
Kirchhain	4,2	21,2	25,0	33,3	71	33	36	33
Mayen	0,0	18,2	5,6	52,8	30	33	36	36
Münster	0,0	0,0	6,3	23,3	15	18	16	30
Veitshöchheim	0,0	0,0	9,5	8,0	58	72	74	75
Total	**7,2**	**14,8**	**18,7**	**32,8**	**346**	**373**	**359**	**369**

	Israel Acute Paralysis Virus (IABPV) in % (only 2007)			
Institute	**Negative**	**Positive**	**Uncertain**	**Total**
Celle	87,2		12,8	39
Halle	100			18
Hohenheim	95,5		4,5	44
Hoh-Neuendorf	100			66
Kirchhain	100			33
Mayen	100			36
Münster	100			30
Veitshöchheim	100			75
Total	**97,7**	**0,0**	**2,4**	**341**

***Nosema* sp.**

During the first two years one third of the analysed spring samples were positive for *Nosema* sp., but only 8 % showed a high infection level (table 5). Surprisingly, during the third year the amount of positive samples was below 20%. Analysis showed that most samples were infected with *Nosema ceranae*. Noticeable is the increase of *Nosema* sp. infections in spring 2008. Remarkable is that in summer 2008 samples the percentage of positive findings was still 25%.

Table 5 Average *Nosema* sp. infection levels in spring (in % of analysed samples; n = number of analysed samples). In 2007/2008 autumn and summer samples were also analysed.

	Spring 2005					Spring 2006				
Institute	**no**	**low**	**medium**	**high**	**n**	**no**	**low**	**medium**	**high**	**n**
Celle	78,8	6,1	11,1	4,0	99	61,4	2,9	27,9	7,9	140
Freiburg	76,0	10,0	12,0	2,0	50	33,3	6,2	33,3	27,2	81
Halle	10,0	70,0	15,0	5,0	40	52,9	44,1	2,9	0,0	34
Hohenheim	42,8	15,2	27,5	14,5	138	66,0	27,9	0,7	5,4	147
Hoh-Neuendorf	75,8	4,3	7,4	12,6	231	70,5	11,0	10,1	8,4	227
Kirchhain	63,0	16,8	11,8	8,4	119	76,0	5,2	1,0	17,7	96
Mayen	74,8	16,8	6,5	1,9	107	56,8	21,2	15,3	6,8	118
Münster	86,0	10,0	4,0	0,0	50	92,7	5,5	1,8	0,0	55
Veitshöchheim	72,8	15,1	9,6	2,5	239	28,2	51,7	15,4	4,6	259
Total	**67,7**	**13,9**	**11,6**	**6,9**	**1073**	**56,3**	**22,2**	**13,1**	**8,5**	**1159**

	Spring 2007					Autumn 2007				
Institute	**no**	**low**	**medium**	**high**	**n**	**no**	**low**	**medium**	**high**	**n**
Celle	86,0	2,3	10,1	1,6	129	81,5	9,2	7,7	1,5	130
Freiburg	79,4	15,7	4,9	0,0	102					
Halle	72,3	27,7	0,0	0,0	47	98,2	1,8	0,0	0,0	57
Hohenheim	83,3	11,9	4,8	0,0	126	70,0	22,7	7,3	0,0	150
Hoh-Neuendorf	75,4	7,0	8,3	9,2	228	93,2	2,3	2,7	1,8	219
Kirchhain	91,8	1,0	5,2	2,1	97	97,3	0,0	0,9	1,8	110
Mayen	88,8	7,5	3,7	0,0	107	90,8	2,5	1,7	5,0	120
Münster	93,5	6,5	0,0	0,0	62	98,4	0,0	0,0	1,6	61
Veitshöchheim	81,2	13,7	2,4	2,7	255	80,2	6,7	8,7	4,0	252
Total	**82,6**	**9,6**	**5,0**	**2,8**	**1153**	**86,4**	**6,6**	**4,7**	**2,3**	**1099**

	Spring 2008					Summer 2008				
Institute	**no**	**low**	**medium**	**high**	**n**	**no**	**low**	**medium**	**high**	**n**
Celle	62,6	22,0	12,2	3,3	123					
Freiburg										
Halle	50,0	11,8	8,8	29,4	34					
Hohenheim	38,7	50,0	5,3	6,0	150					
Hoh-Neuendorf	64,4	8,7	7,2	19,7	208					
Kirchhain	85,7	4,4	4,4	5,5	91					
Mayen	68,9	6,7	6,7	17,6	119	86,1	8,9	5,0	0,0	101
Münster	73,9	8,7	2,2	15,2	46	86,7	6,7	6,7	0,0	30
Veitshöchheim	58,2	28,9	10,0	2,8	249	69,2	24,7	6,1	0,0	247
Total	**61,3**	**20,8**	**7,7**	**10,2**	**1020**	**75,1**	**19,0**	**5,8**	**0**	**378**

Malpighamoeba mellificae

The total amount of positive samples is low. In southern Germany more infections were found (table 6).

Table 6 Average *Malpighamoeba mellificae* infection levels in spring (in % of analysed samples; n = number of analysed samples). In 2007/2008 autumn and summer samples were also analysed.

	Spring 2006					Spring 2007				
Institute	**no**	**low**	**medium**	**high**	**n**	**no**	**low**	**medium**	**high**	**n**
Celle	96,4	3,6	0,0	0,0	140	100,0	0,0	0,0	0,0	129
Freiburg	-	-	-	-	-	0,0	97,1	2,9	0,0	102
Halle	97,1	2,9	0,0	0,0	34	100,0	0,0	0,0	0,0	47
Hohenheim	16,3	66,7	15,6	1,4	147	50,0	46,8	3,2	0,0	126
Hoh-Neuendorf	100,0	0,0	0,0	0,0	227	100,0	0,0	0,0	0,0	228
Kirchhain	100,0	0,0	0,0	0,0	96	100,0	0,0	0,0	0,0	96
Mayen	86,3	12,8	0,9	0,0	117	100,0	0,0	0,0	0,0	109
Münster	100,0	0,0	0,0	0,0	54	-	-	-	-	-
Veitshöchheim	-	-	-	-	-	54,2	45,8	0,0	0,0	236
Total	**82,2**	**14,6**	**2,9**	**0,2**	**815**	**74,6**	**24,8**	**0,7**	**0,0**	**1073**

	Autumn 2007					Spring 2008				
Institute	**no**	**low**	**medium**	**high**	**n**	**no**	**low**	**medium**	**high**	**n**
Celle	99,2	0,8	0,0	0,0	130	71,5	28,5	0,0	0,0	123
Freiburg										
Halle	91,2	8,8	0,0	0,0	57	100,0	0,0	0,0	0,0	34
Hohenheim	100,0	0,0	0,0	0,0	150	88,0	12,0	0,0	0,0	150
Hoh-Neuendorf	100,0	0,0	0,0	0,0	219	100,0	0,0	0,0	0,0	209
Kirchhain	100,0	0,0	0,0	0,0	110	100,0	0,0	0,0	0,0	91
Mayen	100,0	0,0	0,0	0,0	103	98,3	1,7	0,0	0,0	119
Münster	100,0	0,0	0,0	0,0	61	100,0	0,0	0,0	0,0	46
Veitshöchheim	87,3	12,7	0,0	0,0	252	87,3	12,7	0,0	0,0	251
Total	**96**	**4**	**0**	**0**	**1082**	**91**	**9**	**0**	**0**	**1023**

	Summer 2008				
Institute	**no**	**low**	**medium**	**high**	**n**
Celle					
Freiburg					
Halle					
Hohenheim					
Hoh-Neuendorf					
Kirchhain					
Mayen	84,9	15,1	0,0	0,0	119
Münster	100,0	0,0	0,0	0,0	30
Veitshöchheim	85,4	14,6	0,0	0,0	247
Total	**86**	**9**	**0**	**0**	**396**

Residue analysis/residues in bee bread (stored pollen)

In the first project year honey and bee bread samples were specifically analysed for imidacloprid residues of treated oilseed rape sites. In 36 nectar/honey samples no residues were found and in only two out of 48 pollen/bee bread samples residues were found at the limit of quantification (1 ppb).

Bee bread samples were analysed for assessing the basic residue contamination of the colonies. Higher amounts of active ingredients can be expected in pollen than in nectar. Bee bread is consumed by nurse bees and larvae over a longer period which may result in long term effects.

First a method for detecting all relevant active ingredients had to be established. The LUFA in Speyer developed a sensitive multi-method for detecting and quantifying 258 active ingredients in bee bread samples. The limits of quantification are between 3 and 10 in single cases 15 µg/kg bee bread. Thus 215 bee bread samples of 2005 to 2007 were analysed. Only samples collected during or after the flowering of oilseed rape in spring were analysed because this crop is intensively treated with plant protection products and oilseed rape pollen and nectar are very attractive for bees.

In the first test series of 2005 and 2006, 105 bee bread samples from colonies exposed to oilseed rape and showing negative overwintering success were analysed. Here 42 active ingredients with a number of 1 to 46 positive detections were found (figure 1).

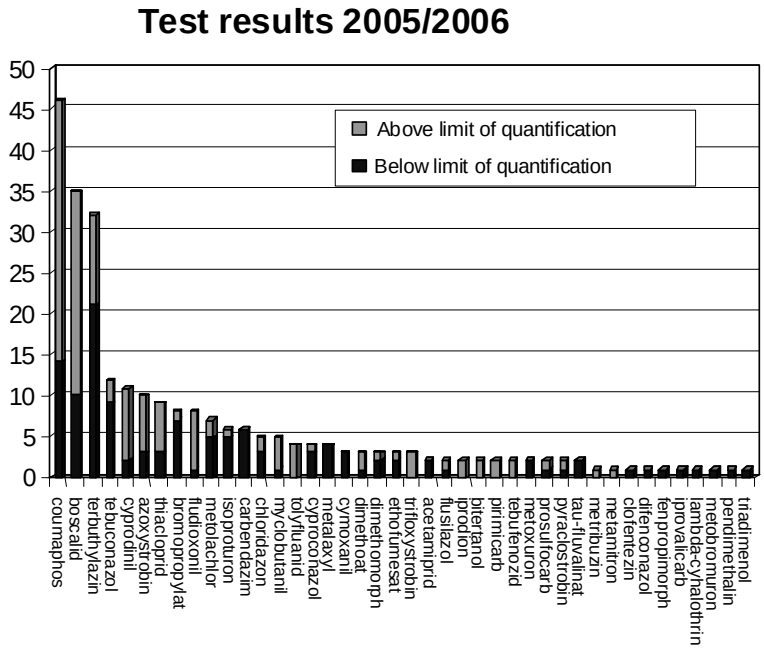

Figure 1 Frequency of positive detections of active ingredients found in 105 bee bread samples of spring 2005 and 2006.

In nearly all samples more than one active ingredient was detected. Only in 25 samples (24%) no residues were found. Coumaphos (46 positive results, varroacide), boscalid (35 detections, fungicide) and terbuthylazin (32 positive results, herbicide) were found most often. And thiacloprid is the insecticide which was found most often (9 positive results, max. 199 µg/kg). Other detected insecticides were dimethoate (3 positive results), azetamiprid (2 positive results), pirimicarb (2 positive results), tau-fluvalinat (2 positive

results) and lambda-cyhalotrine (1 positive result). The amounts of the 5 insecticides were below 10 µg/kg (except dimethoate, 20 µg/kg). Apart of these single results the detected amounts were small: 112 out of 171 positive results were below 10 µg/kg. Imidacloprid was not found in any sample.

In the project year 2007/2008, 110 additional bee bread samples of the season 2007 were analysed. Aliquots of the extracted samples were analysed by BayerCropscience for neonicotinoids. Numbers and quantities of the residues are similar to the results of the years 2005/2006. Again 42 active ingredients were found with a number of 1 to 67 positive detections almost in traces (figure 2). In comparison with the previous years single active ingredients were not found any longer while others were found for the first time. The frequency was different: the active ingredient coumaphos was in 4th position (33 positive results, varroacide). The number of positive samples increased for the active ingredients boscalid (67 positive results, fungicide), thiacloprid (62 positive results, insecticide, classified in Germany as not harmful for bees), and terbuthylazin (48 positive results, herbicide).

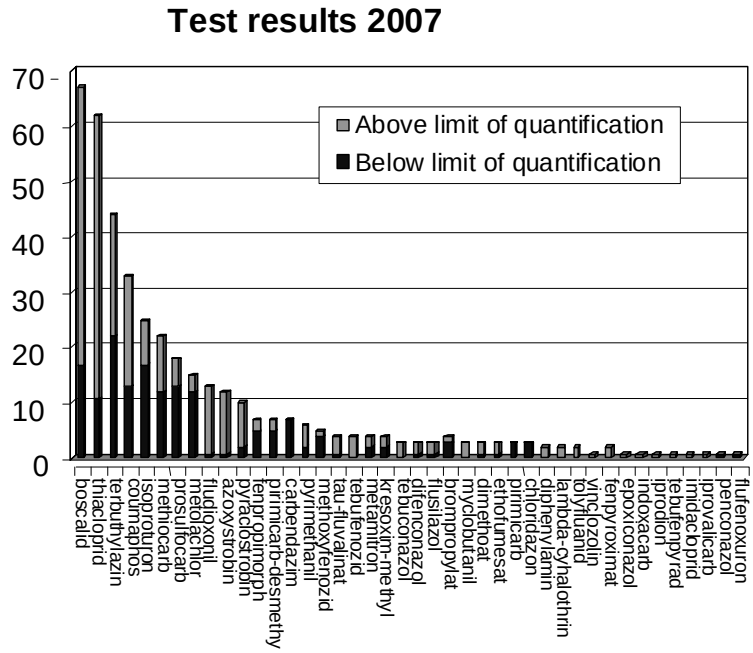

Figure 2 Frequency of positive detections of active ingredients found in 110 bee bread samples of spring 2007.

Of special interest were the active ingredients of the neonicotinoid group, which are classified in Germany as harmful for bees. In 215 analysed samples of 2005 to 2007 clothianidin was not found in any sample while imidacloprid was found in one sample (3 µg/kg).

Preliminary conclusions:

- The results of the residue analysis represent the first evaluations of residue contamination of bee bread in Germany and give important basic data for further evaluations;
- No residues of active ingredients classified as harmful for bees of which acute side effects for bees can be expected were found in bee bread. The same applied for neonicotinoids which were not found in spring samples either with just one exception.

- A considerable contamination with active ingredients was found in the bee bread samples. It is uncertain if this contamination with almost more than one active ingredient per sample will have negative long-term effects on colonies (bee brood, nurse bees).
- For evaluating the effects of the residue contamination on the development and overwintering success of a colony, specific and long term assessments have to be run with colonies being treated with different residue amounts.
- For testing potential sublethal or long term effects a useful test design has to be developed yet.
- Coumaphos should be replaced by other active ingredients in *Varroa* treatments.

Statistical evaluations

In a first step it was statistically analysed if certain parameters (site of the apiary, bee diseases, beekeeping management) were significantly correlated with colony losses or bad overwintering results. Nearly 4400 data sets were statistically analysed with non-parametric tests (U-test, Chi^2-test). Various parameters were tested for significance of differences between surviving colonies and colonies that died. In evaluations still going on the data of different project years and different parameters will be linked. The results will be published in the following months.

During the four years of the project the loss rates were below the threshold of the disaster in 2002/2003. Besides losses also a comparisons of the bee population before and after winter were recorded. This offers the possibility to evaluate sublethal effects which potentially weakened colonies during winter. Factors with negative influence tendencies on wintering and factors to be excluded as triggers could be identified.

Based on the current evaluations it was concluded:

1. Between oilseed rape sites and non-oilseed rape sites no differences were found for colony losses neither for the overwintering quotient (= colony strength in autumn divided by colony strength in spring). The evaluations are based on 2325 data sets of the project years 2005/2006 and 2006/2007. The results indicate even better overwintering success for colonies exposed to oilseed rape sites.
2. Highly significant correlations were found between winter losses and the *Varroa* infestation levels in autumn. The risk for colony loss increases with the number of mites in the colony in autumn.
3. Similarly the correlations between the infection with ABPV and DWV in autumn and winter losses were significant.
4. No significant correlations were found for *Nosema* sp. infections.
5. Surprisingly, the age of the queen was significantly correlated with the winter losses. Young queens were more successful. Not surprisingly, the strength of the colony in autumn is significantly correlated with the winter losses. The risk of winter losses decreases with the strength of the colony in autumn.
6. No significant effects were found for the type of syrup used for feeding before wintering, for the type of boxes (wood/plastic), for the size of frames or young colony/old colony.

The annual interim reports of the project are published on http://www.ag-bienenforschung.de. A detailed report will be published by the German apiculture research institutes in spring 2009.

References

1. AG Bienenforschung, Monitoring-Projekt “Völkerverluste”, Untersuchungsjahre 2004-2008, Zusammenfassung und vorläufige Beurteilung der Ergebnisse, 19.12.2008. http://www.ag-bienenforschung.de.