Report Honeybee Surveillance Program the Netherlands 2016-2017

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Consortium: Bijen@wur, Wageningen Environmental Research, & Naturalis Biodiversity Center



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Justification of the report and its results

The Honeybee Surveillance Program of the Netherlands is initiated to obtain insight in the level of winter mortality in honeybee colonies as well as in the different factors underlying this mortality. The program is commissioned by the ministry of Economic Affairs to Naturalis Biodiversity Center and is a collaboration between the important research parties in the field. This report summarizes the overall conclusions of the program.

The results of the winter mortality questionnaire are robust and representative. A random sample of approximately 500 beekeepers has been questioned about the hive survival in their operation. This has been a coordinated effort in collaboration with the Netherlands Beekeeping Association (NBV). The results of the surveillance study are also robust and representative, as they are based on a large-scale stratified random sample of bee colonies across the Netherlands.

The duration of the program will be four years and this report summarizes findings for year three. The four years are needed to obtain a longer-term view of both winter mortality and the underlying causing factors; and to take into account the substantial inter-annual variation.

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Verantwoording bij het rapport en de resultaten

Het Nederlandse honingbijen-surveillance programma heeft als doel inzicht te krijgen in de wintersterfte van honingbijenvolken in Nederland en in de onderliggende factoren voor de sterfte. Het wordt uitgevoerd in opdracht van het Ministerie van Economische Zaken door Naturalis Biodiversity Center en is een samenwerking van de belangrijkste partijen in het onderzoeksveld. Dit rapport vat de resultaten van verschillende deelprojecten samen.

De resultaten van de Wintersterfte Monitor zijn robuust en representatief. Deze uitvoering is gebaseerd op een a-selecte steekproef van ongeveer 500 imkers die gevraagd zijn naar de sterfte in hun bijenvolken. De winter monitor is uitgevoerd in samenwerking met de Nederlandse Bijenhouders Vereniging (NBV). De resultaten van de Surveillance Studie zijn gebaseerd op een gestratificeerde a-selecte steekproef waaraan een groot aantal imkers heeft meegedaan.

De duur van het programma is vier jaar. Op die manier kan een robuuste analyse gemaakt worden van de sterftepatronen en hun factoren, waarbij variatie tussen jaren meegenomen kan worden. Dit rapport vat de resultaten van jaar drie samen.

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1. Summary of 2016/2017 results

1.1 Executive Summary

- 1 The Honeybee Surveillance Program assesses honeybee winter mortality in the Netherlands and aims to unravel the factors explaining colony losses. To achieve this, two studies are combined: the Honeybee Mortality Monitor, a random online beekeepers' survey and the Honeybee Surveillance Study, a random field survey of honeybee hives, samples of which were analyzed in the lab. This report concerns the third year, 2016-2017.
- **2** National-level hive survival. The Honeybee Mortality Monitor reveals that winter survival in 2016-2017 was, 85.7% (14.3% of hives died). This figure is in line with the normal variation of around 5-15% and the last five years. Survival was higher last year, when mortality was at its lowest point during the last decade. The number of managed honeybee colonies in the Netherlands is estimated to be between 70 and 95 thousand.
- **3** Apiary-level mortality: Hive survival was high all-round and no single factor explains all mortality at apiary level. However, apiaries with lower *Varroa* mite counts and ABPV virus and those occurring in landscapes that are not too fragmented had higher apiary-level survival in the 2016-2017 winter.
- **4 Hive-level mortality:** A large number of variables each contribute just a little to explain hive winter mortality. Several factors are correlated with each other making it difficult to separate them in the analyses. A few factors seem to have slightly more importance, but no single factor comes out as the main driver of winter hive loss. Hives containing substantial amounts of pollen from mustards and impatiens had slightly higher survival as well as, surprisingly, those with *Nosema ceranae* present. Hives foraging in highly diverse, fragmented landscapes and those with residues of the fungicide Tebuconazole in stored honey has slightly higher mortality.
- **5** Summarizing: Honeybee winter loss of 14.3% was within the normal range in the 2016-2017 winter, but no single factor can be pointed to as the main driver of loss. Many factors play small roles in determining hive survival. Among them are aspects of beekeeping practice, disease control, pollen sources, landscape features and chemical residues. However, within these groups of factors most aspects have no significant impact and few factors have been found to affect winter loss in each of the last three years. This indicates that the factors underlying honeybee colony loss in the real world, i.e. hives managed by beekeepers in our Dutch landscapes, are many, are variable in space and time, and are likely to interact to produce the final outcome: survival or not.

1.2 Nederlandse samenvatting

- 1 Het Honingbijensurveillance programma stelt de wintersterfte onder honingbijen in Nederland vast en heeft tot doel de oorzaken te ontrafelen die de wintersterfte kunnen verklaren. Hiervoor gebruiken we twee methoden. Ten eerste de Wintersterfte Monitor, een online vragenlijst die wordt gestuurd naar een aselecte steekproef van imkers. Ten tweede de Honingbijen Surveillance studie. Hierin worden van een steekproef van de Nederlandse bijenhouders in het veld bijenvolken bemonsterd voor nadere analyse in het laboratorium. Dit rapport geeft de resultaten weer van het derde seizoen, 2016-2017.
- **2 Bijensterfte in Nederland**. De Wintersterfte monitor laat zien dat de overleving van bijenvolken in Nederland in 2016-2017 hoog was, namelijk 85.7% (14.3% van de volken ging dood). Deze mate van wintersterfte ligt binnen de als normaal geziene variatie (rond de 5-15%) evenals de afgelopen vijf jaar. Overleving was iets lager dan het vorige jaar, waarin een recordaantal volken de winter overleefde. Op basis van de monitor kunnen we een schatting maken van het aantal bijenvolken in Nederland; dat ligt tussen de 70 en 95 duizend
- **3 Sterfte per bijenstand.** Overleving was overal hoog afgelopen winter en de sterfte van volken per bijenstand lijkt niet het gevolg van één enkele oorzaak. De overlevingskans was hoger in bijenstanden met minder Varroa en minder ABPV virus. Bijenstanden in zeer gefragmenteerde, diverse landschappen hadden iets lagere overleving.
- 4 Sterfte per bijenvolk. Een groot aantal factoren lijkt allemaal een heel kleine bijdrage te leveren aan wintersterfte. Veel van deze factoren zijn gecorreleerd en zijn daardoor in de modellen niet goed te scheiden. Enkele factoren verklaren een iets groter deel van de wintersterfte, maar niet één factor kan aangewezen worden als de belangrijkste. Volken die vooral stuifmeel van mosterdachtigen (koolzaad, herik) en balsemienen (o.a. reuzenbalsemien) verzamelden hadden een iets grotere overlevingskans. Dit gold ook voor volken waarin Nosema ceranae aanwezig was. Dit is verrassend, immers dit betreft een bijenziekte die vooral bekend staan om haar negatieve effecten. Volken die in zeer diverse, gefragmenteerde landschappen staan en volken waarbij residuen van Tebuconazole in honing werd aangetroffen, toonden iets hogere sterftekans.
- 5 Samenvattend: De 2016-2017 wintersterfte onder de in Nederland gehouden honingbijen viel, met 14.3%, binnen de normaal te verwachten spreiding. Er zijn veel factoren (waaronder imkerpraktijken, stuifmeelbronnen, landschapsaspecten en chemische residuen) die elk een (zeer) klein aandeel lijken te hebben in het verklaren van die sterfte, maar niet één factor kan aangewezen worden als meest belangrijke. Als we de afgelopen drie jaar van de studie overzien, kunnen we concluderen dat binnen elk van de groepen factoren die we onderzochten er voor een bepaald aspect in een bepaald jaar wel een correlatie te vinden, maar voor de meeste aspecten vinden we geen significante relatie met wintersterfte. Dit lijkt erop te wijzen dat het bij het verklaren van de wintersterfte in het veld, d.w.z. imkers en hun bijenstanden, er sprake is van vele factoren, die variëren in ruimte en tijd en elkaar beïnvloeden, en tezamen de uitkomst levend of dood opleveren in de winter.

2 Introduction to the surveillance program

The Netherlands Honeybee Surveillance Program has been initiated as a result of the public debate hosted by the former Minister for agriculture and environment, Sharon Dijksma, with many societal partners as participants. The top priority that was identified was to assess the status of bees, particularly honeybees, and unravel the main factors that contribute to honeybee winter mortality in the Netherlands. Such a program requires an integrated approach towards honeybee health and a substantial investment. The Dutch Ministry of Economic Affairs, also dealing with agriculture, approached Prof. Dr. Koos Biesmeijer, Naturalis Biodiversity Center and University of Leiden, to assemble a consortium and program to address this important issue. The consortium consists, besides Naturalis, of Dr. Sjef van der Steen (Bijen@Wur) and Dr. Arjen de Groot (Wageningen Environmental Research), whereas Theo de Rijk (RIKILT, Wageningen UR) is the subcontractor for chemical residue analysis. The financial support for the program, € 1.2M total, is provided by the ministry of Economic Affairs (51%) with Nefyto as co-financer (49%). The program will run from 2014-2018.

2.1 Main objective of the surveillance program

The main objective in this program is to determine the health status of honeybees in the Netherlands: estimate colony winter loss over four years and map drivers that correlate with winter loss, including exposure to agro-chemicals, bee diseases, food availability, landscape configuration and beekeeping practice (Figure 1).

In addition to the main aim, the program aims to meet several other objectives:

1- The results should be representative and be informative for ongoing European initiatives, e.g. the ANSES protocol recently used in the Epilobee project and the CoLoSS colony loss questionnaires that estimate winter mortality in many countries. EFSA is currently making an inventory of different attempts and our consortium will be taken into account there. Our program is more complete (more possible drivers of loss assessed) than the above-mentioned initiatives. Through the EU COST Action Super-B (Sustainable Pollination in Europe, joint research on bees and other pollinators), led by Koos Biesmeijer at Naturalis, the consortium links to all other honeybee surveillance initiatives in Europe, e.g. Austria, Germany, UK, Italy, USA.



Figure 1. Overview of the main risk factors for honeybee colony survival that will be addressed in the surveillance program.

- 2- We use standardized protocols, most of which are applied in other projects and all of which have been validated before. If needed small changes are being incorporated, but these will not be detrimental to the comparability of the results. The results are used in comparative studies on honeybee colony loss. The Super-B network mentioned above strived to explore whether more standardization could be achieved across EU countries to increase the impact of our national programs.
- 3- The knowledge that will be gained from the project should benefit the Dutch honeybees through the close collaboration of consortium partner Bijen@WUR with the Dutch beekeeping community.

2.2 The structure of the surveillance program

The program merges two different approaches to the problem of bee mortality and its causes. The first approach is a beekeeper survey (honeybee survival monitor), the second approach is a field campaign actually sampling and analysing different factors directly (honeybee surveillance study).

The Honeybee Survival Monitor is an annual survey that questions beekeepers about the survival of their hives. The method of monitoring the winter survival in honey bees is based on the international standard, the CoLoSS survey, and was set up by Naturalis, Bijen@WUR and the NBV to replace the monitor of the Netherlands Centre for Bee Research (NCB). This change was needed as a result of NCB's decision not to join our project from 2016 onward. It was decided to conduct an integrated survey together with the Netherlands Beekeeping Association (NBV) and Bijen@wur, because they already conducted a more simple mortality survey in the past few years to be able to obtain an indication of honeybee mortality early in the season. The honeybee survival monitor is a survey based on CoLoSS protocols (www.coloss.org) to facilitate comparison with other countries. The survey is, however, more extended than the previous NBV survey, but more compact than the CoLoSS long-survey (for survey see appendix A). We conducted the survey as follows: To obtain a reliable estimate of honeybee winter mortality in the Netherlands we aim to obtain survival figures from about 500 beekeepers, randomly drawn from association membership lists (>8000 beekeepers in total). Since not all members possess bees and many beekeepers did not respond to our request by e-mail, we continued to approach beekeepers till we reached approximately 500 beekeepers, first digitally by sending a survey created in Google forms to selected beekeepers. Later remaining selected beekeepers were called directly. In total we needed to approach 1400 beekeepers to obtain figures from 500 of them.

The <u>Honeybee Surveillance Study</u> is set-up for this program and consists of a random sampling of hives in apiaries from around the Netherlands. Samples are taken by beekeepers themselves of bees, honey and pollen to identify diseases, chemical residues and food sources. Beekeepers were also questioned about their beekeeping methods. In this way we can assess the influence of the beekeeper (interviews and field survey), diseases (laboratory analysis of bees), food sources (pollen analysis), chemical products (residue analysis of honey), and the local landscape in which the bees live (GIS analysis). In the first year of this study bee health inspectors that were trained by Bijen@wur staff conduct the field survey and collect samples. In the following years beekeepers are instructed by a clear manual with pictures how to take their samples themselves each year in May and August from 3 up to 5 of their hives at the same apiarie. Only a subset of the samples, up to 400 per year, will be analysed (due to limited funds), but all will be stored for future analyses.

The distribution of tasks among the consortium partners (Figure 2) is that Bijen@wur is responsible for the field sampling, distribution of samples and disease analyses; Wageningen Environmental Research is responsible for the pollen analysis; Naturalis is responsible for the landscape GIS analysis and for the integrated analysis of all results. The analysis of chemical residues is conducted by subcontractor RIKILT. RIKILT is the Dutch National Reference Laboratory for pesticides in food of animal origin. Naturalis is in charge of the overall program.



Figure 2. Overview of the main risk factors for honeybee colony survival that will be addressed in the Surveillance Program

3 Results

3.1 Honeybee Survival Monitor 2016/2017

3.1.1. Results from Honeybee survival Monitor

Honeybee hive survival in the winter of 2016-2017 was 85.7%, therefore the mortality was 14.3%. The 470 beekeepers that responded to the electronic questionnaire had 3479 bee hives going into winter (late autumn 2016) of which 2981 hives survived the winter, i.e. were still alive in April 2017. While the increase in mortality relative to the previous year is substantial, variability in mortality between 5 and 15% are regarded as normal. The survival over this winter is in line with the 4 winters before 2016/2017 (table 1) and based on a representative sample (figure 3).

Table 1. Winter survival 2005-2016

Winter	Number of beekeeper s	Number of hives (October)	% winter survival ¹	% winter mortality ¹	Method
2005-2006	737	7.050	73.7	26.3	NBC [CoLoSS]
2006-2007	1422	13.591	84.1	15.9	NBC [CoLoSS]
2007-2008	808	9.616	76.3	23.7	NBC [CoLoSS]
2008-2009	1193	10.678	78.3	21.7	NBC [CoLoSS]
2009-2010	1326	11.265	70.9	29.1	NBC [CoLoSS]
2010-2011	1541	13.726	78.6	21.4	NBC [CoLoSS]
2011-2012	1673	14.915	79.2	20.8	NBC [CoLoSS]
2012-2013	1589	13.920	86.3	13.7	NBC [CoLoSS]
2013-2014	1594	15.280	91.4	8.6	NBC [CoLoSS]
2014-2015	1549	14.650	86.3	13.7	HB-Surv [CoLoSS] 1
2015-2016	580	5919	93.5	6.5	HB-Surv [CoLoSS] 1
2016-2017	470	3479	85.7	14.3	HB-Surv [CoLoSS] ¹

¹based on HB surveillance reports: 14-15 NCB voluntary survey, 15-16 NBV random sample

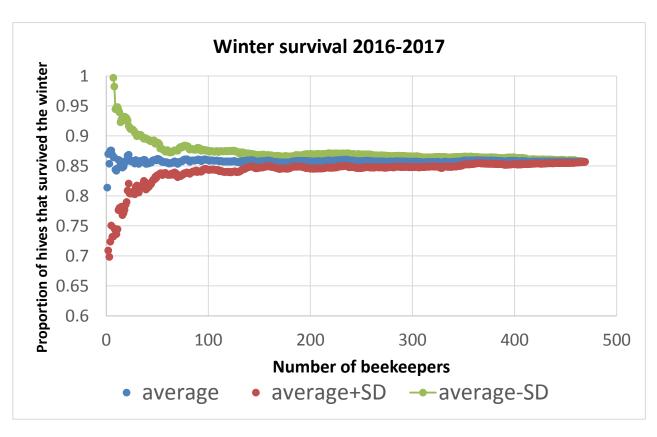


Figure 3. This graph indicates that the percentage of winter survival of 85.7% (14.3% mortality) is a representative figure and that sufficient numbers of beekeepers have been surveyed. The mean survival (blue line) is already indicating the correct survival figure after about 250 beekeepers surveyed. Moreover, the confidence intervals (green line mean + standard deviation, red line mean - standard deviation) are very close to the mean from about 400 beekeepers surveyed.

3.1.2. Estimate of the number of honeybee hives in the Netherlands

The Netherlands needs to submit the estimated number of honeybee hives in the Netherlands annually to the EU. This figure can be estimated using the winter monitor data, given that they represent a random sample of all Dutch beekeepers. The largest source of error in the calculation is the uncertainty about the percentage of Dutch beekeepers that is a member of one of the three main beekeeping associations, the NBV, the ABTB and the ANI. Therefore, we give estimates for various membership percentages in table 2 (superscripts in text below refer to the lines in the table).

Data on the number of hives going into winter 2016-2017 were received from 632 beekeepers¹. In total these beekeepers had 5245² hives in late autumn 2016, while 49 beekeepers (7.7%) had no hives at all. The average number of hives was 8.4 across all beekeepers³ with a few large beekeepers and many with fewer hives. A total of 8399 beekeepers⁷ is registered with one of the three beekeeping associations⁴⁻⁶. The total number of hives of these beekeepers is about 70700 (beekeepers * 8.4 hives on average)⁸.

The question that remains for estimating total beehives in the Netherlands is the percentage of registration of all Dutch beekeepers and also the number of double memberships among the beekeepers. Both are not known although the second issue could be resolved by comparing membership lists. The actual number of beehives critically depends on the share of beekeepers being a member of at least one association. We calculated the population of Dutch bee hives for degrees of registration between 70 and 95% ¹¹⁻¹⁶. The estimate increases from 70 thousand at complete registration to 101 thousand at 70% registration.

In conclusion: there were at least 70700 managed bee hives in the Netherlands in late Autumn 2016. This is certainly an underestimate due to incomplete registration. The best estimate may be between 75,000 and 95,000 beehives.

Table 2. Procedure to estimate the number of bee hives in the Netherlands in 2016. For explanation see text. Line numbers indicate the various steps and numbers taken into account and line numbers are referred to in the text as superscript numbers.

1	Beekeepers in sample		632
2	Total number of hives going into winter		5245
3	Average number of hives per beekeeper	r	8.4
4	Number of beekeepers on NBV list		7349
5	Number of beekeepers member of ABTE according to their website	В	700
6	Number of beekeepers on ANI list		350
7	Total number of beekeeper members		8399
8	Aantal imkerleden zonder honingbijen (4 508 leden in enquete hadden geen bijer	9.6%	
8	Number of hives in associations (beeke * average hives per beekeeper)		70700
10	Number of hives in associations (beeke	epers	70700 Estimated total number of hives
	Number of hives in associations (beeke * average hives per beekeeper) Estimated percentage of beekeepers me	epers	Estimated total
10	Number of hives in associations (beeke * average hives per beekeeper) Estimated percentage of beekeepers me	ember	Estimated total number of hives
10	Number of hives in associations (beeke * average hives per beekeeper) Estimated percentage of beekeepers me	eepers ember 95%	Estimated total number of hives 74421
10 11 12	Number of hives in associations (beeke * average hives per beekeeper) Estimated percentage of beekeepers me	ember 95% 90%	Estimated total number of hives 74421 78556
10 11 12 13	Number of hives in associations (beeke * average hives per beekeeper) Estimated percentage of beekeepers me	ember 95% 90% 85%	Estimated total number of hives 74421 78556 83176

3.2 Honeybee Surveillance Study 2016-2017

3.2.1. Set-up of the field campaign

The field campaign is based on a random selection of beekeepers (apiaries) from across the Netherlands (see appendix B for details). The participating beekeepers are asked to take their own samples, accompanied by an extensive manual with pictures describing exactly what has to be done. samples are taken in May and August and the beekeepers are instructed to keep them cooled and send them to Bijen@WUR by mail. Three to five hives are sampled in one apiary of each beekeeper (maximum number of samples: 200 apiaries x 5 hives x 2 samples (May and August) = 2000 samples). The maximum number is unlikely to be reached for several reasons: (1) Not all beekeepers have five hives that can be sampled; (2) many beekeepers do not want to participate when field visits are conducted even after originally agreeing to join; (3) not all hives sampled have sufficient honey and pollen stored; (4) other circumstances may prevent us from sampling, e.g. American Foulbrood outbreaks. Given the large investment needed for the field campaign, we decided to collect a large number of samples, more than we can analyse, and store all samples for future analysis (e.g. available for follow-up projects).

3.2.2. Selection of samples for analysis

The laboratory analyses are costly, therefore we select a subset of the samples for analysis. In short the procedure is as follows:

- 1- Hive number 1 and 2 per apiary was selected for analysis in Autumn 2016. Samples were distributed from Bijen@wur (pathogen and disease analysis based on bee sample) to Wageningen Environmental Research (food sources analysis based on pollen sample), RIKILT (chemical residue analysis based on honey sample), Naturalis (location information of apiaries for landscape analysis).
- 2- In April 2017, beekeepers were contacted to obtain information on survival of each of their hives.
- 3- The third sample for analysis was selected based on this survival/mortality information. We aim at selecting hives such that we obtain, for every beekeeper, a pair of hives one of which has survived the winter, the other of which has died during winter. In that case we can eliminate the influence of the landscape in general and the beekeeping treatments as explanatory variables. For those apiaries for which this is not possible, i.e. if all hives survived or all died, we did not analyse a third hive. Third hives were only analysed for selected apiaries to make matched pairs of dead-alive hives.
- 4- The samples of the third hive for the selected apiaries are distributed to the partners for analysis in April 2017. After that all data have been integrated and analysed by Naturalis.
- 5- Reporting occurs every year in late June/early July.

3.2.3. Single factor results: pathogens, residues, pollen sources and landscape

Here we first summarize the main findings per possible driver of mortality of the single factor analysis and after that we provide an integrated analysis of all drivers. Comparisons over three years are discussed in chapter 4. Note that the number of analysed samples can be different for each factor. This can have various reasons, for example, insufficient honey/beebread/bees to sample or to analyse.

Parasites and pathogens

Samples of bees (n=314) were analysed on the presence and quantity of the parasite *Varroa* destructor and on the presences of 4 diseases that are all closely linked to *Varroa*; *Nosema apis*

(microsporidian), Nosema ceranae (microsporidian), DWV (deformed wing virus) and ABPV (Acute Bee Paralysis Virus).

In 24 hives Varroa-infestation was 10% or more, whereas 98 hives did not contain any Varroa in late summer. This indicates that Varroa control was generally very effective and that mite levels were low for the bees going into winter. DWV was found in most hives. This indicates that even in hives in which no Varroa mites have been detected at the end of the summer, largely due to adequate control, Varroa mites must have been present previously or still were present at very low numbers as DWV is largely transmitted by Varroa-mites.

Table 3. Presence of various pests and diseases in bee samples in 2014 (n=91), 2015 (n=331) and 2016 (n=314).

Pest/Disease	2014	2015	2016
Varroa present (%hives)	73%	63%	68%
Varroa mites / 100 bees	7	3	5
Nosema ceranae	89%	59%	22%
Nosema apis	0%	0.6%	1%
DWV virus	98%	93%	96%
ABPV virus	0%	1%	9%

Pollen sources used by hives

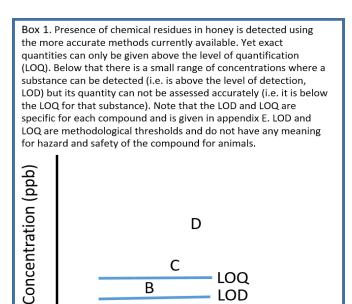
In the 318 samples beebread that were analysed, 72 different pollen types were found (unidentifiable pollen types were found in only two samples). The pollen types were counted only when they consisted of 5% or more of the sample (see table 4 for 10 most found pollen and appendix D for full list). On average 4.4 different types of pollen were found per sample, ranging from 1 to 9 types. Note that not all pollen types indicate the presence of only a single plant species. Some types in fact represent a genus of plants and some even a whole family. Still pollen analysis gives a good indication of the important food plants honeybees collect pollen from and the variety of pollen that the bees have collected.

Table 4. Main pollen sources and their percentages in hives in late 2016. Pollen types that occur in at least 10% of the samples, for the complete list see Appendix D.

Pollen type	found in # samples	% of total	Max % in a single sample	Average % when present
Brassicaceae (mustards, rapeseed)	170	53.3	100	28.4
Trifolium (clovers)	135	42.3	100	24.3
Asteraceae (dandelion family)	102	32.0	100	14.8
Calluna (heather)	96	30.1	100	46.4
Castanea (chestnut)	81	25.4	90	35.1
Rosaceae (rose family)	67	21.0	100	24.0
Fabaceae (legumes)	54	16.9	90	24.2
Cornus- type (Dogwood)	44	13.8	40	11.1
Impatiens (Himalayan balsam & relatives)	43	13.5	95	31.7
Zea (mais)	38	11.9	30	7.2
Heracleum (hogweed)	32	10.0	65	11.6

Chemical residues detected in honey

Honey samples from August were analyzed for the presence of a long list of chemicals including neonicotinoids, other pesticides, acaricides and other chemicals reported to be a potential threat for bees (for complete list see appendix E). The list of tested chemicals is much larger than in previous years as we excluded an expensive method that only detects Cyfluthrin-Beta, Esfenvalerate and Fluvalinate tau. These compounds were not detected regularly in previous years. With the saving on costs, we decided to expand the analysis with a range of agro-chemicals (see appendix E). For the chemical analysis we have taken into account the fact that a chemical is present or not (the LOD or Level of Detection; above LOD = present, below LOD = absent) and the level at which we can tell how much is actually



Chemical compound

present (the LOQ or level of quantification; above LOQ = quantity known, below LOQ = may be present (if above LOD), but level is too low to quantify; see box 1). Note that the LOD and LOQ thresholds are purely methodological thresholds and do not have any relation to the potential hazard and safety of these compounds for any organism.

14 of the tested compounds were encountered in at least one honey sample and mostly at low frequency (see table 5 and 6). Honey samples in 318 hives (92%) did not contain any of the chemical residues we screened for at a level above the LOQ (Level of Quantification) and 86% of hives did not contain any traces above the LOD of any tested chemical, so no chemicals were detected. Neonicotinoids (imidacloprid, thiacloprid or acetamiprid, thiamethoxam) were found in 39 hives (11.0%) of which 26 (7.5%) above LOQ. Acaricides (amitraz, coumaphos) were found in 28 hives (8%). The concentration of all the chemical residues found in the stored honey were (often very far) below the LD50 for oral toxicity for an adult honeybee.

Table 5. Chemical residues present above LOQ level in samples of 2014 (90 hives), 2015 (327 hives) and 2016 (342 hives). Neonicotinoid and their products are indicated with a *, Acaricide used by beekeepers with a ^ and fungicides are indicated with a #. Given are percentage of hives in which each residue has been found above the level of quantification - LOQ (see box 1 for explanation of LOQ; more information

Chemical residue	2014	2015	2016
Acetamiprid *	2.2%	2.8%	3.5%
Amitraz ^ (banned)	8.9%	2.1%	1.8%
Boscalid #	Not tested	Not tested	4.1%
Carbendazim #	Not tested	Not tested	0.6%
Chlorfenvinphos	Not tested	Not tested	0.6%
Coumaphos ^ (banned)	1.1%	2.4%	0%
Dimethoate	0%	0.9%	0.3%
Fluvalinate-tau ^ (banned)	0%	0.9%	Not tested
Fluopyram #	Not tested	Not tested	0.9%
Imidacloprid * (banned)	6.7%	2.8%	0.3%

Permethrin	0%	0.3%	0%
Tebuconazole #	Not tested	Not tested	1.5%
Thiacloprid *	2.2%	9.8%	8.2%
Thiamethoxam/Clothianidin *	0%	0.9%	0.6%
Neonicotinoids total *	7.7%	15.0%	11.3%
Acaricides total ^	7.7%	5.2%	8.1%

Table 6. Chemical residues encountered in 341 honey samples: presence, concentrations and LD50 for honeybees. LOQ=Level of Quantification. Samples are scored as 'absent' (column 2; indicating nothing was found), 'detected but <LOQ' (column 3; indicating very small quantity detected, but not sufficient to quantify it, i.e. below LOQ). LOQs for detected compounds is given in [] after the compound name in column 1. Several compounds can be detected as the compound itself or its metabolites, there values are recalculated according to standard residue definitions. These are indicated with superscript numbers and are: 1 Amitraz (Amitraz + DMA + DMF + DMPF), 2 Dimethoate (Dimethoate + Omethoate), 3 Fipronil (Fipronil + Fipronil-sulfone MB46136), 4 Imidacloprid (Imidacloprid + Imidacloprid_5-Hydroxy + Imidacloprid_olefin + Imidacloprid_desnitro + Imidacloprid_desnitro_olefin + Imidacloprid_urea + 6Chloronicotinic_acid). 5 EcoTox database values from: http://www.ipmcenters.org/ecotox/ . Values are micrograms per bee, with an individual bee weighing about 100 milligrams. Values thus have to be multiplied by 10.000 to be comparable to the detected concentrations in the previous columns.

Compound [LOQ in μg/kg]	number of samples in which absent	samples in which detected but <loq< th=""><th>samples in which detected and >LOQ</th><th>average concentration (μg/kg) if present</th><th>Maximum concentration (μg/kg) if present</th><th>LD50 in µg per kg in 48h tests from USDA EcoTox database</th></loq<>	samples in which detected and >LOQ	average concentration (μg/kg) if present	Maximum concentration (μg/kg) if present	LD50 in µg per kg in 48h tests from USDA EcoTox database
Acetamiprid	328	1	12	3.5	12	810000
Amitraz (+DMF+DMFP)	334	3	6			10000000
Coumaphos	321	20	0	NA	0	
Dimethoate	340	0	1	5.2	5.2	5600
Imidacloprid	340	0	1	0.75	0.75	380
Thiacloprid	313	13	15	7.4	36	1794000
Thiamethoxam/ Clothianidin	340	2	0			3500
Boscalid	321	6	14	4.3	12	16600000
Carbendazim	339	1	1	14	14	5000000
Chlorfenvinphos	339	0	2	5	8.7	
Fluopyram	338	1	2	1.55	1.8	1023000
Tebuconazole	336	3	2	1.8	2	

Landscapes in which bees forage

Landscapes determine in part the health of bee hives. Not only do landscapes provide pollen and nectar sources (pollen sources are assessed in this study through pollen analysis, nectar sources not), they also expose hives to mass-flowering crops and wild plants, year-round provision of foraging and growth conditions and unhealthy components (e.g. agro-chemicals, pollution, drought and water shortage). Information for land use and habitat factors has been compiled from a range of sources to create up-to-date relevant spatially explicit layers for analysis. Data are available on crops and groups of crops grown on each parcel and for each year (2016 data from BRP: basis registratie percelen). Detailed land use data are available from CBS land use database for 2010 (latest version). One important variable that we created was the number of land use classes per area around the hives (1000 or 3000m, see below). All land use classes are included here, not only the bee-friendly classes, but also urban areas, crops, (water)ways, cemeteries and other landscapes. A high value indicates a highly fragmented and heterogeneous landscape containing a mix of many land use types. While heterogeneity in the landscape can generally be regarded as positive to biodiversity, landscapes containing more than 10 different classes within a kilometer are most likely too fragmented for bees and lack large forage areas.

In addition, we created a separate data layer called 'Nature' which aggregates the different categories of land use referring to natural areas, semi-natural areas and other areas under specific nature management schemes. Another layer, we refer to as 'crop', aggregates all cropping types into one layer. This allows us to summarize the combined impact of agriculture. Finally, we created a layer we refer to as 'Bee forage' which aggregates all land use and habitat types that we rate as providing decent to good forage for bees at least part of the year. Note that this is a subjective assessment based on our experience with bees and bee foraging and follows a similar assessment previously carried out for the UK. We calculated all parameters around the apiary for a 1000m and a 3000m circle. Most foraging is expected to take place within 1km from the hive, while good forage opportunities further afield are also readily discovered and exploited.

Landscapes differed substantially in several of the factors that are known to be potentially beneficial or detrimental to honeybee colony health (table 7).

Table 7. Summary of occurrence of important landscape parameters around apiaries (within 1km) in 2014, 2015 and 2016.

Landscape factor	2014 landscapes average (range)	2015 landscapes average (range)	2016 landscapes average (range)
Number of land use classes	9.1 (3-15)	9.4 (4-14)	9.6 (3-17)
% Bee Forage	20.7 (0-72)	18.0 (0-68)	15.4 (0-70)
% Natural habitat	10.7 (0-50)	8.6 (0-57)	9.1 (0-68)
% Crop area	29.1 (0-92)	30.1 (0-91)	29.4 (0-80)
% Maize cultivation	5.9 (0-29)	5.6 (0-32)	6.7 (0-31)

3.2.3. Integrated analysis results from Honeybee Surveillance Study

Integrated analysis: We aim to answer two related, but separate questions in the integrated analysis:

Q1: Is the percentage of survival at apiary level related to specific explanatory variables?

[this may reflect the overall quality of the beekeeper and the landscape pressures (food, diseases]

Q2: Is colony survival related to specific explanatory variables?

[this may reflects the specific conditions of the individual beehive (food, agro-chemicals, diseases found in the hive)]

Both questions have been addressed by applying generalized linear models (Q1: GLMs; Q2: GLMMs), the best current approach for this type of problem. This method relates the focal variable (Q1: percentage of survival of hives in apiary; Q2: survival/mortality of the single hive) to a range of potential factors influencing the survival (see Table 8). Given that there are many possible factors for each of the main categories ('pests and diseases', 'beekeeping aspects', 'agro-chemicals', 'food sources', 'landscape characteristics'), the method first selects the main candidate causes within each category. Next, a full model is constructed using of these selected factors and model selection is performed to find those factors that significantly contribute to the percentage of hives surviving within an apiary (Q1) or to the probability for a single hive to survive (Q2).

Table 8. Factors used in surveillance analysis for questions Q1 and Q2.

Factor use in models	Description	Included in Q1	Q2
% winter survival in apiary	Proportion of colonies in the apiary that survived the winter. This is what we try to explain in Q1.	YES	NO
Winter survival	Colony survived the winter (YES) or died in the winter (NO). This is what we try to explain in Q2.	NO	YES
% Varroa	Number of mites occurring on 80 bees (first sample was 50 bees) of a single hive. For Q1, the maximum value of a single hive in the apiary is included.	YES	YES
Presence of DWV	Presence of deformed wing virus in honeybees (YES/NO)	YES	YES
Presence of ABPV	Presence of ABPV virus in honeybees (YES/NO)	YES	YES
Presence of <i>Nosema</i> apis	Presence of the microsporidian <i>Nosema apis</i> in honeybees (YES/NO)	NO	YES
Presence of <i>Nosema</i> ceranae	Presence of the microsporidian <i>Nosema ceranae</i> in honeybees (YES/NO)	YES	YES
Number of hives going into winter	Indication from the beekeeper how many hives he had before the winter. This is an indication of size of the beekeeping operation	YES	NO
Presence of neonicotinoids	This variable is YES if any neonicotinoids have been detected in the honey sample of a hive, and NO if none have been detected	NO	YES
Presence of individual chemical compounds	Each chemical residue observed at least 5 times in the sample under analysis was included as a separate variable in step 1 of model 2. Only the significant ones at step 1 were used in the full model in step 2. For details see below.	NO	YES
% maize area	Area of maize cultivation around the apiary (we analyzed this at two levels: 1000m and 3000m radius)	YES	YES
% nature	Area of (semi-)natural habitats around the apiary (we analyzed this at two levels: 1000m and 3000m radius). Note that nature as defined here ranges from flower-rich chalk grassland to biodiversity poor dense conifer stands, which makes interpretation difficult.	YES	YES
% cropped area	Area of cropland, all crops summed, around the apiary (we analyzed this at two levels: 1000m and 3000m radius)	YES	YES
Number of land use elements	Sum of the different types of land use around the apiary (we analyzed this at two levels: 1000m and 3000m radius)	YES	YES
Number of pollen sources	The sum of the number of different pollen types detected in the pollen sample of a hive.	NO	YES
% of pollen of plant X	The percentage of pollen grains of plant X in a hive pollen sample. We analyzed the dominant pollen types separately, namely Brassicaeae (mustards and oilseed rape), <i>Impatiens</i> (Himalayan balsam), <i>Heracleum</i> (hogweed)	NO	YES

Q1: Is the percentage of survival at apiary level related to specific explanatory variables? Here we try to explain the % of winter survival (reverse of mortality) using land use, disease and size of the apiary. Factors that were tested in the model are given in table 8. A total of 135 apiaries could be included in this analysis.

Result: Most factors did not have an important contribution to the percentage of hive survival in apiaries and little variation could be explained by the factors taken into account. There is a series of models that is almost equally good (table 9), with some factors appearing in most of these models. This indicates two things: some factors are highly likely to play a role (the ones appearing consistently in best models) and other factors play small, interchangeable roles. Important factors affecting survival at apiary level are: The maximum percentage of *Varroa* mites found in an apiary (higher percentage leads to lower survival), the number of land use classes (high number of classes leads to lower survival) and the presence of the ABPV virus (survival is lower if ABPV is present) (see figure 4).

Table 9. Factors related to the survival percentage of hives in an apiary. Values indicate the estimates from the model with standard error in parentheses. The final model is model 2, whereas a total of 21 models are close to be the best, i.e. within 2 AIC points. We display here 5 of them and in appendix C the full table. The full model is the one with all variables included, after which variables are deleted till the best model is found. Apiary level survival is lower in very diverse landscapes, when higher *Varroa* infection levels are observed and when ABPV virus is present in the apiary.

			_		
	Q	1 LU Mode	ls		
	Full Model 1	Model 2	Model 3	Model 4	Model 5
(Intercept)	4.51***	4.51***	4.28***	4.33***	4.12***
	(1.29)	(0.89)	(0.91)	(0.90)	(0.91)
natuur_J_1K	-0.01			-0.01	-0.01
	(0.00)			(0.00)	(0.00)
gewas_J_1K	-0.00		-0.00		-0.00
	(0.00)		(0.00)		(0.00)
nlanduse_3K	-0.14	-0.17**	-0.13	-0.17**	-0.13
	(80.0)	(0.06)	(0.07)	(0.06)	(0.07)
voedsel_J_3K	0.00			0.00	0.00
	(0.00)			(0.00)	(0.00)
varroa	-0.03*	-0.04*	-0.03*	-0.04*	-0.03*
	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)
Nos cer Present	0.35				
	(0.36)				
ABPV -Present	-0.99 [*]	-0.78*	-0.80*	-0.85*	-0.89*
	(0.40)	(0.37)	(0.37)	(0.37)	(0.38)
IN_LocalLevel	0.03				
	(0.16)				
AIC	311.77	303.13	303.36	303.52	303.67
BIC	346.64				
Log Likelihood	-143.89	-147.57	-146.68	-145.76	-144.83
Deviance	206.31				
Num. obs.	135	135	135	135	135
Delta		0.00	0.23	0.39	0.53
Weight		0.09	0.08	0.07	0.07
p < 0.001, **p <	< 0.01, *p < 0.05				

Other factors play a very small role (i.e. low coefficients in table) in only some of the better models and do not affect honeybee survival substantially (see appendix C). They may of course have been

responsible for the mortality of individual hives or low survival at some apiaries, but are not consistently contributing to mortality across the country's apiaries (table 9).

Conclusion: No single factor explains all survival/mortality at apiary level. However, higher survival is linked to better beekeeping practices such as disease control and leading to lower percentage of mites and lower prevalence of ABPV. The landscape in which the bees forage also has an impact on survival with more heterogeneous landscapes (at 3km scale) leading to slightly lower survival. This is interesting given that habitat diversity is generally seen as positive. However, most landscapes in which Dutch honeybees forage are already quite diverse (on average more than 9 major land use categories within 1km from the hive location), therefore the result should be interpreted as a slight negative effect of extremely diverse, highly fragmented, landscapes compared to less fragmented, but still highly diverse, landscapes.

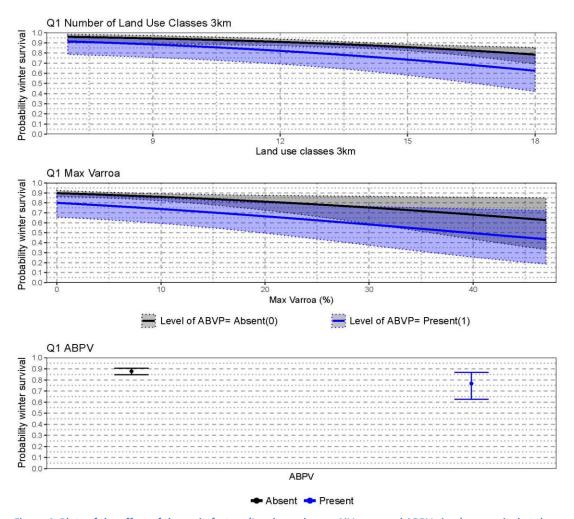


Figure 4. Plots of the effect of the main factors (Land use classes, %Varroa and ABPV virus) on survival at the apiary level. For details on the model outcomes see table 9.

Q2: Is colony survival related to specific explanatory variables?

Here we assess whether the winter survival of an individual colony can be explained by any of the main factors assessed in the surveillance study. In this mixed model apiary was included as a random factor, whereas we assessed all other variables. Given the large number of variables within each category (land use, chemicals, diseases, pollen), we perform the analysis in two steps (figure 5). In step one we constructed models for each category separate to identify the main variables within each category (details in appendix C). Step two analyzed the final model using all the relevant variables resulting from the step 1 models.

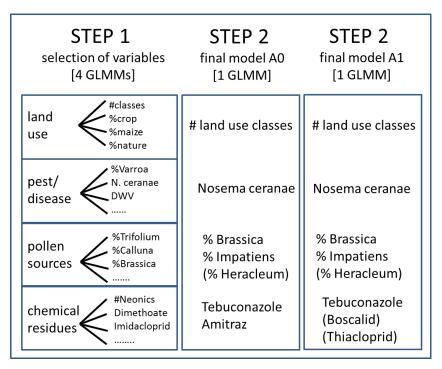


Figure 5. Schematic overview of analysis for question 2. STEP 1 selects the main variables within each of the four variable categories (boxes on the left; for details on all variables that were included see appendix C) using GLMM models. STEP 2 uses the variables selected in STEP 1 (indicated in the two other boxes) in a final GLMM model. Difference between A0 and A1 models is explained in the text. '---' indicates that none of the variables in that subcategory explained significant amount of variation in colony survival.

This procedure is the same as was performed last year. Again we have taken into account the fact that a chemical is present or not (the LOD or Level of Detection; above LOD = present, below LOD = absent) and the level at which we can tell how much is actually present (the LOQ or level of quantification; above LOQ = quantity known, below LOQ = may be present (if above LOD), but level is too low to quantify; see box 1). We have now added an analysis in which all cases above LOD (compounds B,C,D in Box 1) but cannot take the quantity into account in that case. Results of the two main models (Q2 A0 model = below LOQ recorded as 0, in Box 1: A=B=0, C and D actual concentration; Q2 A1 model = above LOD recorded as 1, in Box 1: A=0, B=C=D=1) are given below. The main reason for adding this complication is that one may argue that even the presence of chemical at very low levels may have an effect. Also note that the LOD and LOQ thresholds are purely methodological thresholds and do not have any relation to the potential hazard and safety of these compounds for any organism.

<u>Result</u>: The first observation is that a large number of different models are explaining almost the same level of variance in winter survival. This shows that a large number of variables have only very small contributions and that they are interchangeable. However, a few factors play a more important role as they occur consistently in many models and have higher coefficient values (Tables 9 and 10; figures 6 and 7.

Two pollen types occurring in stored bee bread consistently have a positive effect on winter survival particularly when occurring at high quantities, namely brassicaceae (oil-seed rape, mustards), Impatiens (most likely the invasive species *Impatiens glandulifera*, an important nectar and pollen source). A small positive effect was found for a third pollen type, hogweed (most likely the invasive Heracleum mantegazzianum, 'reuzenbereklauw').

Two chemical compounds were found to affect honeybee winter survival. Amitraz is used by beekeepers to combat mites and is very effective, meaning that hives containing higher concentrations of Amitraz had higher survival probability. Tebuconazole, a fungicide used widely in both open and covered crops, had a consistent negative effect when present (found in 5 samples). If we include the cases in which minute traces were found (including those below LOQ level; Q2A0 models) we find that the presence of boscalid (n=14 in A0, and n=20 in A1) and thiacloprid (n=15 in A0, and n=28 in A1) both had negative effects in some of the models. Boscalid is a widely-used systemic fungicide, whereas Thiacloprid is a neonicotinoid insectide. Both are used broadly in agriculture (open and closed crops) and horticulture.

As on previous occasions and in Q1 above, the number of land use classes had a negative effect on winter survival. This indicates that highly fragmented and diversified landscapes are not good for honeybee survival. Other landscape factors, such as area of maize cultivation, had virtually no impact.

Bee diseases affected winter survival in a surprising way. The presence of *Nosema ceranae* had a positive effect on survival, whereas the incidence of *Varroa* in single hives in autumn did not have a negative effect. It is remarkable that the presence of *Nosema ceranae* seems to be declining consistently over the year, now down to 22% of hives infected in the autumn. Incidence of *Varroa* mites at apiary level increased mortality (Q1 models), but it had no effect on single hive survival. Neither DWV nor ABPV levels in single hives could explain mortality over the winter.

<u>Conclusion</u>: Only a small part of the mortality of bee hives could be explained by the main factors that were analyzed. There are many potential influences on honeybee hives next to the ones we measure: every beekeeper uses slightly different methods, queen quality and replacement varies a lot and not all aspects of the landscape can be included. Finally, interactions between different factors may be of importance. Interactions of factors occur regularly in field studies and is not unlike what is found in human cohort studies.

Mortality was slightly higher in very heterogeneous landscapes and for colonies in which Tebuconazole was detected. Survival was higher for colonies that had stored a large amount of Brassicaceae or Impatiens pollen and those that were infected with *Nosema ceranae*. The main factors in the Q2A0 and Q2A1 models were very similar, there were some differences in the minor factors (see tables 9 and 10)

Table 10. Summary of factors related to the survival (only 5 of 31 models are shown, full table in appendix C)

	Q2 A0 Fi	nal overal	models		
	Full Model	Model 2	Model 3	Model 4	Model 5
(Intercept)	3.19	3.66	3.79	4.11	3.99
	(1.84)	(1.50)	(1.41)	(1.41)	(1.52)
nlanduse_3K	-0.18	-0.15	-0.16	-0.17	-0.16
	(0.10)	(0.10)	(0.10)	(0.10)	(0.10)
opp_mais_1K	0.00				
	(0.01)				
Tebuconazole1	-2.60	-2.84	-2.69	-2.52	-2.67
	(1.61)	(1.60)	(1.39)	(1.37)	(1.58)
Amitraz_DMF_DMPF1	9.29		13.84	29.49	
	(87.49)		(951.74)	(2370881.46)	
Brassicaceae	1.87	1.71	1.67		
	(1.35)	(1.28)	(1.30)		
Impatiens	9.60	9.41	9.72	9.49	9.16
	(9.51)	(9.50)	(10.06)	(9.55)	(8.97)
Heracleum	8.61				
	(10.09)				
Nosema_ceranae1	1.07	1.03	1.06	1.08	1.05
	(0.62)	(0.62)	(0.62)	(0.62)	(0.62)
varroa	-0.00				
	(0.04)				
DWV1	0.72				
	(1.28)				
AIC	189.32	183.16	183.19	183.34	183.34
BIC	230.73				
Log Likelihood	-82.66	-84.58	-83.60	-84.67	-85.67
Num. obs.	233	233	233	233	233
Num. groups: Imker	131				
Var: Imker (Intercept)	0.31				
Delta		0.00	0.04	0.18	0.18
Weight		0.06	0.06	0.06	0.06

Table 11. Summary of factors related to the survival (only 5 of 42 are shown, full table in appendix C)

Q2 A1 Final overall models									
	Model 1	Model 2	Model 3	Model 4	Model 5				
(Intercept)	3.21*	3.70 [*]	4.09**	3.62 [*]	1.54***				
	(1.33)	(1.48)	(1.50)	(1.48)	(0.43)				
nlanduse_3K	-0.13	-0.16	-0.18	-0.16					
	(0.10)	(0.09)	(0.09)	(0.09)					
opp_mais_1K	0.00								
	(0.01)								
Boscalid1	-0.73				-1.18				
	(0.72)				(0.69)				
Tebuconazole1	-1.72	-1.90		-1.78	-2.06				
	(1.06)	(1.22)		(1.21)	(1.16)				
Thiacloprid1	-0.48								
	(0.63)								
Brassicaceae	1.58	1.87		1.84	1.72				
	(1.21)	(1.26)		(1.25)	(1.23)				
Impatiens	8.85	9.39	9.57	9.32	9.18				
	(9.33)	(9.41)	(8.95)	(9.34)	(10.21)				
Heracleum	8.84			9.33					
	(9.82)			(10.00)					
varroa	-0.01								
	(0.03)								
Nosema_ceranae1	1.06	1.02	0.94	1.07	1.03				
	(0.58)	(0.61)	(0.58)	(0.61)	(0.61)				
AIC	190.84	184.61	184.94	185.08	185.13				
BIC	232.25								
Log Likelihood	-83.42	-85.30	-87.47	-84.54	-85.57				
Num. obs.	233	233	233	233	233				
Num. groups: Imker.x	131								
Var: Imker.x (Intercept)	0.00								
Delta		0.00	0.33	0.47	0.52				
Weight		0.04	0.03	0.03	0.03				

A dominant idea is that agrochemicals, particularly neonicotinoids, are the main causes of honey bee colony loss in the winter. However their presence could not explain loss in winter 2016-17 and neither did they show a significant relation to loss in the previous two winters. Residues of neonicotinoids are found in the samples, but not systematically in those of colonies that died.

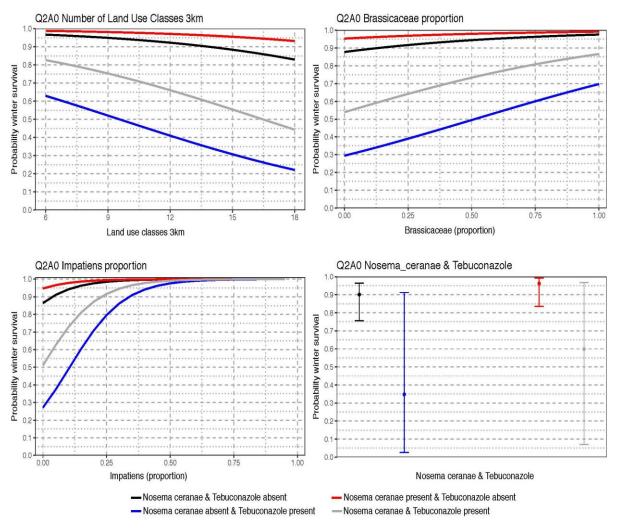


Figure 6. Graphic representation of the influence of the main effects on colony survival for models using LOQ as threshold for chemical presence (Q2A0) for the four different conditions in which *Nosema ceranae* is present/absent and residues of Tebuconazole is found/absent.. When both are present survival is lower than when both are absent. When Tebuconazole is present survival is lower than when it is absent, however, for Nosema ceranae survival is slightly higher when it is present.

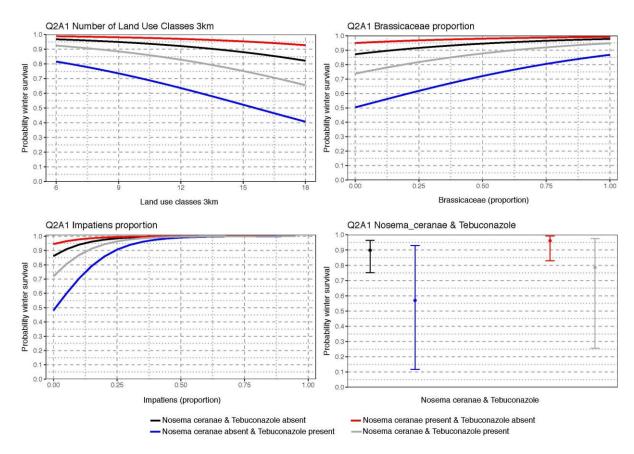


Figure 7. relationships with colony survival of the major factors explaining colony survival in the Q2A1 models. Findings are very similar to those found in Q2A0 models, depicted in figure 6.

3.3. Other results and planning

<u>Honeybee winter mortality monitor</u>: As in the previous year, the collaboration between the NBV and our consortium has led to a single broadly-supported national winter mortality figure for the Netherlands, that was published in April 2017. This collaboration will be continued the coming year and will deliver the official figure for annual honeybee winter mortality.

<u>Honeybee Surveillance:</u> To get to a response level of 200 beekeepers, what we aim at in this study, we needed to approach more than 800 beekeepers. Many registered beekeepers do not reply, do not want to collaborate, and some do not have bees. This makes it a huge effort, but it is the only way to obtain a solid dataset, i.e. a random selection of apiaries.

Several procedures have been automated by Naturalis since the 2015-2016 sampling (both the monitoring and the surveillance). Google email systems are used to approach beekeepers, while other beekeepers are reached by telephone. Also the recruitment of beekeepers for the new season has been partly automated by Naturalis, which saves substantial time.

The sampling of bees, pollen and honey was organized differently in 2016 than before. Selected beekeepers were asked directly to collaborate, they were sent the sampling materials and full instructions. Materials were sent back to Bijen@wur for further processing and distribution to the consortium partners for further analysis.

4. Discussion: Comparison across the three years of the study

With this report, the third year of the study has been concluded and data are starting to accumulate so that a first comparison between years can be made. First, we compare findings for individual factors, second we compare findings of the integrated model.

4.1. Single factor comparison

Parasites and pathogens

Over three years the most important trend is that *Nosema ceranae* is decreasing strongly from 89% in 2014 to 22% in 2016. Presence of Varroa is with 68% not very different from 2015 (63%) and a bit lower than in 2014 (73%). *Nosema apis* is slightly increasing, whereas ABPV has increased considerably. It is well known that both the *Nosema* species can have negative impact on honeybee hives and are sometimes blamed for colony loss. Therefore, a positive effect of *Nosema ceranae* presence is a surprise and needs further exploration. It could be that hives that were previously heavily affected have disappeared, which may mean that hives with *Nosema* still present are strong hives that, for other reasons than *Nosema*, have a good chance to survive. Another speculative explanation could be that *Nosema* disappears from hives that are treated against disease and that this may have negative side-effects. At this moment we do not know.

Pollen sources used by hives

A comparison over three years (table 12) shows that the plant species most often used as pollen source, sampled as bee bread in late summer, are largely the same across years. Brassicaceae and *Trifolium* are found in a large part of the hives in all years. Some differences can be explained by differences in sampling period. In 2014 Hedera was found in more than 50% of the hives, in the two years after that it was 16.7 resp. 8.5 percent, which can be explained by the earlier sampling in later years. Impact of pollen sources was positive for mustards and Himalayan balsam relatives in year three, but negative for clover pollen in years 1 and 2. At the end of the study we will assess the overall influence of pollen types on survival, also in relation to the landscapes where these pollen types have been found.

Table 12. Overview of most important pollen types found in 2014, 2015 and 2016. Figures indicate the percentage of samples each pollen type was present in. The table is a composition of all species that were found in more than 10% of the hives in at least one of the sampled years.

Pollen type	English name	% of total 2014	% of total 2015	% of total 2016	Average % over 3 years
Brassicaceae	mustards, rapeseed	33.3	49.8	53.3	45.5
Trifolium	Clovers	25.6	47.1	42.3	38.3
Hedera	ivy	52.6	16.7	8.5	25.9
Calluna	heather	15.4	21.6	30.1	22.4
Asteraceae	dandelion family	3.8	20.4	32	18.7
Rosaceae	rose family	10.3	23.1	21	18.1
Lotus	birds foot trefoil	11.5	26.7	0.3	12.8
Caryophyllaceae	ragged robin and relatives	8.9	15.3	9.1	11.1
Impatiens	Himalayan balsam and relatives	10.3	8.6	13.5	10.8

Phacelia	phacelia	11.5	12.1	8.2	10.6
Castanea	chestnut	0	0	25.4	8.5
Fabaceae	legumes	2.6	2.4	16.9	7.3
Zea	mais	0	8.6	11.9	6.8
Heracleum	hogweed	3.8	3.5	10	5.8
Fagopyrum	buckwheat type	1.3	14.5	0.6	5.5
Cornus	dogwood	0	2.4	13.8	5.4
Hypericum	St. John's wort and relatives	3.8	5.9	4.1	4.6
Rubus	bramble	0	11.4	1.9	4.4

Chemical residues detected in honey

While the presence of acaricides in 2016 is slightly higher than in 2015, the presence of neonicotinoids in honey samples was lower than in 2015 (see table 5). Of the neonicotinoids, thiacloprid was present most often (also the highest presence of any chemical compound) and at similar level as in 2015, while imidacloprid was continuing its downward trend and was only found in 0.3% of honey samples. Note that analysis was expanded in 2016 due to a slight change in the method being available at Rikilt, leading to the possible detection of more chemical substances. Like in previous years, the majority of the honey samples in the hives did not contain any trace, above level of detection of our method, of the chemical compounds we tested for.

Landscapes in which bees forage

Dutch landscapes do not change a lot from year to year. Crop rotations probably make up a large part of change. In addition, our sample is large and representative of the Dutch landscape. This can be seen from the very similar numbers for different aspects of the landscape across the years (table 7).

4.2. Integrated analysis results from Honeybee Surveillance Study

In this study we aim at explaining honeybee winter survival and mortality by analyzing various factors that have been indicated previously to be responsible for hive loss in at least some cases: beekeeping practice, pathogens and parasites, pollen sources, chemical residues and the forage landscape. In each of the years, variation between the hive that die is large and the list of factors that we analyse seems to be able to explain just a small part of hive loss. Field trials are notoriously difficult to show similar effects as those found under the controlled circumstances in the laboratory. Yet it is very important to assess the real world situation, which is what we aim for.

The main factors we analyze can explain only a small part of the hive loss. This indicates that there are many potential influences on honeybee hives next to the ones we measure. Every beekeeper uses slightly different methods, queen age and quality varies a lot, queen can die or be replaced and not all aspects of the landscape can be included. Finally, interactions between different factors may be of importance. By building up the dataset across the years we will reach a critical mass of data at

the end to obtain the best, broadest, most detailed picture of the factors influencing honeybee winter mortality in the Netherlands.

For our integrated models, we first identify the most important specific factors from within the different factor groups (e.g. Varroa presence from the disease group, mustard pollen presence from the pollen types). The selected factors differ each year, due to natural variation. These main factors are included in the integrated model.

In the first year, 2014-2015, the main factors found to explain winter mortality were related to beekeeping practice (better Varroa control. i.e. lower mite counts), with a second surprising finding of a negative influence of clover pollen on survival. In the second year, 2015-2016, again beekeeping related factors explained some of the hive loss (Varroa counts, DWV presence). In addition, we found a negative impact of Dimethoate, which was only found in a small subset of the hives. As in the previous year, the presence of substantial amounts of clover pollen had a negative effect on hive survival. Very diverse, and therefore fragmented, landscapes have a negative effect on survival in both the second and third year (and a similar tendency in year 1). The current year, 2016-2017, reveals some different patterns with the presence of Nosema ceranae having a slight positive effect on hive survival as have two pollen sources (mustards and Himalayan-balsam relatives).

If we compare the impact of neonicotinoids (as a group or as individual compounds), we see that they do not appear in the final models, which indicates that they do not explain a significant part of winter hive loss.

Following results of the coming year, we will pool all data from across years into a single large analysis where also the variability between years can be taken into account. We trust that the dataset will be substantial enough to reveal the influence of the main factors in honeybee colony winter loss.

5. Conclusions

- **1.** The Honeybee Mortality Monitor reveals that winter mortality was not extremely high and within the normal range in 2016-2017 (14.3%).
- **2.** The number of managed honeybee colonies in the Netherlands is estimated to be between 70,000 and 95,000 depending on the number of unregistered beekeepers.
- **3. Apiary-level mortality**: No single factor explains the proportion of survival or mortality at apiary level. However, higher mortality was found in apiaries with higher *Varroa* levels and presence of ABPV virus, as well as those occurring in highly diverse landscapes.
- **4. Hive-level mortality**: Looking at individual colonies, survival chances decreased in highly diverse landscapes, and when residues of Tebuconazole were detected in the stored honey. Survival was higher for colonies that had stored large amounts of Brassicaceae or Impatiens pollen and those in which residues of the Varroacide Amitraz was detected. Surprisingly, presence of *Nosema ceranae* was found to have some positive impact on survival. *Nosema* is a bee disease that occurred, overall, much less in 2016 than in the years before.

Neonicotinoids and other chemical residues did not have any significant relation with colony winter mortality in our study.

5. Summarizing: Hive survival was high all-round (85.7% national figure) and no single factor explains the proportion of hive survival or mortality. Impact of the five main factors that have been analyzed can be summarized as follows [Note that for interpretation of all findings in this study, as in other studies, it is important to note that the absence of a significant correlation does not prove the absence of any effect]: **Bee management practice:** Honeybee colonies survive best if beekeepers keep *Varroa*-mite infestation levels low, which was the case for most beekeepers in 2016.

Pests and diseases: Varroa infection levels before winter were up a bit compared to 2015 to 5%, had negative effect on apiary level mortality, but could not be confirmed as a negative factor at the level of single hives. Levels of ABPV virus were much higher than in previous years and had a negative effect on survival. DWV presence was, unlike last year, not correlated to survival or mortality, whereas *Nosema ceranae* seems on the way down, and even had a slight positive effect.

Chemical residues: Of the several dozen chemical compounds and their metabolites we screened for, including all neonicotinoids, 14 were detected in stored winter food in autumn. Two of these substances are used by beekeepers for Varroa-control. The fungicide Tebuconazole was detected in 1.5% of samples, but when present had a negative impact on survival. Similar, but much smaller, effects were detected for Thiacloprid and Boscalid presence. Other chemical residues were rarely found and when present were not related to colony mortality.

Pollen sources: Hives with abundant Brassica or Impatiens pollen stored in bee bread had higher survival than other hives. Unlike in the previous two years, no effect of Clover pollen was detected. **Landscape conditions:** Highly diverse, fragmented, landscapes led to a decrease in hive survival. Most Dutch landscapes where apiaries are positioned are quite diverse (on average more than nine different land use categories within 1km of the apiary). In landscapes with even more land use types, i.e. rather fragmented landscapes, hive survival was slightly lower.

6. Appendices

- A Winter mortality survey based on CoLoSS questionnaire
- B Set-up of stratified Random Field Campaign [in Dutch]
- C Overview of results from GLMM analyses surveillance study
- D List of food plants found in stored pollen
- E List of chemical residues and their detection limits used for screening honey samples

Appendix A

Winter mortality Survey based on CoLoSS questionnaire

Enquête uitwintering bijenvolken NBV/Bijen@wur/Naturalis

14-06-17 20:18

Enquête uitwintering bijenvolken NBV/Bijen@wur/Naturalis

Voor het 5e jaar op rij organiseren de NBV en Bijen@WUR een wintersterfte monitor, dit jaar in samenwerking met Naturalis. Waar we 5 jaar geleden uitsluitend telefonisch zijn begonnen gaan we met onze tijd mee en doen we het grootste gedeelte van de enquête nu online. We houden elk jaar deze enquête om de overleving van de Nederlandse bijenvolken in de gaten te kunnen houden. Graag horen we van u hoe het uw bijen is vergaan deze winter. U bent random geselecteerd uit de ledenlijst van de NBV om een goede representatie van alle imkers in Nederland te krijgen.

Wij zouden u dan ook vriendelijk willen verzoeken deze enguête in te vullen!

*Vereist

Imker gegevens

1. Naam imker

Wij vragen u om uw naam en e-mail adres om u te kunnen bereiken. Het adres van de bijenstand vragen we om te kijken of er regionale verschillen zijn in sterfte. Als uw volken niet niet op een postadres staan vragen we u een adres zo dichtbij mogelijk te noemen zodat onze analyses zo precies mogelijk zijn.

2.	Adres bijenstand (zo precies mogelijk) *	
3.	E-mail adres imker *	
Wij uitw asp	brte/Lange enquete? bieden u de mogelijkheid te kiezen voor een kor vinteringsgegevens doorgeven) of een meer uitg ecten gevraagd wordt (zoals varroa bestrijding e	tgebreide enquête, waarin ook naar andere g etc.)
	langere enquête bestaat uit een aantal extra vra	- N
4.	Wilt u de korte of iets langere enquete invul Markeer slechts één ovaal.	ullen?*
	Kort - alleen de vraag over de uitwinteri	NO NO NO NOT
	Langer - extra vragen over onder ander	are wijze van varroabestrijding

In- en uitwinteringscijfers

5.	Hoeveel volken heeft u ingewinterd in 2016? *
6.	Hoeveel volken heeft u uitgewinterd in 2017? *
7.	Op hoeveel ramen heeft u gemiddeld genomen ingewinterd? * Markeer slechts één ovaal.
	<5
	5-10
	10-15
	15-20
De vra	arroabestrijding behandeling van varroa kan van belang zijn voor het overwinteren van uw bijen, daarom gen wij u hoe uw bijen tegen varroa behandeld zijn. Bij al deze vragen zijn meerdere woorden mogelijk.
8.	Welke manier(en) van varroabestrijding past u toe?* Vink alle toepasselijke opties aan.
	Geen varroabestrijding
	Voorjaar darrenbroed verwijderen
	Vóór zomerdracht (combinatie van zwermverhindering en oxaalzuur)
	Na de zomerdracht
	Winterhehandeling

9.	Indien u na de zomerdracht behandeld heeft, welke middelen heeft u gebruikt? Vink alle toepasselijke opties aan.
	Mierenzuur behandeling (Liebig / Nassenheider / anders)
	Thymovar
	Apistol
	Apistan
	Amitraz
	Apivar
	Thymol
	Api Life Var
	Api guard
	Anders:
10.	Indien u winterbehandeling(en) heeft toegepast, welke methoden heeft u gebruikt? Vink alle toepasselijke opties aan. Oxaalzuur (druppelmethode)
	Oxaalzuur (verdampingsmethode)
	Anders:
De dez naja wint wor	ajaarsdracht najaarsdracht is de laatste dracht voor de inwintering. Afhankelijk van de imkermethode wordt e najaarsdracht 1) gebruikt als wintervoer en wordt er dus niet geslingerd; 2) wordt de aarshoning deels gebruik als wintervoer en aangevuld met suikeroplossing om voldoende tervoer aan de volken te geven en 3) de najaarshoning wordt geheel geslingerd en de volken den vervolgens ingewinterd met suikeroplossing.
11.	welke najaarsdracht hebben uw volken bezocht? Markeer slechts één ovaal.
	Geen
	Heide
	Balsemien
	Anders:
12.	De najaarshoning Markeer slechts één ovaal.
	is geheel gebruikt voor inwinteren en niet aangevuld met suikeroplossing
	is deels gebruikt voor inwinteren en wel aangevuld met suikeroplossing
	is geheel / grotendeels geslingerd en daarna zijn de bijen ingewinterd

Herkomst Koningin

13.	Wat is de herkomst van uw koningin(nen) ? Vink alle toepasselijke opties aan.
	Uit eigen teelt, op eigen stand bevrucht
	Uit eigen teelt, op een andere stand bevrucht
	Gekocht
	Anders:
	Allueis.
14.	Indien u uw koningin gekocht heeft, waar komt deze vandaan?
15.	Wat is het ras van de door u gekochte koningin?
Ηοι	e publiceren de resultaten van deze enquête op 15 april udt hiervoor de website en de nieuwsbrief van de NBV in de gaten! p://www.bijenhouders.nl/)
Via	de onderstaande link kunt u meer informatie vinden over het Surveillance programma.
http	://www.wageningenur.nl/nl/Expertises-Dienstverlening/Onderzoeksinstituten/plant-research-
inte	rnational/Over-Plant-Research-International/Organisatie/Biointeracties- ntgezondheid/Bijen/Surveillanceprogramma-Honingbijen.htm
riai	ngezondneid/bjjen/Sdrveinanceprogramma-noningbjjen.ntm
Sto	o met het invullen van dit formulier.
ln.	- en uitwinteringscijfers
	3,
16.	Hoeveel volken heeft u ingewinterd in 2016? *
	2010.
17.	Hoeveel volken heeft u uitgewinterd in 2017? *
18.	Op hoeveel ramen heeft u gemiddeld genomen ingewinterd?* Markeer slechts één ovaal.
	<5
	5-10
	10-15
	15-20

Appendix BSet-up of stratified Random Field Campaign [in Dutch]

Stappenplan voor Selectieprocedure van imkers voor surveillance onderzoek.

	1	ctieprocedure van imkers voor surveillance onderzoek.
STAPPE		
N	WIE	WAT
STAP 1	allen	Besloten is om gestratificeerd random te selecteren, gebaseerd op 4 jaar gemiddelde sterfte per postcodegebied. De 90 postcodegebieden worden in 5 groepen gedeeld op basis van sterftecijfers en per groep worden 5 postcodes random geselecteerd. Daar worden monsters genomen.
STAP 2	Koos	Romee heeft gegevens aangeleverd voor 2011-2014: residual effects van het beste mixed-model. DWZ in hoe verhoudt de sterfte zich in het betreffende postcodegebied t.o.v. het gemiddelde (mixed model).
STAP 3	Koos	Gemiddelden van 4 jaar residuals zijn berekend en in rangorde gezet. Zo worden 5 groepen gemaakt (quintiles) variërend in residual effect en dus in sterfte.
STAP 4	Koos	Randomgetallen aan elke postcode toegekend, daarna gesorteerd en hoogste 5 genomen voor selectie. Postcode gebieden met minder dan 5 responses worden niet meegenomen omdat daar wellicht niet voldoende imkers zijn. In zo'n geval wordt de volgende in de rij genomen.
		Per postcode gebied worden imkers (met 5 of meer volken) geselecteerd op basis van toekennen random getallen, dan sorteren en vanaf hoogste getal per postcode 5 imkers selecteren per postcode gebied. Mocht een imker afvallen c.q. niet mee willen werken, dan wordt nummer 6 op de
STAP 5	Sjef/Koos	lijst benaderd enzovoorts.

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Appendix C: Overview of results from GLMM analyses surveillance study **Full model for Q1**

Q1 LU Models																						
	FM 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	M 12	M 13	M 14	M 15	M 16	M 17	M 18	M 19	M 20	M 21	M 22
(Intercept)	4.51***	4.51***	4.28***	4.33***	4.12***	4.20***	4.41***	4.42***	4.17***	2.87***	4.45***	4.25***	4.50***	4.82***	4.43***	4.84***	4.26***	4.30***	4.62***	4.05***	4.69***	2.64***
	(1.29)	(0.89)	(0.91)	(0.90)	(0.91)	(0.91)	(0.91)	(0.89)	(0.91)	(0.32)	(0.90)	(0.91)	(0.89)	(1.17)	(0.90)	(1.16)	(0.90)	(0.91)	(1.15)	(0.91)	(1.09)	(0.37)
nlanduse_1K	0.04														-0.04							
	(0.08)														(0.07)							
opp_mais1K	-0.00						-0.01					-0.01						-0.01				
	(0.01)						(0.01)					(0.01)						(0.01)				
natuur_J_1K	-0.01			-0.01	-0.01	-0.00				-0.00		-0.01	-0.00				-0.01		-0.01	-0.01		-0.01
	(0.00)			(0.00)	(0.00)	(0.00)				(0.00)		(0.00)	(0.00)				(0.00)		(0.00)	(0.00)		(0.00)
gewas_J_1K	-0.00		-0.00		-0.00	-0.00			-0.00	-0.01**				-0.00					-0.00	-0.00		-0.01**
	(0.00)	**	(0.00)	**	(0.00)	(0.00)	*	**	(0.00)	(0.00)	**	*	**	(0.00)		**	**	*	(0.00)	(0.00)	**	(0.00)
nlanduse_3K	-0.14	-0.17**	-0.13	-0.17**	-0.13	-0.11	-0.15*	-0.17**	-0.13		-0.17**	-0.16	-0.16**	-0.12	-0.13	-0.17**	-0.17**	-0.15	-0.12	-0.13	-0.17**	
	(0.08)	(0.06)	(0.07)	(0.06)	(0.07)	(0.07)	(0.06)	(0.06)	(0.07)		(0.06)	(0.06)	(0.06)	(0.07)	(80.0)	(0.06)	(0.06)	(0.06)	(0.07)	(0.07)	(0.06)	
voedsel_J_3	0.00			0.00	0.00						0.00	0.00					0.00		0.00	0.00		0.00
K	(0.00)			(0.00)	(0.00)						(0.00)	(0.00)					(0.00)		(0.00)	(0.00)		(0.00)
varroa	(0.00) -0.03 [*]	-0.04*	-0.03 [*]	(0.00) -0.04*	(0.00) -0.03 [*]	-0.03 [*]	-0.03 [*]	-0.04*	-0.03 [*]	-0.03 [*]	(0.00) -0.04*	(0.00) -0.03*	-0.04*	-0.03 [*]	-0.03 [*]	-0.03 [*]	(0.00) -0.04*	-0.03 [*]	(0.00) -0.03*	(0.00) -0.03*	-0.04*	(0.00) -0.03 [*]
varroa	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Nos cer	0.35	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	0.31	0.34	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	0.26	0.35	(0.01)	0.27	(0.01)	(0.01)
present																						
	(0.36)							(0.34)	(0.34)								(0.34)	(0.34)		(0.34)		
DWV present	-0.70													-0.61		-0.35			-0.59			
A D D) /	(0.81)	0.70*	0.00*	0.05*	0.00*	0.06*	0.00*	0.76*	0.70*	0.05*	0.77*	0.07*	0.04*	(0.79)	0.72	(0.77)	0.04*	0.70*	(0.79)	0.07*	0.77*	0.06*
ABPV -	-0.99 [*]	-0.78 [*]	-0.80*	-0.85 [*]	-0.89 [*]	-0.86 [*]	-0.80*	-0.76	-0.79 [*]	-0.85 [*]	-0.77*	-0.87	-0.81	-0.83	-0.73	-0.79 [*]	-0.84	-0.79 [*]	-0.93 [*]	-0.87*	-0.77*	-0.86
Present	(0.40)	(0.37)	(0.37)	(0.37)	(0.38)	(0.38)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.38)	(0.38)	(0.37)	(0.38)	(0.37)	(0.38)	(0.38)	(0.37)	(0.37)
IN hives	0.03	(0.37)	(0.37)	(0.37)	(0.38)	(0.38)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.37)	(0.38)	(0.38)	(0.37)	(0.38)	(0.37)	(0.38)	(0.38)	-0.04	(0.57)
III IIIVES	(0.16)																				(0.15)	
AIC	311.7	303.1	303.3	303.5	303.6	303.8	304.0	304.2	304.3	304.5	304.5	304.5	304.6	304.6	304.6	304.9	304.9	304.9	305.0	305.0	305.0	305.1
AIC	7	303.1	6	2	7	9	8	4	0	1	2	8	4	9	9	2	4	9	2	4	6	3
BIC	346.6	3	U		,	J	0	-	U		2	0	7	,	J		-	,		-	U	3
DIC .	4																					
Log	-	-	_	-	-	-	-	-	-	-	-	-	_	-	-	-	_	_	-	-	_	_
Likelihood	143.8	147.5	146.6	145.7	144.8	145.9	147.0	147.1	146.1	147.2	147.2	145.2	147.3	146.3	147.3	147.4	145.4	146.4	144.5	144.5	147.5	146.5
	9	7	8	6	3	4	4	2	5	5	6	9	2	4	5	6	7	9	1	2	3	6
Deviance	206.3 1																					
Num. obs.	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135	135
Delta		0.00	0.23	0.39	0.53	0.75	0.95	1.10	1.17	1.37	1.38	1.44	1.51	1.55	1.56	1.78	1.81	1.85	1.89	1.91	1.92	2.00
Weight		0.09	0.08	0.07	0.07	0.06	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03
****p < 0.001, ***	p < 0.01, *																					
	,																					

STEP 1 for Q2 A0 models (analysis at hive level, residues considered present when above LOQ)

	C	2 A0 LU	Models	2016			
	LU Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6
(Intercept)	2.14	2.14	2.14	2.14	2.14	2.14	2.14
	(0.46)	(0.46)	(0.46)	(0.46)	(0.46)	(0.46)	(0.46)
opp_mais_1K	0.07			0.07			
	(0.25)			(0.22)			
natuur_J_1K	-0.06				-0.05		
	(0.29)				(0.21)		
gewas_J_1K	-0.00					0.05	
	(0.28)					(0.24)	
nlanduse_3K	-0.42	-0.41		-0.43	-0.40	-0.43	-0.41
	(0.28)	(0.24)		(0.25)	(0.25)	(0.27)	(0.25)
voedsel_J_3K	0.02						-0.02
	(0.31)						(0.22)
AIC	196.60	188.74	189.94	190.64	190.69	190.71	190.73
BIC	220.75						
Log Likelihood	-91.30	-91.37	-92.97	-91.32	-91.34	-91.35	-91.36
Num. obs.	233	233	233	233	233	233	233
Num. groups: Imker	131						
Var: Imker (Intercept)	0.68						
Delta		0.00	1.20	1.90	1.95	1.96	1.99
Weight		0.33	0.18	0.13	0.12	0.12	0.12

									Q	2 A0 Pc	ollen M	odels										
	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	M 12	M 13	M 14	M 15	M 16	M 17	M 18	M19	M 20	M 21	M 22
(Intercept)	1.68	1.69	1.90	1.61	1.77	1.81	1.97	1.64	1.62	1.66	2.00	1.64	2.25	1.84	1.87	1.87	1.93	1.86	1.71	1.67	1.69	1.70
	(0.89)	(0.44)	(0.45)	(0.44)	(0.44)	(0.46)	(0.46)	(0.43)	(0.45)	(0.43)	(0.70)	(0.44)	(0.71)	(0.46)	(0.44)	(0.44)	(0.46)	(0.45)	(0.46)	(0.45)	(0.44)	(0.46)
nrpollen	-0.10										-0.07		-0.07									
	(0.13)										(0.11)		(0.11)									
Asteraceae	-0.86				-1.66		-1.57													-1.54		
D	(1.69)	4.62		4.62	(1.61)		(1.60)	4.00	1.64	4.60	1.64	4.60					1.66		1.62	(1.58)	1.64	1.62
Brassicacea	2.08	1.63		1.62	1.69			1.80	1.64	1.68	1.64	1.68					1.66		1.62	1.67	1.64	1.63
е	(4.26)	(4.24)		(4.20)	(4.22)			(4.25)	(4.20)	(4.24)	(4.24)	(4.24)					(4.24)		(4.24)	(4.22)	(4.22)	(4.24)
Callina	(1.36)	(1.21)		(1.20)	(1.23)			(1.25)	(1.20)	(1.21)	(1.24)	(1.21)			0.38		(1.24)		(1.21)	(1.22)	(1.22)	(1.21)
Calluna	0.53 (0.95)							0.59 (0.82)							(0.80)							
Castanea	0.21							(0.82)							(0.80)							-0.00
Castallea	(1.11)																					(1.03)
Cornustyp	3.41									2.43						1.94						(1.03)
e e	5.41									2.43						1.54						
	(4.64)									(4.27)						(4.22)						
Fabaceae	0.42									(1.27)						(1.22)					0.11	
	(1.39)																				(1.33)	
Heracleum	8.40			7.14		7.21														6.79	(/	
	(9.49)			(9.08)		(9.09)														(9.02)		
Impatiens	11.02	9.15	8.80	9.23	10.45	8.92	9.85	9.30	8.53	9.40	9.63	9.41	9.29	8.37	8.88	8.96		8.98	9.06	10.44	9.17	9.15
	(11.68	(9.71)	(9.19)	(9.79)	(11.25	(9.28)	(10.49	(9.74)	(9.32)	(9.88)	(10.19	(9.86)	(9.72)	(8.88)	(9.17)	(9.27)		(9.29)	(9.69)	(11.19	(9.74)	(9.72)
)))))		
Rosaceae	0.95											0.69						0.54				
	(1.32)											(1.22)						(1.22)				
Trifolium	0.17																		-0.13			
	(1.12)																		(1.01)			
Zea	8.39								4.76					4.38								
	(9.27)								(8.26)					(8.32)								
AIC	203.14	187.8	188.0	188.7	188.85	188.9	189.15	189.2	189.4	189.4	189.49	189.5	189.6	189.7	189.8	189.8	189.8	189.8	189.8	189.84	189.8	189.8
510	254.45	5	4	2		3		7	5	8		0	0	2	1	1	1	3	3		4	5
BIC	251.45	00.00	04.02	00.26	00.42	00.46	00.50	00.64	00.70	00.74	00.74	00.75	00.00	00.00	00.00	00.00	04.00	00.03	00.03	00.03	00.02	00.02
Log Likelihood	-87.57	-89.92	-91.02	-89.36	-89.42	-90.46	-90.58	-89.64	-89.73	-89.74	-89.74	-89.75	-90.80	-90.86	-90.90	-90.90	-91.90	-90.92	-89.92	-88.92	-89.92	-89.92
Num. obs.	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233
Num.	131	233	233	233	255	255	233	233	255	255	233	255	255	233	233	233	233	233	233	233	255	233
groups:	131																					
Imker																						
Var: Imker	0.25																					
(Intercept)	0.23																					
Delta		0.00	0.19	0.87	1.00	1.08	1.30	1.42	1.60	1.63	1.64	1.65	1.75	1.87	1.96	1.96	1.96	1.98	1.98	1.99	1.99	2.00
Weight		0.10	0.09	0.06	0.06	0.06	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
		0.10	0.00	0.00	0.00	0.00	0.03	3.03	0.01	0.01	5.01	0.01	0.0 1	0.01	5.01	3.01	3.0 1	3.0 1	3.0 1	3.0 1	0.01	5.01

	Q2 A	0 Virus N	/lodels			
				Post 2	Doct /	Post F
(1	Virus Full	Best 1	Best 2	Best 3	Best 4	Best 5
(Intercept)	1.80	1.93	2.14	1.94	1.74	1.93
	(1.21)	(0.46)	(0.46)	(0.47)	(1.21)	(0.47)
varroa	-0.02			-0.02		
	(0.03)			(0.03)		
Nosema_ceranae1	0.91	0.94		0.92	0.93	0.94
	(0.59)	(0.59)		(0.59)	(0.59)	(0.59)
DWV1	0.14				0.21	
	(1.22)				(1.24)	
ABPV1	0.04					0.01
	(0.70)					(0.71)
AIC	194.73	189.00	189.94	190.75	190.97	191.00
BIC	215.44					
Log Likelihood	-91.37	-91.50	-92.97	-91.37	-91.49	-91.50
Num. obs.	233	233	233	233	233	233
Num. groups: Imker	131					
Var: Imker (Intercept)	0.58					
Delta		0.00	0.94	1.75	1.97	2.00
Weight		0.36	0.22	0.15	0.13	0.13

	Chemicals Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6	Best 7	Best 8
(Intercept)	2.11	2.25	2.20	2.32	2.22	2.09	2.28	2.16	2.25
	(0.23)	(0.49)	(0.23)	(0.51)	(0.49)	(0.22)	(0.24)	(0.23)	(0.49)
Acetamiprid1	-1.19			-1.02			-1.20	-1.16	
	(0.93)			(1.02)			(0.99)	(0.93)	
Thiacloprid1	0.54								0.18
	(1.20)								(1.18)
Amitraz_DMF_DMPF1	29.88		28.33			29.47	29.64	29.11	
	(1794950.87)		(2131587.98)			(2144237.48)	(3380424.91)	(1318092.63)	
Boscalid1	-1.65				-1.02	-1.51		-1.56	
	(1.02)				(1.00)	(1.00)		(1.01)	
Tebuconazole1	-2.93	-3.23	-3.16	-3.32	-3.14	-2.93	-3.25	-3.01	-3.23
	(1.34)	(1.70)	(1.45)	(1.73)	(1.66)	(1.35)	(1.47)	(1.36)	(1.70)
AIC	190.90	187.26	188.24	188.32	188.35	188.45	189.00	189.13	189.24
BIC	215.06								
Log Likelihood	-88.45	-90.63	-90.12	-90.16	-90.17	-89.22	-89.50	-88.57	-90.62
Num. obs.	233	233	233	233	233	233	233	233	233
Num. groups: Imker	131								
Var: Imker (Intercept)	0.39								
Delta		0.00	0.97	1.06	1.09	1.18	1.73	1.87	1.97
Weight		0.22	0.14	0.13	0.13	0.12	0.09	0.09	0.08

		Q2 A1 L	U Model	S			
	LU Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6
(Intercept)	4.42**	4.41**	2.14***	4.46**	4.39**	4.46**	4.42**
	(1.59)	(1.55)	(0.46)	(1.56)	(1.54)	(1.57)	(1.55)
opp_mais_1K	0.00			0.00			
	(0.01)			(0.01)			
natuur_J_1K	-0.00				-0.00		
	(0.01)				(0.00)		
gewas_J_1K	-0.00					0.00	
	(0.00)					(0.00)	
nlanduse_3K	-0.17	-0.16		-0.17	-0.16	-0.17	-0.16
	(0.11)	(0.10)		(0.10)	(0.10)	(0.11)	(0.10)
voedsel_J_3K	0.00						-0.00
	(0.00)						(0.00)
AIC	196.60	188.74	189.94	190.64	190.69	190.71	190.73

BIC	220.75						
Log Likelihood	-91.30	-91.37	-92.97	-91.32	-91.34	-91.35	-91.36
Num. obs.	233	233	233	233	233	233	233
Num. groups: Imker.x	131						
Var: Imker.x (Intercept)	0.68						
Delta		0.00	1.20	1.90	1.95	1.96	1.99
Weight		0.33	0.18	0.13	0.12	0.12	0.12
****p < 0.001, **p < 0.01, *p	< 0.05						

									02	Λ1 Βο	llen M	odols										
									Q2	AIPU	ileli ivi	oueis										
	Pollen	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6	Best 7	Best 8	Best 9	Best	Best	Best	Best	Best	Best	Best	Best	Best	Best	Best	Best
	Full	***	***	***	***	***	***	***	***	***	10	11	12	13	14	15	16	17	18	19	20	21
(Intercept)	1.68	1.69	1.90	1.61	1.77	1.81	1.97	1.64	1.62***	1.66***	2.00**	1.64	2.25	1.84	1.87	1.87	1.93	1.86		1.67***		1.70
and allera	(0.89)	(0.44)	(0.45)	(0.44)	(0.44)	(0.46)	(0.46)	(0.43)	(0.45)	(0.43)	(0.70)	(0.44)	(0.71)	(0.46)	(0.44)	(0.44)	(0.46)	(0.45)	(0.46)	(0.45)	(0.44)	(0.46)
nrpollen	-0.10 (0.13)										-0.07		-0.07									
Asteraceae	-0.86				-1.66		-1.57				(0.11)		(0.11)							-1.54		
Asteraceae	(1.69)				(1.61)		(1.60)													(1.58)		
Brassicacea	2.08	1.63		1.62	1.69		(1.00)	1.80	1.64	1.68	1.64	1.68					1.66		1.62	1.67	1.64	1.63
e	2.00	1.03		1.02	1.03			1.00	1.01	1.00	1.01	1.00					1.00		1.02	1.07	1.01	1.03
	(1.36)	(1.21)		(1.20)	(1.23)			(1.25)	(1.20)	(1.21)	(1.24)	(1.21)					(1.24)		(1.21)	(1.22)	(1.22)	(1.21)
Calluna	0.53	,		(- /	(- /			0.59	(- /	,	,	,			0.38		, ,		()	(/	,	
	(0.95)							(0.82)							(0.80)							
Castanea	0.21																					-0.00
	(1.11)																					(1.03)
Cornustyp	3.41									2.43						1.94						
е																						
	(4.64)									(4.27)						(4.22)						
Fabaceae	0.42																				0.11	
	(1.39)																				(1.33)	
Heracleum	8.40			7.14		7.21														6.79		
Immotions	(9.49)	9.15	8.80	(9.08)	10.45	(9.09)	9.85	9.30	8.53	9.40	9.63	0.41	9.29	8.37	8.88	8.96		8.98	9.06	(9.02) 10.44	0.17	9.15
Impatiens	11.02 (11.68	(9.71)	(9.19)	9.23 (9.79)	10.45 (11.25	8.92 (9.28)	(10.49	(9.74)	(9.32)	(9.88)	(10.19	9.41 (9.86)	(9.72)	(8.88)		(9.27)		(9.29)	(9.69)	(11.19	9.17 (9.74)	(9.72)
	(11.00	(9.71)	(9.19)	(9.79)	(11.25	(9.20)	(10.49	(9.74)	(9.52)	(3.00)	(10.19	(9.00)	(9.72)	(0.00)	(9.17)	(9.27)		(9.29)	(9.09)	(11.19	(9.74)	(9.72)
Rosaceae	0.95				1		1				1	0.69						0.54		1		
Hosacuc	(1.32)											(1.22)						(1.22)				
Trifolium	0.17											(/						(2.22)	-0.13			
	(1.12)																		(1.01)			
	(1.12)																		(1.01)			

Zea	8.39								4.76					4.38								
	(9.27)								(8.26)					(8.32)								
AIC	203.14	187.8	188.0	188.7	188.85	188.9	189.15	189.2	189.4	189.4	189.49	189.5	189.6	189.7	189.8	189.8	189.8	189.8	189.8	189.84	189.8	189.8
		5	4	2		3		7	5	8		0	0	2	1	1	1	3	3		4	5
BIC	251.45																					
Log	-87.57	-89.92	-91.02	-89.36	-89.42	-90.46	-90.58	-89.64	-89.73	-89.74	-89.74	-89.75	-90.80	-90.86	-90.90	-90.90	-91.90	-90.92	-89.92	-88.92	-89.92	-89.92
Likelihood																						
Num. obs.	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233
Num.	131																					
groups:																						
Imker.x																						
Var: Imker.x	0.25																					
(Intercept)																						
Delta		0.00	0.19	0.87	1.00	1.08	1.30	1.42	1.60	1.63	1.64	1.65	1.75	1.87	1.96	1.96	1.96	1.98	1.98	1.99	1.99	2.00
Weight		0.10	0.09	0.06	0.06	0.06	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
***p < 0.001, **	p < 0.01, *p	o < 0.05																				

	Q2 A1	. Virus N	/lodels			
	Virus Full	Best 1	Best 2	Best 3	Best 4	Best 5
(Intercept)	1.80	1.93***	2.14***	1.94***	1.74	1.93***
	(1.21)	(0.46)	(0.46)	(0.47)	(1.21)	(0.47)
varroa	-0.02			-0.02		
	(0.03)			(0.03)		
Nosema_ceranae1	0.91	0.94		0.92	0.93	0.94
	(0.59)	(0.59)		(0.59)	(0.59)	(0.59)
DWV1	0.14				0.21	
	(1.22)				(1.24)	
ABPV1	0.04					0.01
	(0.70)					(0.71)
AIC	194.73	189.00	189.94	190.75	190.97	191.00
BIC	215.44					
Log Likelihood	-91.37	-91.50	-92.97	-91.37	-91.49	-91.50
Num. obs.	233	233	233	233	233	233
Num. groups: Imker.x	131					

Var: Imker.x (Intercept) 0.58					
Delta	0.00	0.94	1.75	1.97	2.00
Weight	0.36	0.22	0.15	0.13	0.13
*** p < 0.001, ** p < 0.01, * p < 0.05					

						Q2 A	1 Chem	icals M	odels								
	Chemicals Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6	Best 7	Best 8	Best 9	Best 10	Best 11	Best 12	Best 13	Best 14	Best 15	Best 16
(Intercept)	2.26	2.13	2.16	2.05	2.05	2.29	2.08	2.22***	2.14	1.98	2.29	2.25	2.17	2.35	2.21	2.04	2.23
	(0.52)	(0.53)	(0.50)	(0.51)	(0.80)	(0.52)	(0.48)	(0.48)	(0.46)	(0.21)	(0.50)	(0.51)	(0.49)	(0.55)	(0.52)	(0.78)	(0.50)
Acetamiprid1	-0.70														-0.54		-0.74
	(0.98)														(0.97)		(0.94)
Thiacloprid1	-0.82		-1.06		-0.77		-1.00			-0.72	-1.09	-0.80					-1.06
	(0.69)		(0.62)		(0.62)		(0.61)			(0.61)	(0.68)	(0.69)					(0.63)
Amitraz_DMF_DMPF1	0.76															0.51	
	(1.37)															(1.33)	
Coumaphos1	1.22					1.26					1.18	1.20	0.98	1.28			
	(1.32)					(1.32)					(1.30)	(1.32)	(1.26)	(1.32)			
Boscalid1	-1.14	-1.33		-1.27	-1.01	-1.44				-0.97		-1.12	-1.35		-1.28	-1.38 [*]	
	(0.82)	(0.72)		(0.71)	(0.72)	(0.81)				(0.69)		(0.81)	(0.78)		(0.75)	(0.70)	
Tebuconazole1	-2.20	-1.71	-1.74		-1.65	-2.06		-1.78			-2.06	-2.03		-2.12	-1.78	-1.72	-1.80
	(1.24)	(1.11)	(1.11)		(1.16)	(1.26)		(1.19)			(1.25)	(1.21)		(1.35)	(1.13)	(1.16)	(1.13)
AIC	193.69	188.76	189.20	189.20	189.40	189.58	189.67	189.68	189.94	189.99	190.14	190.34	190.45	190.46	190.46	190.61	190.61
BIC	221.29																
Log Likelihood	-88.84	-90.38	-90.60	-91.60	-89.70	-89.79	-91.84	-91.84	-92.97	-90.99	-90.07	-89.17	-91.23	-91.23	-90.23	-90.30	-90.31
Num. obs.	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233
Num. groups: Imker.x	131																
Var: Imker.x (Intercept)	0.62																
Delta		0.00	0.44	0.44	0.64	0.82	0.91	0.92	1.18	1.23	1.38	1.58	1.69	1.69	1.70	1.85	1.85
Weight		0.11	0.09	0.09	0.08	0.07	0.07	0.07	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.04	0.04
****p < 0.001, **p < 0.01, *	p < 0.05																

								Q2 A	1 Final	overa	ll mod	els (pa	rt 1)									
(Intercept)	M 1 3.21 [*]	M 2 3.70 [*]	M 3 4.09**	M 4 3.62 [*]	M 5 1.54***	M 6 3.80 [*]	M 7 3.99**	M 8 3.24 [*]	M 9 4.04**	M 10 3.71**	M 11 3.64**	M 12 3.80**	M 13 3.45**	M 14 1.73**	M 15 3.49 [*]	M 16 1.43***	M 17 3.13 [*]	M 18 3.67 [*]	M 19 3.64**	M20 1.56***	M 21 3.96**	M 22 1.59**
nlanduse_3K	(1.33) -0.13	(1.48) -0.16	(1.50) -0.18	(1.48) -0.16	(0.43)	(1.48) -0.17	(1.31) -0.18 [*]	(1.46) -0.12	(1.51) -0.16	(1.31) -0.17	(1.30) -0.14	(1.31) -0.15	(0.01) - 0.13**	(0.50)	(1.50) -0.13	(0.26)	(1.30) -0.12	(1.47) -0.14	(1.32) -0.15	(0.44)	(1.50) -0.17	(0.01)
opp_mais_1K	(0.10) 0.00 (0.01)	(0.09)	(0.09)	(0.09)		(0.09)	(0.09)	(0.09)	(0.10)	(0.09)	(0.09)	(0.09)	(0.01)		(0.10)		(0.09)	(0.10)	(0.09)		(0.10)	
Boscalid1 Tebuconazole	-0.73 (0.72) -1.72	-1.90		-1.78	-1.18 (0.69) -2.06			-0.89 (0.72) -1.89	-1.48		-0.87 (0.69)		-	-1.28 (0.69) -1.70	-0.98 (0.72) -1.50	-1.17 (0.67) -1.96	-0.88 (0.70) -1.75	-1.51	-0.87 (0.69)	-2.16	-1.35	-2.08 [*]
1	(1.06)	(1.22)		(1.21)	(1.16)			(1.18)	(1.18)				1.89**	(1.12)	(1.14)	(1.01)	(1.05)	(1.13)		(1.21)	(1.17)	(0.99)
Thiacloprid1	-0.48											-0.73	0.67**					-0.82				0.87**
Brassicaceae	(0.63) 1.58	1.87		1.84	1.72	1.43		1.72		1.41		(0.59)	(0.01) 1.65**			1.71	1.69	(0.61)		1.94		(0.01) 1.65**
Impatiens	(1.21) 8.85	(1.26) 9.39	9.57	(1.25) 9.32	(1.23) 9.18	(1.17) 9.75	9.52	(1.24) 9.14	9.17	(1.13) 9.62	9.30	9.18	(0.01) 9.02**	8.77	8.90	(1.19) 9.38	(1.20) 9.12	8.76	9.35	(1.27) 9.21	9.20	(0.01) 8.95
	(9.33)	(9.41)	(8.95)	(9.34)	(10.21	(9.35)	(8.84)	(9.44)	(8.88)	(9.21)	(9.01)	(8.88)	(0.01)	(9.46)	(8.93)	(10.42)	(9.43)	(8.78)	(9.03)	(10.13	(8.84)	(9.86)
Heracleum	8.84 (9.82)			9.33 (10.00)			9.94 (9.88)			9.92 (9.92)						8.35 (9.30)	9.25 (9.76)		9.95 (9.84)		9.41 (9.94)	
varroa	-0.01 (0.03)																					

													**									**
Nosema_cera	1.06	1.02	0.94	1.07	1.03	0.92	1.00	1.02	1.01	0.99	0.94	0.96	1.03	1.00	1.00	1.08	1.06	1.02	1.01	1.04	1.06	1.03
nae1													*									*
	(0.58)	(0.61)	(0.58)	(0.61)	(0.61)	(0.58)	(0.57)	(0.60)	(0.60)	(0.57)	(0.57)	(0.57)	(0.01)	(0.61)	(0.60)	(0.58)	(0.58)	(0.60)	(0.57)	(0.62)	(0.60)	(0.01)
AIC	190.8	184.6	184.9	185.0	185.1	185.1	185.1	185.2	185.3	185.3	185.4	185.5	185.5	185.5	185.6	185.6	185.6	185.6	185.6	185.6	185.7	185.8
	4	1	4	8	3	5	6	3	0	6	7	2	5	9	1	4	6	6	9	9	6	7
BIC	232.2																					
	5																					
Log Likelihood	-83.42	-85.30	-87.47	-84.54	-85.57	-86.57	-86.58	-84.62	-86.65	-85.68	-86.73	-86.76	-84.78	-86.79	-85.80	-84.82	-83.83	-85.83	-85.84	-86.85	-85.88	-85.94
Num. obs.	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233
Num. groups:	131																					
Imker.x																						
Var: Imker.x	0.00																					
(Intercept)																						
Delta		0.00	0.33	0.47	0.52	0.54	0.55	0.62	0.69	0.75	0.86	0.91	0.94	0.98	1.00	1.03	1.05	1.05	1.08	1.08	1.15	1.26
Weight		0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

										Q2 A	1 Final	overa	ıll mod	dels (p	art 2)										
M 22	M 23	M 24	M 25	M26	M 27	M 28	M 29	M 30	M 31	M 32	M 33	M34	M 35	M 36	M 37	M 38	M 39	M 40	M 41	M 42	M 43	M 44	M 45	M 46	M 47
1.59*	3.80*	3.41*	1.76*	3.40*	3.93*	1.77*	1.63*	4.30*	3.41*	1.58**	1.66*	3.39*	3.59*	4.01*	3.61*	1.44**	3.68*	3.30*	1.58*	1.49**	3.74*	3.56*	3.59*	3.10*	4.08*
(0.01)	(1.32)	(1.30)	(0.46)	(1.31)	(1.49)	(0.23)	(0.23)	(1.51)	(1.32)	(0.27)	(0.22)	(1.46)	(1.46)	(1.49)	(1.48)	(0.44)	(0.42)	(1.30)	(0.23)	(0.25)	(1.50)	(1.31)	(1.32)	(1.45)	(1.32)
	-0.16	-0.14		-0.13	-0.16			-0.18	-0.14			-0.14	-0.15	-0.17	-0.14		- 0.15 [*]	-0.11			-0.16	-0.13	-0.16	-0.11	-0.17
	(0.09)	(0.09)		(0.09)	(0.09)			(0.10)	(0.09)			(0.09)	(0.09)	(0.09)	(0.10)		(0.01)	(0.09)			(0.10)	(0.09)	(0.09)	(0.09)	(0.09)
																					0.00				
																					(0.01)				
		-0.79		-0.97		-1.01	-1.27		-0.79	-0.96	-1.20							-0.79	-1.21	-1.11		-0.71		-0.74	
		(0.69)		(0.70)		(0.69)	(0.66)		(0.69)	(0.70)	(0.66)							(0.71)	(0.66)	(0.66)		(0.71)		(0.72)	
- 2.08 [*]			-1.74	-1.37	-1.67	-1.75	-1.62			-2.04		-1.78			-1.39	-2.04	-1.80	-1.53			-1.88			-1.88	
(0.99)			(1.11)	(1.02)	(1.16)	(0.97)	(0.97)			(1.01)		(1.18)			(1.13)	(1.19)	(1.22)	(1.01)			(1.22)			(1.16)	
- 0.87 [*]	-0.68		-1.03			-0.78				-0.64		-0.61	-0.60		-0.76			-0.67				-0.59	-0.54	-0.52	
(0.01)	(0.59)		(0.60)			(0.61)				(0.62)		(0.62)	(0.60)		(0.60)			(0.61)				(0.61)	(0.60)	(0.63)	
1.65*	,	1.30	,		1.86	,			1.31	1.52		1.63	1.22	1.52		1.92	1.80	,		1.35	1.90	,	1.24	1.55	
(0.01)		(1.14)			(1.26)				(1.14)	(1.18)		(1.24)	(1.16)	(1.19)		(1.25)	(1.25)			(1.14)	(1.27)		(1.14)	(1.23)	
8.95	9.23	9.52	8.48	8.98	8.91	8.80	9.00	9.17	9.50	9.15	9.18	8.99	9.39	9.30	8.84	9.33	9.18	8.79	9.42	9.57	9.35	9.20	9.37	9.01	9.44
(9.86)	(8.88)	(9.39)	(9.20)	(8.94)	(8.90)	(9.73)	(9.68)	(8.71)	(9.42)	(10.3	(9.67)	(9.25)	(9.23)	(9.00)	(8.79)	(10.2	(9.13)	(9.04)	(9.91)	(10.2	(9.35)	(9.13)	(9.23)	(9.48)	(8.78)

										1)						5)				6)					
	9.31			9.26			8.32		9.97			8.64			8.58	8.21			8.83				9.38		
	(9.82)			(9.70)			(9.22)		(9.90)			(9.79)			(9.69)	(9.43)			(9.31)				(9.86)		
																	-								-0.02
																	0.01^{*}								
																	(0.01)								(0.03)
1.03*	1.02	0.93	1.02	1.04		1.04	1.05		1.00	1.04	0.92	1.08	0.94		1.06	1.09	0.99	1.02	0.99	0.91	1.01	0.98	1.01	1.03	0.92
**																									
(0.01)	(0.57)	(0.57)	(0.60)	(0.57)		(0.58)	(0.58)		(0.57)	(0.59)	(0.57)	(0.60)	(0.57)		(0.60)	(0.61)	(0.60)	(0.58)	(0.57)	(0.57)	(0.61)	(0.57)	(0.57)	(0.60)	(0.56)
185.8	185.9	185.9	186.0	186.0	186.0	186.1	186.1	186.1	186.1	186.1	186.1	186.1	186.2	186.2	186.3	186.3	186.4	186.5	186.5	186.5	186.5	186.5	186.5	186.5	186.5
7	3	4	3	4	5	0	2	4	5	5	6	7	0	1	0	0	8	2	3	3	5	7	8	8	9
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85.94	85.97	85.97	87.02	85.02	87.03	86.05	86.06	89.07	85.07	85.08	88.08	84.09	86.10	88.10	85.15	86.15	85.24	85.26	87.26	87.26	85.27	86.28	85.29	84.29	87.30
233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233	233
1.26	1.32	1.34	1.42	1.43	1.44	1.49	1.51	1.53	1.54	1.54	1.56	1.56	1.59	1.60	1.69	1.69	1.87	1.91	1.92	1.92	1.94	1.96	1.97	1.97	1.98
0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01

Appendix D

List of food plants found in stored pollen

Pollen type sorted by pollen most frequently found. Total of 73 different pollen types (of which 2unidentifiable) were found , of which 16 occurred only once.

P ollen type	found in # samples	% of total	max	average when present
Brassicaceae	170	53.3	100	28.4
Trifolium	135	42.3	100	24.3
Asteraceae	102	32.0	100	14.8
Calluna	96	30.1	100	46.4
Castanea	81	25.4	90	35.1
Rosaceae	67	21.0	100	24.0
Fabaceae	54	16.9	90	24.2
Cornus- type	44	13.8	40	11.1
Impatiens	43	13.5	95	31.7
Zea	38	11.9	30	7.2
Heracleum	32	10.0	65	11.6
Persicaria	31	9.7	15	6.5
Caryophylaceae	29	9.1	65	18.4
Amaryllidaceae	28	8.8	100	36.8
Hedera	27	8.5	100	18.5
Phacelia	26	8.2	65	19.8
Verbascum-type	25	7.8	50	9.2
Taraxacum-type	24	7.5	40	10.8
Ranunculaceae	21	6.6	90	23.1
Centaurea	20	6.3	85	21.8
Solanaceae	20	6.3	90	22.3
Tilia	20	6.3	45	9.5
Asparagus	19	6.0	90	42.9
pratensis Trifolium	18	5.6	85	26.9
Ligustrum	16	5.0	90	12.2
Acer	14	4.4	10	6.1
sporen	14	4.4	90	37.5
Hypericum	13	4.1	50	25.8
Lythrum	13	4.1	15	7.3
Geranium	12	3.8	35	8.8
Robinia	12	3.8	55	12.5
Daucus	11	3.4	40	12.3
Cirsium	8	2.5	10	5.6
Lamiaceae	8	2.5	20	10.0
Melilotus	8	2.5	70	36.9

Potentilla	7	2.2	25	10.7
Campanula	6	1.9	35	10.8
Poaceae	6	1.9	40	11.7
Rubus	6	1.9	30	10.0
Clematis	5	1.6	50	28.0
Aesculus	4	1.3	10	6.3
Chenopodiaceae	4	1.3	5	5.0
Symphoricarpus	4	1.3	10	6.3
Campanulaceae	3	0.9	20	13.3
Epilobium	3	0.9	5	5.0
Lathyrus	3	0.9	10	8.3
Pisum	3	0.9	50	36.7
Berberis	2	0.6	40	22.5
Erica	2	0.6	90	52.5
Fagopyrum-type	2	0.6	55	30.0
Ilex	2	0.6	35	22.5
Medicago	2	0.6	10	7.5
Oreganum	2	0.6	10	7.5
Ricinus-type	2	0.6	5	5.0
Skymmia	2	0.6	10	7.5
Vicia	2	0.6	25	15.0
Unknown	2	0.6	65	47.5
Apiaceae	1	0.3	5	5.0
Curcubita	1	0.3	5	5.0
Datura	1	0.3	5	5.0
Filipendula	1	0.3	5	5.0
Fragaria	1	0.3	5	5.0
Gaura	1	0.3	5	5.0
Liliaceae	1	0.3	25	25.0
Linum	1	0.3	15	15.0
Lotus	1	0.3	20	20.0
Mirabilis	1	0.3	5	5.0
Oenoethera	1	0.3	5	5.0
Pastinaca	1	0.3	50	50.0
Rosa	1	0.3	30	30.0
Skimmia	1	0.3	5	5.0
Symphytum	1	0.3	5	5.0
Thymus	1	0.3	5	5.0

Appendix E

List of chemical residues and their detection limits used for screening honey samples

LOQ = Limit Of Quantification in the analytical methods we apply (see also box 1 in main text). This value does not have anything to do with the hazard and safety threshold for any organism. Marked components are found in at least one of the samples, others are not found. Three components that have been tested in the previous two years are also listed here (Cyfluthrin-Beta, Esfenvalerate and Fluvalinate tau) but have not been tested due to costly tests and no relevant detections in the previous years.

Component	LOQ (µg/kg) 2016	Component	LOQ (µg/kg) 2016	Component	LOQ (μg/kg) 2016
4-HSA	5	Etofenprox	1	Omethoate	1
6-Chloronicotinic_acid	10	Famoxadone	5	Paraoxon-methyl	1
Abamectin	10	Fenpropidin	5	Pendimethalin	5
Acetamiprid	0.5	Fenpropimorph	1	Permethrin	5
Aldicarb sulfon	5	Fensulfothion	1	Phorate	1
Azamethifos	1	Fensulfothion-O	1	Phorate sulfon	1
Bendiocarb	1	Fensulfothion-O sulfon	1	Phorate sulfoxide	1
Bifenazate	1	Fensulfothion-sulfon	1	Phorate-O sulfoxide	1
Bifenthrin	1	Fenthion	1	Phosmet	1
Bixafen	1	Fenthion sulfon	1	Phoxim	1
Boscalid	1	Fenthion sulfoxide	1	Pirimiphos-methyl	1
Carbaryl	1	Fenthion-O sulfon	1	Prochloraz	1
Carbendazim	1	Fenthion-O sulfoxide	1	Profenofos	1
Chlorfenvinphos	1	Fipronil	0.5	Propetamphos	1
Chlorpyriphos	1	Fipronil-carboxamide	0.5	Propiconazole	5
Chlorpyriphos-methyl	5	Fipronil-desulfinyl	0.5	Propoxur	1
Clothianidin	2	Fipronil-sulfide	0.5	Prothioconazole-desthio	1
Coumaphos	2	Fipronil-sulfone	0.5	Pyrazophos	1
Cyfluthrin-Beta	-	Fluazifop-P-butyl	1	Pyridaben	1
Cypermethrin	5	Fluopyram	1	Pyridate	1
Cyproconazole	1	Fluquinconazole	1	Rotenone	1
Deltametrin	5	Flusilazole	1	Spinosyn A	1
Deltametrin	1	Fluvalinate tau	-	Spinosyn D	5
Diazinon	1	Haloxyfop-methyl	5	Spiroxamine	1
Dichlorprop	5	Imidacloprid	0.5	Tebuconazole	1
Dichlorvos	5	Imidacloprid_5-Hydroxy	5	Teflubenzuron	1
Disulfoton-sulfone	1	Imidacloprid_desnitro	0.5	Tepraloxydim	5
Disulfoton-sulfoxide	5	Imidacloprid_desnitro_olefin	0.5	Tetrachlorfenvinphos	5
DMA	25	Imidacloprid_olefin	5	Tetraconazole	5
DMF	5	Imidacloprid_urea	0.5	Thiabendazole	1
DMPF	5	Indoxacarb	2	Thiabendazole, 5-OH	5
Edifenphos	1	Ioxynil	1	Thiacloprid	1

Emamectin	2	Malathion	1	Thiamethoxam	2
Epoxiconazole	1	Metaflumizone	1	Thiophanate-methyl	1
Esfenvalerate	-	Metazachlor	1	Triazophos	1
Ethiofencarb	1	Methidathion	5	Triflumizole	1
Ethiofencarb sulfon	1	Methomyl	1		
Ethiofencarb sulfoxide	1	Novaluron	5		