Report Honeybee Surveillance Program the Netherlands

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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, but have been conducted differently. A random sample of 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 good-size stratified random sample of bee colonies across the Netherlands.

The duration of the program will be four years. This is needed to obtain a longer-term view of both winter mortality and the underlying causing factors; and can take into account the substantial inter-annual variation. The past winter (2015-2016) was, for example, very mild, which may have led to lower mortality rates.

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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 gepresenteerde resultaten van de Wintersterfte Monitor zijn robuust en representatief, maar de monitoring is anders uitgevoerd dan vorig jaar. Deze uitvoering is gebaseerd op een a-selecte steekproef van 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 goed aantal imkers heeft meegedaan.

De duur van het programma is vier jaar ook omdat op die manier wel een robuuste analyse gemaakt kan worden van de sterftepatronen en hun factoren, waarbij variatie tussen jaren meegenomen kan worden. Afgelopen winter (2015-2016) was bijvoorbeeld een bijzonder milde winter en dat kan hebben bijgedragen aan de lage sterfte.

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1. Summary of 2015/2016 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. This is done by means of the Honeybee Mortality Monitor, a random beekeepers' survey and the Honeybee Surveillance Study, a stratified random field survey among Dutch beekeepers. This report concerns the second year, 2015-2106.

2 **National-level mortality**. The Honeybee Mortality Monitor reveals that winter mortality was very low in 2015-2016 (6.5%), which is the lowest since large-scale monitoring started in 2004. This may be a result from the mild autumn and winter weather and increase in adequate *Varroa* control, but this cannot be inferred from a one-year correlative survey. The number of managed honeybee colonies in the Netherlands is estimated to be at least 86,000.

3 **Apiary-level mortality**: Hive survival was very high all-round and no single factor explains the proportion of survival or mortality at apiary level. However, beekeepers with more hives and performing better *Varroa* control had slightly higher apiary-level survival in the 2015-2016 winter. Survival was slightly lower in more heterogeneous landscapes and those with less maize cultivation.

4 **Hive-level mortality:** Looking at individual colonies, survival chances decreased in highly diverse landscapes, when substantial amount of clover pollen was stored and when deformed wing virus (DWV) was detected. *Varroa* mite infestation levels were much lower than in the previous year and were not contributing to mortality. However, DWV is transmitted by *Varroa* and indicates previous infections. Neonicotinoids and other chemical residues did not have any significant relation with colony winter mortality in our study. The acaricide/insecticide Dimethoate was occasionally found in honey (in only 9 of 318 samples) and its presence led to slightly lower survival in a few cases. Dimethoate is used commonly in private gardens and allotments for mite and aphid control as well as in glasshouse flower production. More study is needed to interpret this finding.

5 **Summarizing**: Hive survival was very high all-round (93.5% 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 2015. Larger bee operations tend to have slightly higher hive survival than smaller operations.
Pests and diseases: *Varroa* infection levels before winter were half that of 2014 and did not have a direct effect on hive survival. However, the presence of the DWV virus (deformed wing virus), which is transmitted by *Varroa* mites, led to slightly lower survival. DWV was omnipresent in hives. This indicates that *Varroa* and the viruses it transmits are an important factor in colony mortality.
Chemical residues: Of the twenty-four chemical compounds and their metabolites we screened for, including all neonicotinoids, nine were detected in stored in winter food (honey, sugar, syrup etc.) in autumn. Three of these substances are used by beekeepers for *Varroa*-control. Presence

of chemical residues was not related to colony mortality (only a link with Dimethoate presence was found in a few cases, see point 4 above, which needs more in depth analysis.).
Pollen sources: Hives with abundant clover pollen stored in bee bread had slightly lower survival than other hives. Clovers are well-known to be a preferred food source of bees. This effect is still unexplained and needs more research. It is most likely not linked to the clover pollen per se, but to other conditions of the landscape surrounding these hives, e.g. scarcity of food sources).
Landscape conditions: Highly diverse, fragmented, landscapes led to a slight 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.

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 vragenlijst die wordt gestuurd naar een aselecte steekproef van imkers. Ten tweede de Honingbijen Surveillance studie. Hierin worden van een gestratificeerde steekproef van de Nederlandse bijenhouders in het veld bijenvolken bemonsterd voor nadere analyse in het laboratorium. Dit rapport geeft de resultaten weer van het tweede seizoen, 2015-2016.

2 **Bijensterfte in Nederland**. De Wintersterfte monitor laat zien dat de bijensterfte in Nederland in 2015-2016 zeer laag was (6.5%). Dit is het laagste sterftecijfer sinds grootschalige monitoring is begonnen in 2004. Dit zou het gevolg kunnen zijn van het milde weer in najaar en winter en een adequatere *Varroa*-bestrijding door imkers, maar dit kan niet met zekerheid uit een eenjarige survey gehaald worden. Op basis van de monitor kunnen we een schatting maken van het aantal bijenvolken in Nederland. Dit is tenminste 86 duizend volken.

3 **Sterfte per bijenstand.** Overleving was overal zeer hoog afgelopen winter en de sterfte van volken lijkt niet het gevolg van één enkele oorzaak. Een aantal factoren hadden wel invloed op de het percentage sterfte in bijenstanden: overleving was iets hoger voor imkers met meer volken en minder *Varroa* infectie in de bijenstand. Overleving was lager in landschappen die zeer divers zijn en waar meer maisteelt is in de omgeving.

4 **Sterfte per bijenvolk.** De kans op overleving van een bijenvolk (vergeleken met andere volken in dezelfde bijenstand) was lager in landschappen die zeer divers zijn en als er meer klaverstuifmeel opgeslagen was in het bijenbrood en als er DWV (deformed wing virus), een door *Varroa*-mijten verspreid bijenvirus, werd aangetroffen. Het niveau van *Varroa*-infectie zelf had dit jaar geen invloed, waarschijnlijk door de algeheel lage infectieniveaus. Neonicotinoiden en andere chemische residuen hadden geen relatie met sterfte van de bijenvolken. Het acaricide/insecticide bestrijdingsmiddel dimethoate werd af en toe gevonden in opgeslagen honing (in 9 van de 318 monsters), maar had, in deze paar gevallen wel een klein negatief effect op de overleving van het volk. Dimethoate wordt gebruikt door particulieren in (moes)tuinen voor luizen en mijtenbestrijding en tevens in de bedekte bloementeelt, maar dan alleen als er geen bijen in de kas rondvliegen. Om deze bevinding te kunnen duiden moeten we meer in detail kijken naar individuele gevallen.

5 Samenvattend kunnen we zeggen: Honingbijvolk overleving was zeer hoog afgelopen winter (93.5%) en de sterfte van volken lijkt niet het gevolg van één enkele oorzaak. De invloed van de vijf hoofdfactoren die we onderzocht hebben kan als volgt worden samengevat [NB: net als in vergelijkbare studies betekent het niet vinden van een significant effect niet per sé dat die factor helemaal geen effect heeft]:
De imker: Bijenvolken beter overleven als de *Varroa*-infectie laag is, en dat was het geval in de meeste bijenstanden die we bemonsterd hebben in 2015. Imkers met meer volken lijken een iets hogere overleving van volken te hebben.
Ziekten en plagen: Het niveau van *Varroa*-infectie voor inwintering was veel lager dan in 2014 en had geen direct effect of de overleving van het volk. Echter, volken waarin het virus DWV (deformed wing virus) aanwezig was hadden een licht kleinere overlevingskans. Dit virus wordt overgebracht door de Varroamijt en is dus een indirecte aanwijzing voor *Varroa* infectie. Het virus is gevonden in de meeste volken. De resultaten geven aan dat goede bestrijding van *Varroa* een belangrijke voorwaarde is voor de overleving van honingbijenvolken.

Chemische middelen: Van de 24 chemische stoffen (en hun metabolieten) waarvan we de aanwezigheid in opgeslagen honing voor inwinteren hebben geanalyseerd, werden er negen aangetroffen in tenminste één monster. Drie van deze negen stoffen worden door imkers gebruikt voor mijtbestrijding. Aanwezigheid van chemische residuen in de opgeslagen honing had geen effect op overleving (behalve wanneer dimethoate gevonden werd; dat was in enkele gevallen, zie punt 4 hierboven, en voor interpretatie is meer gedetailleerd onderzoek nodig).
Stuifmeelbronnen: Volken waarin het opgeslagen stuifmeel voor een groot deel bestond uit klaverstuifmeel hadden een iets lagere overleving dan de andere volken. Klaversoorten zijn zeer goede voedselbronnen voor bijen en klaverbloemen worden zeer veel bezocht. Deze bevinden behoeft derhalve meer onderzoek. Het is vrijwel onmogelijk dat het klaverstuifmeel zelf een negatief effect heeft. Het is waarschijnlijker dat andere aspecten van de landschappen waarin volken afhankelijk zijn van vooral klaver verantwoordelijk zijn voor deze bevinding. Het zou bijvoorbeeld kunnen dat de hoeveelheid voedsel in die landschappen niet toereikend is of dat de plaatsen waar de klaver groeit anderszins nadelige effecten opleveren voor honingbijen.
Het foerageerlandschap: Het landschap om de bijenstanden is van belang voor de overleving. Zeer diverse landschappen met versplinterd landgebruik had een licht negatief effect op de overleving van het volk. De meeste Nederlandse landschappen waarin bijenstanden staan hebben een zeer gevarieerd landgebruik (gemiddelde meer dan negen verschillende categorieën landgebruik binnen een straal van 1km). In landschappen die extreem diverse, en dus zeer gefragmenteerd, zijn bleken de honingbijen het iets minder goed te doen dan in andere landschappen.

2 Introduction to the surveillance program

The Netherlands Honeybee surveillance Program has been initiated as a result of the public debate hosted by the 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 Amsterdam, 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 (ALTERRA), 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:

Determine the health status of honeybees in the Netherlands: estimate colony winter loss and map drivers that correlate with winter loss, including exposure to agro-chemicals, bee diseases, food availability, landscape configuration and beekeeping practice (Figure 1).

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

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. First initiative is the ANSES protocol recently used in the Epilobee project, in which the Netherlands was not a partner. Note that this project only addresses disease and beekeeping practice as risk factors. Second initiative is the CoLoSS colony loss questionnaires that estimate winter mortality in many countries and use beekeeper responses to assess potential risk factors. Our program includes field assessments of disease and completely incorporates the CoLoSS beekeeper survey. It is therefore, informative for both initiatives, but addresses more factors than both of them together. 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. A recent workshop organized by our consortium in march 2016 in Graz Austria, as part of Super-B, confirmed that our approach is state-of-the-art and compatible with initiatives in Germany, Austria, Italy, France, the USA, to a lesser extent to the UK and to the EU reference laboratory activities across Europe.

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 workshop 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 beekeeping industry in the Netherlands about the importance of adequate honeybee management practices. In addition, it should provide valuable information on the causes of honeybee colony losses in the Netherlands.

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 mortality monitor), the second approach is a field campaign actually sampling and analyzing different factors directly (honeybee surveillance study).

The Honeybee Mortality Monitor is an annual survey, the structure of which was different in 2016, but conducted in such a way that the results are comparable to the previous year results.
The new method of monitoring the winter mortality 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 for 2016 (note that the 2015 monitor was conducted by NCB as part of our program). It was decided to conduct an integrated survey together with the Netherlands Beekeeping Association (NBV) and Bijen@wur. These organizations have been conducting a simple mortality survey in the past few years to be able to obtain an indication of honeybee mortality early in the season. The new honeybee mortality monitor is based on surveys conducted by CoLoSS (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). The results of this collaboration is that there is now a single mortality survey instead of two separate ones last year.

We conducted the survey as follows: To obtain a reliable estimate of honeybee winter mortality in the Netherlands we randomly selected 500 beekeepers from beekeeper membership lists (>8000 beekeepers in total). Since not all members possess bees and many beekeepers did not respond to our request, we continued to approach beekeepers till we reached 500 beekeepers. This survey was conducted in two different ways: digitally, by sending a survey created in Google forms to selected beekeepers. Those selected beekeepers for whom we did not have an e-mail address or that had not responded to the e-mail, were called directly. The same questions were asked in both questionnaires. Given that the new method is based on a random sample of Dutch beekeepers, the number of 500 beekeepers is sufficient to provide a sound estimate. The voluntary survey conducted by NCB tend to use responses from a higher number of beekeepers. It also given a good estimate, because the larger number of responses removes the potential bias due to the non-randomness of these responses.

The Honeybee Surveillance Study is set-up specifically for this program and consists of a stratified random sampling of hives in apiaries from around the Netherlands. Hives are surveyed for disease, beekeepers are interviewed and samples (of bees, honey and pollen) are taken for further analysis. In this way we can assess the influence of the beekeeper (interviews and field survey), diseases (field and 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). Bee health inspectors that were trained by Bijen@wur staff conduct the field survey and collect samples each year in May and August from 5 hives from each of 200 apiaries. Only a subset of the samples, up to 400 per year, will be analyzed (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 and disease analyses; ALTERRA 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, the contacts with the ministry and the co-financer and reporting.

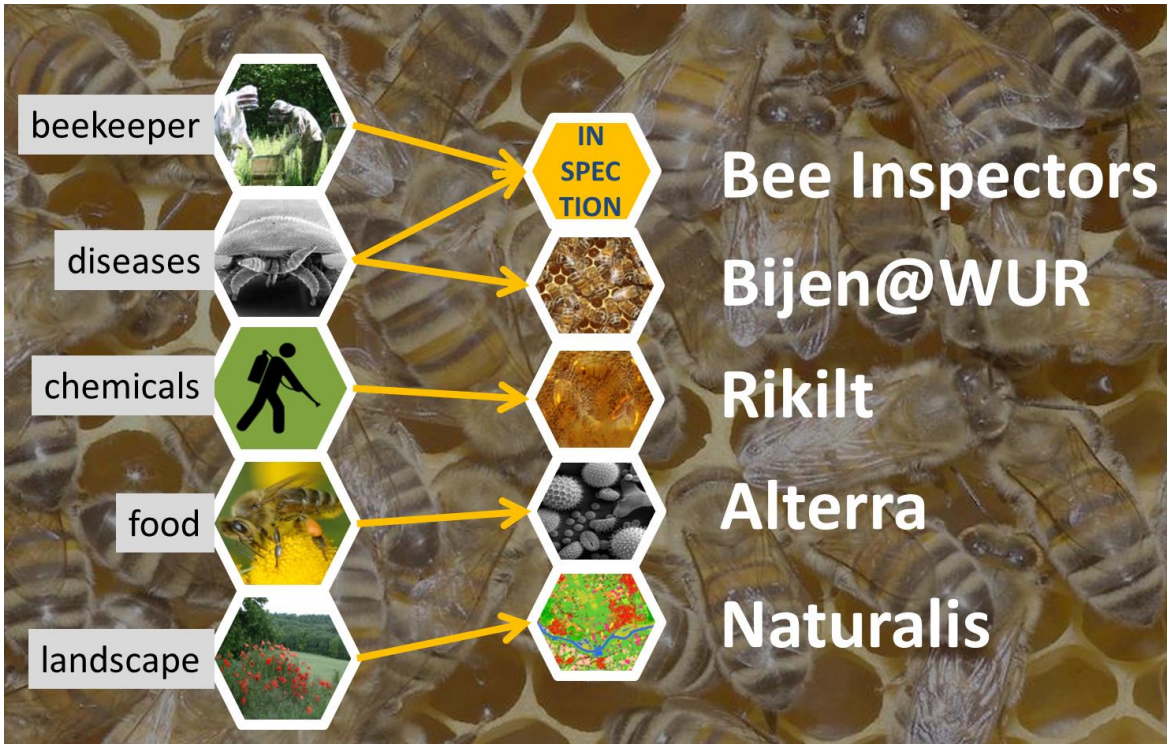


Figure 2. The possible causes of honeybee winter mortality are being assessed based on field sampling of hives, bees, honey and pollen.

3 Results and conclusions

3.1 Honeybee Mortality Monitor 2015-2016

3.1.1. Results from Honeybee Mortality Monitor 2015-2016

Honeybee hive mortality in the 2015-2016 winter was 6.5%. Of the 580 beekeepers that responded 46 did not have any bee hives currently. The remaining 534 beekeepers had 5919 bee hives going into winter (late autumn 2015) of which 5537 hives survived the winter, i.e. were still alive in April 2016. The 6.5% mortality was the lowest mortality level of the last 10 years (Table 3). Because this survey is primarily aimed at obtaining the winter mortality figure, we cannot detect the causes for the low mortality. The beekeepers' association, NBV, stated that the high survival is likely a result of the increase in adequate, well-timed *Varroa*-control combined with the good autumn weather and very mild winter. While this is the most obvious explanation, this cannot be supported from the 2015-2016 data alone.

Table 3. Winter mortality figure 2005-2016.

Winter	Number of beekeepers	Number of hives (October)	% winter mortality[1]	Method
2005-2006	737	7.050	26.3	NBC [CoLoSS]
2006-2007	1422	13.591	15.9	NBC [CoLoSS]
2007-2008	808	9.616	23.7	NBC [CoLoSS]
2008-2009	1193	10.678	21.7	NBC [CoLoSS]
2009-2010	1326	11.265	29.1	NBC [CoLoSS]
2010-2011	1541	13.726	21.4	NBC [CoLoSS]
2011-2012	1673	14.915	20.8	NBC [CoLoSS]
2012-2013	1589	13.920	13.7	NBC [CoLoSS]
2013-2014	1594	15.280	8.6	NBC [CoLoSS]
2014-2015	1549	14.650	13.7	HB-Surv [CoLoSS] [1]
2015-2016	**580**	**5919**	**6.5**	HB-Surv [CoLoSS] [1]

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

Table 4. Procedure to estimate the number of bee hives in the Netherlands in 2015. For explanation see text below. 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	580
2	Total number of hives going into winter	5919
3	Average number of hives per beekeeper	10,2
4	Number of beekeepers on NBV list	7350
5	Number of beekeepers member of ABTB according to their website	700
6	Number of beekeepers on ANI list	360
7	Total number of beekeeper members	8410
8	Number of hives in associations (beekeepers * average hives per beekeeper)	**85826**
10	Estimated percentage of beekeepers member of one of the three associations	Estimated total number of hives
11	95%	90343
12	90%	95362
13	85%	100971
14	80%	107282
15	75%	114434
16	70%	122608

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 4 (superscripts in text below refer to the lines in the table).

Data on the number of hives going into winter 2015-2016 were received from 580 beekeepers[1]. In total these beekeepers had 5919[2] hives in late autumn 2015, while 46 beekeepers had no hives at all. The average number of hives was 10 across all beekeepers[3] with a few large beekeepers and many with fewer hives. A total of 8410 beekeepers[7] is registered with one of the three beekeeping associations[4-6]. The total number of hives of these beekeepers is about 85826 (beekeepers * 10.2 hives on average)[8].
The question that remains for estimating total bee hives in the Netherlands is the percentage of registration of all Dutch beekeepers. This is not known and it is also not known to us how this has been estimated earlier. Therefore we calculated the population of Dutch bee hives for degrees of registration between 65 and 95%[11-16]. The estimate increases from 85 thousand at complete registration to 130 thousand at 65% registration.

In conclusion: there were at least 85000 managed bee hives in the Netherlands in late Autumn 2015. This is certainly an underestimate due to incomplete registration. The best estimate may be the one including 15% unregistered beekeepers making the bee hive total for the Netherlands about 100,000.

3.2 Honeybee Surveillance Study 2015-2016

3.2.1. Set-up of the field campaign

The field campaign is based on a stratified random selection of beekeepers (apiaries) from across the Netherlands (see appendix B for details). The selected beekeepers are visited by Bee Health Inspectors, that have been trained in all skills necessary by Bijen@wur staff. These visits take place in May and August each year. 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 analyze, and store all samples for future analysis (e.g. available for follow-up projects).
Bee Health Inspectors were trained at Bijen@wur to conduct the interviews and follow the standard sampling protocols. This guarantees a high standard of bee, honey and pollen samples and comparable assessments of the health of colonies in the field. Protocols follow recommended procedures (e.g. from CoLoSS bee book) and best practices for the Netherlands beekeeping situation (based on Bijen@wur experience).

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 from the 5 hives samples per apiary was selected for analysis in Autumn 2015. Samples were sent to Bijen@wur (pathogen and disease analysis based on bee sample), Alterra (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 2016, beekeepers were contacted to obtain information on survival of each of their hives.
3- The second sample for analysis is 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 randomly select a second hive for analysis.
4- The samples of the second hive for each apiary are distributed to the partners for analysis in May 2016. After that all data have been integrated and analyzed by Naturalis.
5- Reporting will occur every year in late June/early July.

3.2.3. Results from Honeybee Surveillance Study 2015

Here we first summarize the main finding of the integrated analysis and after that provide a short summary of the separate findings per possible driver of mortality.

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 bee hive (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 5). 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 5. Factors used in surveillance analysis for questions Q1, 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)	NO	YES
Presence of ABPV	Presence of ABPV virus in honeybees (YES/NO)	NO	YES
Presence of *Nosema apis*	Presence of the microsporidian *Nosema apis* in honeybees (YES/NO)	NO	YES
Presence of *Nosema ceranae*	Presence of the microsporidian *Nosema ceranae* in honeybees (YES/NO)	NO	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), *Calluna* (heather), *Trifolium* (clover)	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 5. A total of 105 apiaries could be included in this analysis.

Result: Most factors did not explain the percentage of hive survival in apiaries and little variation could be explained by the factors taken into account. The best model, the one explaining most variance after model selection, included the percentage *Varroa* mites, the number of land use classes and the extent of Maize area within 1km of the apiary, and the size of the beekeeping

operation ('hives into winter'). All factors only had marginal effect on the survival and in different direction (table 5).
The maximum percentage *Varroa* found in the apiary was negatively related to survival [more *Varroa* leads to slightly lower survival]. In addition, survival is lower in more heterogeneous landscapes [more diverse landscape leads to lower survival]. Apiaries in landscapes with more maize show slightly higher survival as do larger beekeeping operations [beekeepers with more hives going into winter show slightly higher survival].

Q1 LU Models

	LU Full	Best 1	Best 2	Best 3	Best 4	Best 5
(Intercept)	3.52***	3.46***	3.10***	3.52***	2.97***	3.46***
	(0.46)	(0.44)	(0.39)	(0.46)	(0.38)	(0.44)
LUclasses1k	-0.14***	-0.12***	-0.08*	-0.14*	-0.08*	-0.12***
	(0.05)	(0.04)	(0.03)	(0.05)	(0.03)	(0.04)
Natu_Perc1k	0.00					0.00
	(0.01)					(0.01)
Crop_Perc1k	0.00			0.00		
	(0.01)			(0.01)		
Mais_Perc1k	0.04***	0.05***	0.05***	0.04***	0.05***	0.05***
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
VarroaPerc_Max	-0.02*	-0.02*	-0.01	-0.02*		-0.02*
	(0.01)	(0.01)	(0.01)	(0.01)		(0.01)
Hives IN	0.0004	0.0004		0.0004		0.0004
	(0.00)	(0.00)		(0.00)		(0.00)
AIC	400.20	396.58	397.74	398.36	398.40	398.45
BIC	418.78					
Log Likelihood	-193.10	-193.29	-194.87	-193.18	-196.20	-193.22
Deviance	264.23					
Num. obs.	105	105	105	105	105	105
Delta		0.00	1.16	1.78	1.82	1.87
Weight		0.36	0.20	0.15	0.15	0.14

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

Table 6. 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 best model 1, whereas models 2-5 are close to be the best, i.e. within 2 AIC points. 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, and when higher *Varroa* infection levels are observed in the apiary. Larger beekeeping operation leads to slightly better survival as does the area of maize around the apiary.

Conclusion: No single factor explains survival/mortality at apiary level. However, beekeepers with more hives and performing better *Varroa* control had higher hive survival in the 2015-2016 winter. The landscape in which the bees forage also has an impact on survival with more heterogeneous landscapes and less maize 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. The finding that maize has a slight positive effect on colony survival is relevant. Maize is a good source of pollen for bees, however it has been suggested that the presence of neonicotinoid chemicals may cause a hazard. In the Netherlands only about 10% of the maize fields was treated with neonicotinoids before the European moratorium, which should have gone down to 0% since the ruling. Note that the main challenge is to find the actual relationships. For example, landscapes with more maize occur on sandy soils, where there may be better conditions for beekeeping in general, but not specifically linked to maize cultivation. The project will accumulate sufficient data over the four years to analyze these results in great detail.

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 3). 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.

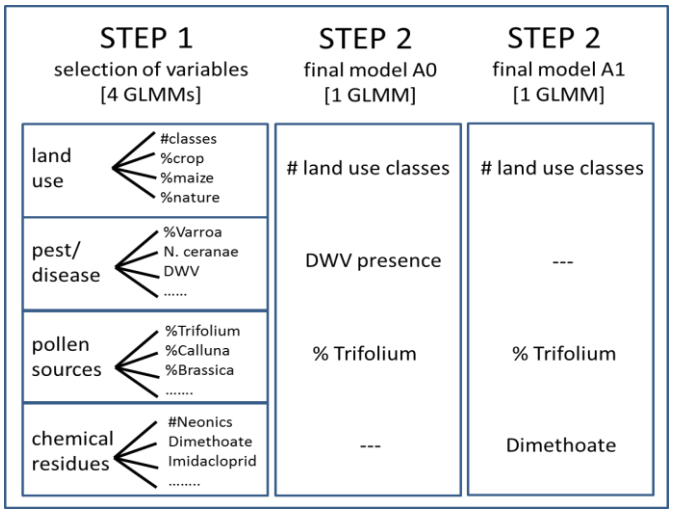
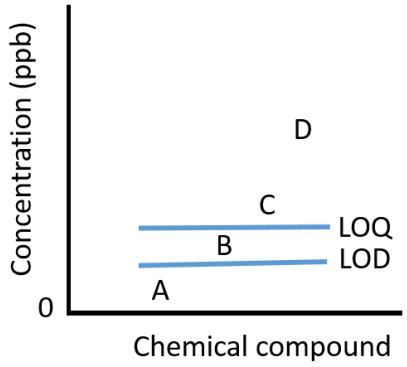


Figure 3. Schematic overview of analysis for question 2. STEP 1 selects the main variables within each of the four variable categories (boxes on the left; for details on all variables that were included see appendix C) using GLMM models. STEP 2 uses the variables selected in STEP 1 (indicated in the 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.

Box 1. Presence of chemical residues in honey is detected using the more accurate methods currently available. Yet exact quantities can only be given above the level of quantification (LOQ). Below that there is a small range of concentrations where a substance can be detected (i.e. is above the level of detection, LOD) but its quantity can not be assessed accurately (i.e. it is below the LOQ for that substance). Note that the LOD and LOQ are specific for each compound and is given in appendix E. LOD and LOQ are methodological thresholds and do not have any meaning for hazard and safety of the compound for animals.



This procedure is the same as was performed last year. We have added one analysis to be able to give a complete overview of the data. This is to take 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.

Result: Two factors consistently appear in the models assessing causes of colony survival, namely the number of land use classes in the surrounding environment (LUclasses1K) and the presence of clover pollen in the stored bee bread (*Trifolium*). Both of these factors have a slight negative effect on colony survival. In the 'conservative' analysis using the LOQ as the threshold for the presence of

chemical compounds (the normal procedure), no chemical compounds showed any significant relation with survival (table 7; figure 4). Not even the newly calculated summary variable 'neonicotinoids' (indicating a presence of at least one neonicotinoid in a sample). When using the LOD as the threshold (Q2 A1 best model; table 8; figure 4) Dimethoate is included. This compound was found only 9 times in the 327 samples, however its presence was linked to a slight decrease in colony survival.
Of the bee pests and diseases only deformed wing virus (DWV) has a significant (negative) effect on colony survival, but only in the Q2 A0 2nd best model (table 7; figure 4). Unlike last year, the percentage *Varroa* did not have a significant negative effect on colony survival. This may be due to the overall much lower *Varroa* infestation (3% average compared to 7% last year; see table 9). None of the other factors were included in the final models after the two step model selection. In other words they did not contribute to explain colony survival or mortality.

Conclusion: Only a small part of the mortality of bee hives could be explained by the main factors that were analyzed. This may be partly due to the fact that mortality was very low this year, only 6.5% of colonies died. This indicates that conditions, including beekeeping practices and weather, were favorable for wintering bees and that even weaker colonies survived.
Mortality was slightly higher in very complex landscapes, for colonies depending on large amounts of clover pollen and for colonies in which deformed wing virus (DWV) was present. The latter virus is transmitted by *Varroa* mites and thus indicates (previous) *Varroa* infection. DWV was present in most hives even in many hives in which *Varroa* mites were not found. The level of *Varroa* infestation per sé was less than half that of the 2014-2015 winter (now only 3%), probably indicating that better control measures were applied by more beekeepers.

Q2 A0 Final overall models

	Final Full	**Final Best 1**	**Final Best 2**
(Intercept)	7.58**	5.98***	7.58**
	(2.48)	(1.74)	(2.48)
LUclasses1k	-0.28	-0.27*	-0.28
	(0.15)	(0.14)	(0.15)
Trifolium_ALL	-2.96*	-2.92*	-2.96*
	(1.40)	(1.31)	(1.40)
DWV1	-1.46		-1.46
	(1.35)		(1.35)
AIC	169.78	169.25	169.78
BIC	186.91		
Log Likelihood	-79.89	-80.63	-79.89
Num. obs.	227	227	227
Num. groups: Imker	85		
Var: Imker (Intercept)	3.90		
Delta		0.00	0.53
Weight		0.57	0.43

*** p < 0.001, ** p < 0.01, * p < 0.05

Table 7. Factors related to the survival of individual bee hives. Values indicate the estimates from the model with standard deviation in parentheses. The final model is best model 1, whereas model 2 is close to be the best, i.e. within 2 AIC points. The full model is the one with all variables included, after which variables are deleted until the best model is found.
Colony survival decreases in very diverse landscapes (LUclasses1k), when clover pollen is collected (*Trifolium*) and when deformed wing virus (DWV) is present (only second best model). In these models (Q2A0) chemical residues were scored as present when occurring above the level of quantification (LOQ).

Q2 A1 Final overall models

	Final Full	**Final Best 1**	**Final Best 2**	**Final Best 3**
(Intercept)	5.03***	5.03**	3.19***	5.98***
	(1.52)	(1.52)	(0.65)	(1.74)
# Land use classes	-0.19	-0.19		-0.27*
	(0.13)	(0.13)		(0.14)
Dimethoate	-2.08	-2.08	-2.56*	
	(1.08)	(1.08)	(1.05)	
Trifolium	-2.70*	-2.70*	-2.76*	-2.92*
	(1.17)	(1.17)	(1.17)	(1.31)
AIC	167.81	167.81	168.39	169.25
BIC	184.94			
Log Likelihood	-78.91	-78.91	-80.19	-80.63
Num. obs.	227	227	227	227
Num. groups: beekeeper	85			
Var: beekeeper (Intercept)	2.14			
Delta		0.00	0.58	1.44
Weight		0.45	0.33	0.22

*** p < 0.001, ** p < 0.01, * p < 0.05

Table 8. Results of GLMM models similar to Table 7 above. Only difference is that these models (Q2A1) chemical residues were scored as present when occurring above the level of detection (LOD), which is lower than the LOQ, but not quantifiable. Colony survival decreases in very diverse landscapes (LUclasses1k), when clover pollen is collected (*Trifolium*) and when Dimethoate is present (this chemical was found in just 9 samples).

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 2015-2016 and neither did they show a significant relation to loss in the previous winter. Residues of neonicotinoids are found in the samples, but not systematically in those of colonies that died. Scientific evidence (e.g. Rundlof et al. 2015) suggests that the impact of neonicotinoids (and possibly other chemicals) on honeybee colony survival is much lower than on survival of solitary bees and bumblebees. This may be a result of the highly social life-style where everything is diluted among the thousands of worker bees and where the death of a few thousand bees will not necessarily lead to colony demise. When also the samples in which minute amounts of compounds were found (above LOD, but below LOQ; see Box 1) were considered, presence of the organophosphate acaricide-insecticide Dimethoate was linked to a slight increase in colony mortality. This compound is present in several aphid and mite control products for sale in garden centers for private use as well as in some control products used in glasshouse flower production. It is well known to be harmful to bees and can therefore not be used in professional activities when bees are foraging. The compound was found at very low quantities and only in a few samples. More analysis is needed to understand what the conditions are in which this has been found. It could be linked to private use in garden or allotment, to glass/covered flower production or possibly even to erroneous beekeeper mite control practice. Presence of none of the other chemical compounds that were found in the winter food honey stored in the colonies was linked to colony mortality in the winter.
As in the previous year the presence of clover pollen is linked to colony mortality. This is unlikely related to these food sources directly, as clover is an excellent bee/food with high concentration of amino acids. It may indicate a lack of pollen variety in those hives or may reflect other, yet unexplained, impact of those landscapes. For example, it seems that the high amounts of clover pollen in bee brood at least partly occur in highly intensive agriculture/grassland areas (see Figure 5).

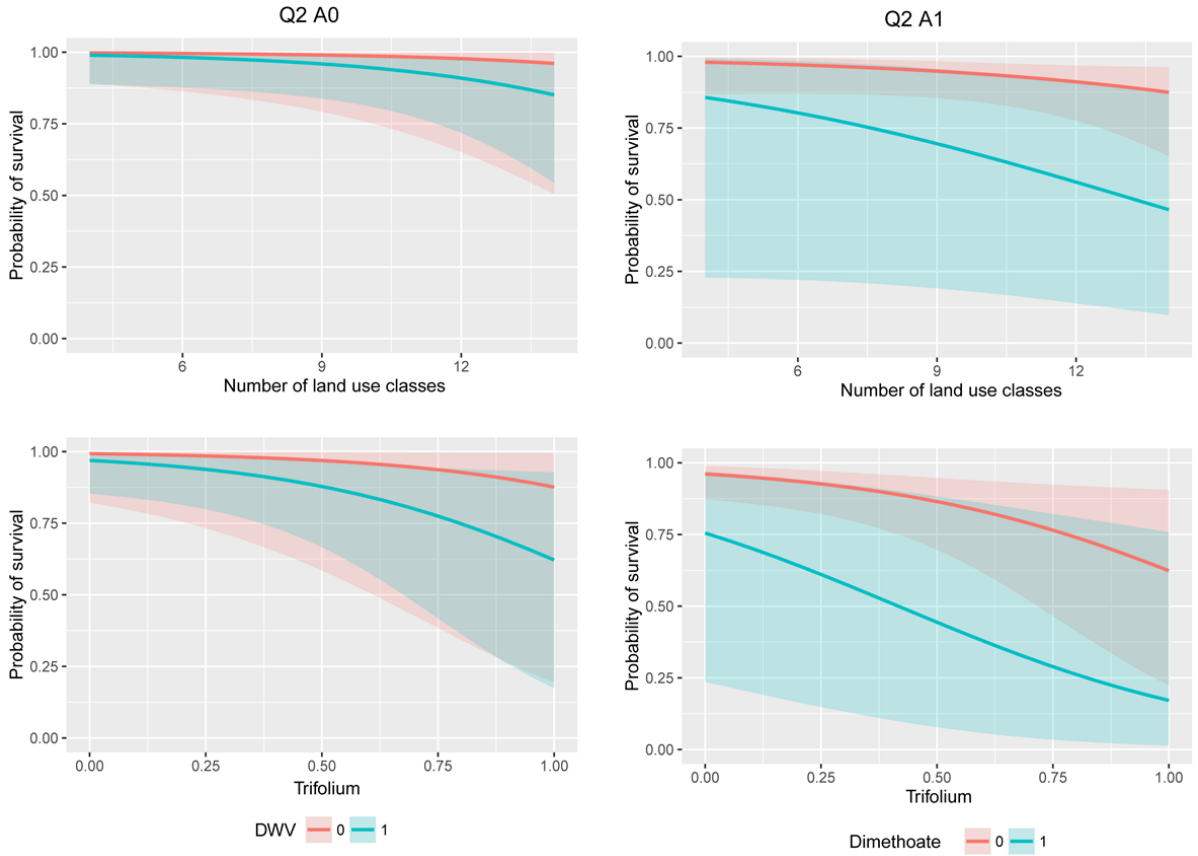


Figure 4. Graphic representation of the influence of the main effects on colony survival for models using LOQ as threshold for chemical presence (left panels; Q2 A0) and using LOD as threshold for chemical presence (right

panels). Upper left panel: influence of land use diversity on colony survival in absence (red line) and presence (blue) of DWV virus for Q2 A0 models; Lower left panel: influence of clover (*Trifolium* spp) pollen amount on colony survival in absence (red line) and presence (blue) of DWV virus for Q2 A0 models; Upper right panel: influence of land use diversity on colony survival in absence (red line) and presence (blue) of Dimethoate for Q2 A1 models; lower right panel: influence of clover (*Trifolium* spp) pollen amount on colony survival in absence (red line) and presence (blue) of Dimethoate for Q2 A1 models. Coloured areas give 95% confidence intervals for each line of corresponding colour. Note that confidence intervals overlap considerable which indicates the relatively small contribution of DWV presence (left panels) and Dimethoate presence (right panels) on colony survival.

Summary of results of single factors: pathogens, residues, pollen sources and landscape.

Parasites and pathogens: Several diseases, pathogens and parasites were found in the bee samples (table 9). Some occur very frequently in hives, *Varroa*-mites, the microsporidian *Nosema cerana* and the *Varroa*-transmitted virus DWV (Deformed Wing Virus), whereas others occur very infrequently, the microsporidian *Nosema apis*, and the ABPV (Acute Bee Paralyses Virus) virus, and in the same pattern as in 2014. The level of *Varroa* infection decreased from 7 mites per 100 bees to 3 mites per 100 bees. In only 21 hives *Varroa*-infestation was 10% or more, whereas 124 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 in those hives or still be present at very low numbers as DWV is largely transmitted by *Varroa*-mites.

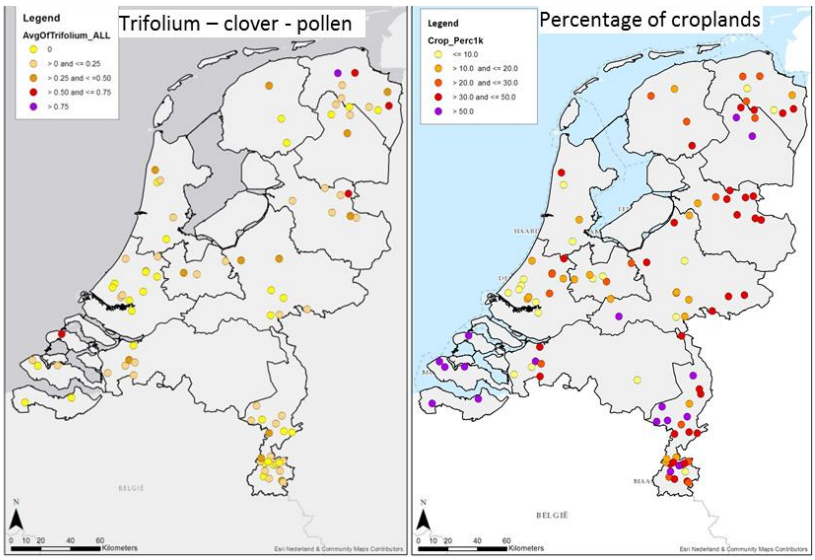


Figure 5. Presence of hives per apiary with substantial amount of *Trifolium* pollen (various clover species) in bee brood (left) and the percentage of cropland within 1km of the apiary. The highest occurrence of *Trifolium* pollen tends to be in intensive agriculture areas. *Trifolium* pollen was found to have a negative impact on colony survival.

Table 9. Presence of various pests and diseases in bee samples 2015 and 2014.
The number of samples analyzed was 331 in 2015 up from 91 in 2014.

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

Pollen sources used by hives: A total of 65 different pollen types were found to be present in pollen stored in bee hives in August 2015, i.e. those represented by 5% or more in a single sample, in pollen samples (for main sources see table 10; full list in appendix D). On average 3.8 (sd 1.2) different types were present in a sample (ranging from 1-7 pollen 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.

Table 10. Main pollen sources and their percentages in hives in late 2015 (for complete list see Appendix D).

Pollen type	**found in # samples**	**% of samples**	**Max % in sample**	**Average % In sample**
Brassicaceae (mustards, rapeseed)	127	49.80	100	42.36
Trifolium (clovers)	120	47.06	100	29.59
Lotus (birds foot trefoil)	68	26.67	85	25.44
Rosaceae (rose family)	59	23.14	95	25.42
Calluna (heather)	55	21.57	100	32.82
Asteraceae (dandelion family)	52	20.39	35	10.10
Hedera (ivy)	43	16.86	100	46.98
Caryophyllaceae (ragged robin family)	39	15.29	60	12.18
*Fagopyrum*_type (buckwheat type)	37	14.51	95	21.76
Phacelia (Phacelia)	31	12.16	80	25.81
Rubus (Bramble)	29	11.37	45	18.28

Note that table 10 indicates pollen sources found in more than 10% of the samples. Several pollen sources not on this list, can contribute a large share of the pollen of a single hive. Most notably among these are Himalayan balsam (*Impatiens glandulifera*; 8.6% of samples but making up 34% of sample if found and sometimes 100%), Lilies (Liliaceae; in 18 samples, but making up 46% of pollen grain on average and sometimes 100%), Asparagus and Verbena (both occur in just 3 samples, but makeup >60% of pollen grains in sample when found). If a hive's pollen is limited to cultivated sources, such as asparagus and lilies, it could potentially be exposed to agro-chemicals used in production fields.

Chemical residues detected in honey: Honey samples 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). Only few of these residues were encountered in any honey samples and mostly at low frequency (see table 11). Honey samples in 186 hives (56.9%) did not contain any of the chemical residues we screened for at a level above our LOQ (Level of Quantification). Neonicotinoids (imidacloprid, thiacloprid or acetamiprid, thiamethoxam) were found in 49 hives (15.0%). Acaricides (amitraz, coumaphos, tua fluvalinate) were found in 17 hives (5.2%). While the presence of acaricides in 2015 is slightly lower than in 2014, the presence of neconicotinoids in honey samples has almost doubled compare to 2014 (see table 11). Particularly thiacloprid was present much more than in 2014, while imidacloprid was found much less.

While our threshold for quantification (LOQ) is very low for all compounds, we can also detect the presence of compounds below that (LOD) (see table 12; box 1). [Note that in all cases, however, observed levels are well below the regulatory risk and safety levels for chemical residues.] Particularly the acaricides coumaphos and amitraz and the neonicotinoids acetamiprid and thiacloprid were detected to be present in many hives at very low quantities below quantification threshold. These very low values may indicate application of *Varroa* control measures using these products earlier in the season (acaricides). In the case of thiacloprid and acetamiprid it may indicate

the broad presence of these compounds in the landscapes where bee hives occur and/or its presence in the winter food (e.g. corn-derived sugar) which beekeepers introduce into the hive. 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. Only two samples (from different hives from a single beekeeper) recorded a value above the honeybee LD50 and both for imidacloprid.

Table 12. Chemical residues present above LOQ level in samples of 2014 (90 hives) and 2015 (327 hives). 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 in table 11 and appendix E). Note that the percentages for total neonicotinoid and acaricide presence do not simple constitute the sum of compound presences, because multiple compounds may be found in a single hive.

Chemical residue	2014	2015
Acetamiprid	2.2	2.8
Amitraz	8.9	2.1
Coumaphos	1.1	2.4
Dimethoate	0	0.9
Fluvalinate-tau	0	0.9
Imidacloprid	6.7	2.8
Permethrin	0	0.3
Thiacloprid	2.2	9.8
Thiamethoxam/Clothianidin	0	0.9
Neonicotinoids total	7.7	15.0
Acaricides total	7.7	5.2

Table 11. Chemical residues encountered in 327 honey samples. Groups according to acaricides used by beekeepers for Varroa control, Neonicotinoid pesticides, other chemicals.

Compound [LOQ in µg/kg]	**number of samples in which absent**	**samples in which detected but <LOQ**	**samples in which detected and >LOQ**	**average concentration (µg/kg) if present**	**Maximum concentration (µg/kg) if present**	**LD50 in µg per bee in 48h tests from USDA EcoTox database**
Acaricides for Varroa control						
Amitraz[1]^ [80]	303	17	7	274.3	660.0	100
Coumaphos^ [2]	279	40	8	3.1	5.3	
Fluvalinate_tau^ [2]	320	4	3	4.6	7.1	0.20
Neonicotinoid						
Acetamiprid* [0.5]	300	18	9	1.06	3.8	8.1
Imidacloprid[4]* [0.5]	318	0	9	2	7.0	0.0038
Thiacloprid* [1]	204	91	32	4.6	22.9	17.94
Thiamethoxam[5]* [2]	320	7	3			0.035
Other chemicals						
Abamectin	327	0	0			0.408
Bendiocarb	327	0	0			0.4280
Bifenazate	327	0	0			7.93
Chlorpyriphos	327	0	0			0.114

Cyfluthrin-Beta	327	0	0			0.037
Cypermethrin	327	0	0			0.023
Deltametrin	327	0	0			0.0015
Dimethoate[2] [1]	318	6	3	17	37.4	0.056
Emamectin	327	0	0			0.0035
Esfenvalerate	327	0	0			0.0172
Fipronil[3]	327	0	0			0.0040
Indoxacarb	327	0	0			0.18
Permethrin [0.5]	326	0	1	2.7	2.7	0.024
Propiconazole	327	0	0			25
Pyridaben	327	0	0			1.81
Triflumizole	327	0	0			160

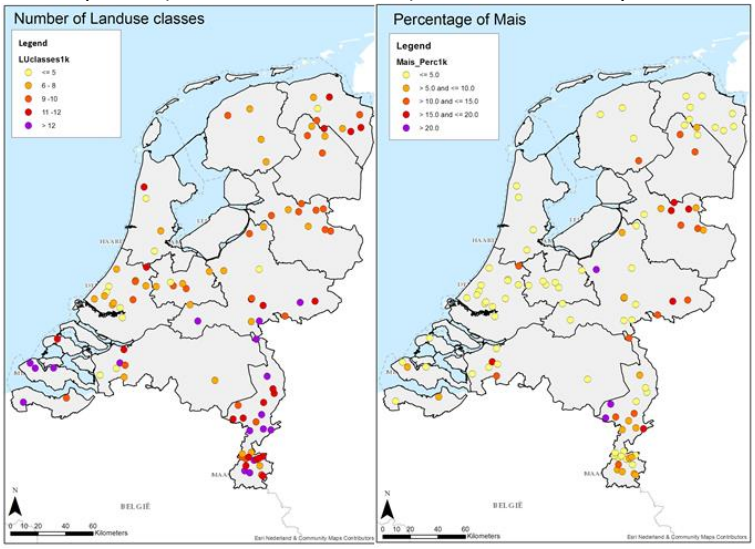
Neonicotinoid and their products are indicated with a *, Acaricide used by beekeepers with a ^. LOQ=Level of Quantification. Samples are scored are 'absent' (column 2; indicating nothing was found), 'detected but <LOQ' (column 3; indicating very small quantity detected, but not sufficient to quantify 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 100milligrams. Values thus have to be multiplied by 1000 to be comparable to the detected concentrations in the previous columns.

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 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). Different land use and habitat factors have been collated and created (from other data sources) so that the role of landscape factors in hive mortality can be assessed. Data are available on crops and groups of crops grown on each parcel and for each year (2015 data from BRP: basis registratie percelen). Detailed land use data are available from CBS land use database for 2010. 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 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 13). The intensity of these factors is spread across the country (figure 6). Only the factor number of land use classes was a significant contributor to colony survival with highly diverse landscapes being less favorable to bees.

Table 13. Summary of occurrence of important landscape parameters around apiaries (within 1km).

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

Figure 6. Landscape diversity (i.e. number of land use classes) and the percentage maize cultivation in areas around the apiaries (i.e. within 1 km radius) varies considerably across sites.



3.3. Other results and planning

Honeybee winter mortality monitor: The decision of the Netherlands bee research center (NCB) not to join the consortium in 2016 was unfortunate, but has been countered by installing very efficient alternative procedures for obtaining winter mortality figures, in collaboration with the Netherlands Beekeeping Association, NBV.
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. This collaboration will be continued in the coming years and will deliver the official figure for annual honeybee winter mortality (for more details on the new approach and the comparability with previous years see section 2.2.

Honeybee Surveillance: To get to a response of the 200 apiaries we aim at in this study, we need to approach almost 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 stratified random selection of apiaries.
Several procedures have been automated by Naturalis for 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 will be organized differently in 2016. While in 2014 and 2015 the Bee Health Coordinators (BHC) were trained to collect all samples, this did not result in high enough sample sizes and appeared to be very complex in the field. This is mainly because both beekeepers and BHCs mostly have jobs and activities besides their beekeeping activities. Selected beekeepers are now asked directly to collaborate, they are sent the sampling materials and full

instructions. Materials are sent back to Bijen@wur for further processing. The spring sampling has been successful and we have already samples from more apiaries than in the previous year.

Planning for 2016/2017

Month	Action	Status*
2016		
March	Selection beekeepers 2016	complete
April	Winter Mortality Monitor	complete
	Retrieval survival/mortality results hives sampled in 2015	complete
May/June	1st round of apiary inspections 2016	complete
June	Analysis 2nd round of samples 2015	complete
July	Report 2015-2016 submitted to funders	Draft report completed, discussed in August
August/September	2nd round of apiary inspections 2016	In progress
September	Samples stored and 1st set of samples distributed for analysis	
October/December	Laboratory analysis of 1st round of samples	
2017		
April	Winter Mortality Monitor 2016	
	Retrieval survival/mortality results hives sampled 2016	
May	1st round of apiary inspections 2017	
June	Analysis 2nd round of samples 2016	
July	Report year 3 (2016-2017) submitted to funders	

4. Conclusions

1. The Honeybee Mortality Monitor reveals that winter mortality was very low in 2015-2016 (6.5%), which is the lowest since large-scale monitoring started in 2004. This may be a result of the mild autumn and winter weather and/or of an increase in adequacy of *Varroa* control, but this cannot be inferred from a one-year correlative survey.

2. The number of managed honeybee colonies in the Netherlands is estimated to be at least 86,000 and may be as high as 100,000 depending on the number of unregistered beekeepers.

3. Apiary-level mortality: Hive survival was very high all-round and no single factor explains the proportion of survival or mortality at apiary level. However, beekeepers with more hives and performing better *Varroa* control had slightly higher apiary-level survival in the 2015-2016 winter. Survival was slightly lower in more heterogeneous landscapes and those with less maize cultivation.

4. Hive-level mortality: Looking at individual colonies, survival chances decreased in highly diverse landscapes, when substantial amount of clover pollen was stored and when deformed wing virus (DWV) was detected. *Varroa* mite infestation levels were much lower than in the previous year and were not contributing to mortality. However, DWV is transmitted by *Varroa* and indicates previous infections. Neonicotinoids and other chemical residues did not have any significant relation with colony winter mortality in our study. The acaricide/insecticide Dimethoate was occasionally found in honey (in only 9 of 318 samples) and its presence led to slightly lower survival in a few cases.

Dimethoate is used commonly in private gardens and allotments for mite and aphid control as well as in glasshouse flower production. More study is needed to interpret this finding.

5. Summarizing: Hive survival was very high all-round (93.5% 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 2015. Larger bee operations tend to have slightly higher hive survival than smaller operations.

Pests and diseases: Varroa infection levels before winter were half that of 2014 and did not have a direct effect on hive survival. However, the presence of the DWV virus (deformed wing virus), which is transmitted by *Varroa* mites, led to slightly lower survival. DWV was omnipresent in hives. This indicates that *Varroa* and the viruses it transmits are an important factor in colony mortality.

Chemical residues: Of the twenty-four chemical compounds and their metabolites we screened for, including all neonicotinoids, nine were detected in stored in winter food (honey, sugar, syrup etc.) in autumn. Three of these substances are used by beekeepers for Varroa-control. Presence of chemical residues was not related to colony mortality (only a link with Dimethoate presence was found in a few cases, see point 4 above, which needs more in depth analysis.).

Pollen sources: Hives with abundant clover pollen stored in bee bread had slightly lower survival than other hives. Clovers are well-known to be a preferred food source of bees. This effect is still unexplained and needs more research. It is most likely not linked to the clover pollen per se, but to other conditions of the landscape surrounding these hives, e.g. scarcity of food sources).

Landscape conditions: Highly diverse, fragmented, landscapes led to a slight 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.

5 Appendices

A Winter mortality survey based on CoLoSS questionnaire

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

Algemene enquête wintersterfte Surveillance programma

Voor het honingbijen surveillance programma van Bijen@WUR, Naturalis en Alterra onderzoeken wij de wintersterfte van bijenvolken in Nederland. Met deze (zeer) korte vragenlijst vragen naar de wintersterfte van uw bijenvolken. Mogelijk heeft u in 'bijenhouden' of via de nieuwsbrief van PRI bijen@wur hier al meer over vernomen.

*Required

Imker gegevens

Wij vragen u om uw naam, adres en e-mail adres om u te kunnen bereiken.

1. **Naam imker**

2. **Adres imker**

3. **E-mail adres imker** *

In- en uitwinteringscijfers

4. **Hoeveel volken heeft u ingewinterd in 2015?** *

5. **Hoeveel volken heeft u uitgewinterd in 2016?** *

6. **Op hoeveel ramen heeft u ingewinterd?** *

   Mark only one oval.

   - ◯ <5
   - ◯ 5-10
   - ◯ 10-15
   - ◯ 15-20

Varroabestrijding

De behandeling van varroa kan van belang zijn voor het overwinteren van uw bijen, daarom vragen wij u hoe uw bijen tegen varroa behandeld zijn. Bij al deze vragen zijn meerdere antwoorden mogelijk.

7. **Welke manier(en) van varroabestrijding past u toe?** *
Tick all that apply.

- [ ] Geen varroabestrijding
- [ ] Voorjaar darrenbroed verwijderen
- [ ] Vóór zomerdracht (combinatie van zwermvermindering en oxaalzuur)
- [ ] Na de zomerdracht
- [ ] Winterbehandeling

8. **Indien u na de zomerdracht behandeld heeft, welke middelen heeft u gebruikt?**
Tick all that apply.

- [ ] Mierenzuur behandeling (Liebig / Nassenheider / anders)
- [ ] Thymovar
- [ ] Apistol
- [ ] Apistan
- [ ] Amitraz
- [ ] Apivar
- [ ] Thymol
- [ ] Api Life Var
- [ ] Api guard
- [ ] Other: .....

9. **Indien u winterbehandeling(en) heeft toegepast, welke methoden heeft u gebruikt?**
Tick all that apply.

- [ ] Oxaalzuur (druppelmethode)
- [ ] Oxaalzuur (verdampingsmethode)
- [ ] Other: .....

Najaarsdracht

De wintervoorraad wordt voor een belangrijk deel bepaald door de najaarsdracht, deze is dan ook van belang voor de overwintering van uw volken

10. **welke najaarsdracht hebben uw volken bezocht?**
Mark only one oval.

- ◯ Geen
- ◯ Heide
- ◯ Balsemien
- ◯ Other: .....

Herkomst Koningin

11. **Wat is de herkomst van uw koningin(nen)**
Tick all that apply.

- [ ] Uit eigen teelt, op eigen stand bevrucht
- [ ] Uit eigen teelt, op een andere stand bevrucht
- [ ] Gekocht
- [ ] Other: .....

Herkomst Koningin (II)

12. **Indien u uw koningin gekocht heeft, waar komt deze vandaan?**

.....

13. **Wat is het ras van de door u gekochte koningin?**

.....

Wilt u meer weten over het surveillance programma?

Via de onderstaande link kunt u meer informatie vinden over het Surveillance programma.

http://www.wageningenur.nl/nl/Expertises-Dienstverlening/Onderzoeksinstituten/plant-research-international/Over-Plant-Research-International/Organisatie/Biointeracties-Plantgezondheid/Bijen/Surveillanceprogramma-Honingbijen.htm

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Appendix C Overview of results from GLMM analyses surveillance study

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

Q2 A0 Land use Models

	LU Full	**Best 1**	**Best 2**	**Best 3**	**Best 4**
(Intercept)	5.19***	5.16***	5.16***	5.30***	5.12***
	(1.51)	(1.47)	(1.47)	(1.52)	(1.46)
Natu_Perc1k	0.01				0.01
	(0.03)				(0.03)
Mais_Perc1k	0.04		0.05		
	(0.07)		(0.06)		
Crop_Perc1k	0.00			0.01	
	(0.02)			(0.02)	
LUclasses1k	-0.30	-0.26*	-0.29*	-0.31	-0.26*
	(0.16)	(0.12)	(0.13)	(0.16)	(0.13)
AIC	178.86	173.70	174.92	175.43	175.63
BIC	199.41				
Log Likelihood	-83.43	-83.85	-83.46	-83.72	-83.82
Num. obs.	227	227	227	227	227
Num. groups: Imker	85				
Var: Imker (Intercept)	2.49				
Delta		0.00	1.22	1.73	1.93
Weight		0.43	0.23	0.18	0.16

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A0 Pollen Models

	Pollen Full	**Best 1**	**Best 2**	**Best 3**	**Best 4**	**Best 5**	**Best 6**
(Intercept)	4.05***	3.54***	3.75***	3.64***	3.47***	3.66**	3.51***
	(1.20)	(0.93)	(1.02)	(0.89)	(0.95)	(1.12)	(0.95)
PollenTypes	-0.05					-0.03	
	(0.14)					(0.14)	
Brassicaceae	-0.99			-0.84			
	(1.02)			(0.93)			
Calluna	-1.68		-1.50				
	(1.69)		(1.64)				
Lotus	-0.16						0.26
	(1.98)						(1.89)
Rosaceae	0.80				1.28		
	(2.08)				(2.02)		
Trifolium_ALL	-3.60*	-3.18*	-3.41*	-3.36*	-3.15*	-3.17*	-3.17*
	(1.51)	(1.43)	(1.50)	(1.42)	(1.44)	(1.44)	(1.42)
AIC	179.55	171.70	172.88	172.93	173.25	173.66	173.68
BIC	206.95						
Log Likelihood	-81.77	-82.85	-82.44	-82.46	-82.62	-82.83	-82.84
Num. obs.	227	227	227	227	227	227	227
Num. groups: Imker	85						
Var: Imker (Intercept)	3.93						
Delta		0.00	1.18	1.23	1.55	1.96	1.98
Weight		0.30	0.17	0.16	0.14	0.11	0.11

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A0 Chemicals Models

	Chemicals Full	**Best 1**	**Best 2**	**Best 3**	**Best 4**	**Best 5**	**Best 6**
(Intercept)	2.53***	2.77***	2.70***	2.66***	2.57***	2.74***	2.77***
	(0.33)	(0.63)	(0.33)	(0.32)	(0.31)	(0.64)	(0.63)
Neonics1	0.68						0.08
	(1.70)						(0.78)
Amitraz1	24.22			22.37	51.82		
	(217276.14)			(116486.22)	(27397079.00)		
Coumaphos1	46.17		18.57		24.27		
	(25364766.42)		(14172.24)		(234955.18)		
NumberChemicals	-0.49					0.29	
	(1.25)					(0.58)	
AIC	181.61	176.57	176.94	177.48	177.78	178.30	178.56
BIC	202.16						
Log Likelihood	-84.81	-86.29	-85.47	-85.74	-84.89	-86.15	-86.28
Num. obs.	227	227	227	227	227	227	227
Num. groups: Imker	85						
Var: Imker (Intercept)	2.44						
Delta		0.00	0.36	0.91	1.20	1.73	1.99
Weight		0.26	0.22	0.17	0.14	0.11	0.10

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A0 Virus Models

	Virus Full	**Best 1**	**Best 2**	**Best 3**	**Best 4**	**Best 5**	**Best 6**	**Best 7**	**Best 8**
(Intercept)	4.23**	4.51**	2.77***	4.29*	2.46***	4.23**	4.49**	2.75***	2.41***
	(1.62)	(1.62)	(0.63)	(1.70)	(0.67)	(1.62)	(1.56)	(0.55)	(0.59)
NosemaCeranae1	0.72			0.67	0.63	0.72			0.68
	(0.51)			(0.52)	(0.50)	(0.51)			(0.49)
DWV1	-1.74	-1.66		-1.75		-1.74	-1.66		
	(1.34)	(1.31)		(1.36)		(1.34)	(1.30)		
VarroaPercent_najaar_2015	-0.05					-0.05	-0.04	-0.04	-0.04
	(0.04)					(0.04)	(0.04)	(0.04)	(0.04)
AIC	177.35	176.46	176.57	176.69	176.91	177.35	177.42	177.58	177.60
BIC	194.47								
Log Likelihood	-83.68	-85.23	-86.29	-84.35	-85.46	-83.68	-84.71	-85.79	-84.80
Num. obs.	227	227	227	227	227	227	227	227	227
Num. groups: Imker	85								
Var: Imker (Intercept)	3.33								
Delta		0.00	0.12	0.24	0.46	0.89	0.97	1.13	1.14
Weight		0.17	0.16	0.15	0.13	0.11	0.10	0.09	0.09

***p < 0.001, **p < 0.01, *p < 0.05

STEP 1 for Q2 A1 models (analysis at hive level, residues considered present when above LOD)

Q2 A1 Land Use Models

	LU Full	Best 1	Best 2	Best 3	Best 4
(Intercept)	5.19***	5.16***	5.16***	5.30***	5.12***
	(1.51)	(1.47)	(1.47)	(1.52)	(1.46)
Natu_Perc1k	0.01				0.01
	(0.03)				(0.03)
Mais_Perc1k	0.04		0.05		
	(0.07)		(0.06)		
Crop_Perc1k	0.00			0.01	
	(0.02)			(0.02)	
LUclasses1k	-0.30	-0.26*	-0.29*	-0.31	-0.26*
	(0.16)	(0.12)	(0.13)	(0.16)	(0.13)
AIC	178.86	173.70	174.92	175.43	175.63
BIC	199.41				
Log Likelihood	-83.43	-83.85	-83.46	-83.72	-83.82
Num. obs.	227	227	227	227	227
Num. groups: Imker	85				
Var: Imker (Intercept)	2.49				
Delta		0.00	1.22	1.73	1.93
Weight		0.43	0.23	0.18	0.16

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A1 Pollen Models

	Pollen Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6
(Intercept)	4.05***	3.54***	3.75***	3.64***	3.47***	3.66**	3.51***
	(1.20)	(0.93)	(1.02)	(0.89)	(0.95)	(1.12)	(0.95)
PollenTypes	-0.05					-0.03	
	(0.14)					(0.14)	
Brassicaceae	-0.99			-0.84			
	(1.02)			(0.93)			
Calluna	-1.68		-1.50				
	(1.69)		(1.64)				
Lotus	-0.16						0.26
	(1.98)						(1.89)
Rosaceae	0.80				1.28		
	(2.08)				(2.02)		
Trifolium_ALL	-3.60*	-3.18*	-3.41*	-3.36*	-3.15*	-3.17*	-3.17*
	(1.51)	(1.43)	(1.50)	(1.42)	(1.44)	(1.44)	(1.42)
AIC	179.55	171.70	172.88	172.93	173.25	173.66	173.68
BIC	206.95						
Log Likelihood	-81.77	-82.85	-82.44	-82.46	-82.62	-82.83	-82.84
Num. obs.	227	227	227	227	227	227	227
Num. groups: Imker	85						
Var: Imker (Intercept)	3.93						
Delta		0.00	1.18	1.23	1.55	1.96	1.98
Weight		0.30	0.17	0.16	0.14	0.11	0.11

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A1 Chemicals Models

	Full	Best 1	Best 2	Best 3	Best 4	Best 5	Best 6	Best 7	Best 8
(Intercept)	2.53***	2.62***	2.52***	2.56***	2.46***	2.70***	2.68***	2.72***	2.67***

	(0.50)	(0.49)	(0.48)	(0.48)	(0.47)	(0.54)	(0.50)	(0.54)	(0.50)
Acetamiprid	0.67								-0.40
	(1.43)								(0.77)
Amitraz	3.64		1.52		1.54	2.27		2.06	
	(2.23)		(1.36)		(1.35)	(1.49)		(1.49)	
Coumaphos	1.30						-0.41		
	(1.57)						(0.63)		
Dimethoate	-0.80	-2.44*	-2.59*	-2.39*	-2.54*	-1.90	-2.41*	-2.10	-2.44*
	(1.78)	(1.01)	(1.02)	(1.00)	(1.01)	(1.14)	(1.01)	(1.14)	(1.02)
Fluvalinate_tau	1.13								
	(2.17)								
Thiamethoxam	6.13			30.04	23.29	21.58			
/clothianidin	(9.46)			(1024.00)	(512.00)	(1024.00)			
Neonics	1.48								
	(1.53)								
NumberChemicals	-1.60					-0.38		-0.29	
	(1.44)					(0.33)		(0.32)	
AIC	181.37	173.21	173.59	173.60	173.90	174.59	174.79	174.80	174.94
BIC	215.62								
Log Likelihood	-80.68	-83.60	-82.79	-82.80	-81.95	-81.30	-83.39	-82.40	-83.47
Num. obs.	227	227	227	227	227	227	227	227	227
Num. groups: Imker	85								
Var: Imker (Intercept)	1.37								
Delta		0.00	0.38	0.39	0.69	1.38	1.58	1.59	1.73
Weight		0.14	0.12	0.12	0.10	0.07	0.06	0.06	0.06

***p < 0.001, **p < 0.01, *p < 0.05

Q2 A1 Virus Models

	Full	**Best 1**	**Best 2**	**Best 3**	**Best 4**	**Best 5**	**Best 6**	**Best 7**	**Best 8**
(Intercept)	4.23**	4.51**	2.77***	4.29*	2.46***	4.23**	4.49**	2.75***	2.41***
	(1.62)	(1.62)	(0.63)	(1.70)	(0.67)	(1.62)	(1.56)	(0.55)	(0.59)
NosemaCeranae1	0.72			0.67	0.63	0.72			0.68
	(0.51)			(0.52)	(0.50)	(0.51)			(0.49)
DWV1	-1.74	-1.66		-1.75		-1.74	-1.66		
	(1.34)	(1.31)		(1.36)		(1.34)	(1.30)		
VarroaPercent 2015	-0.05					-0.05	-0.04	-0.04	-0.04
	(0.04)					(0.04)	(0.04)	(0.04)	(0.04)
AIC	177.35	176.46	176.57	176.69	176.91	177.35	177.42	177.58	177.60
BIC	194.47								
Log Likelihood	-83.68	-85.23	-86.29	-84.35	-85.46	-83.68	-84.71	-85.79	-84.80
Num. obs.	227	227	227	227	227	227	227	227	227
Num. groups: Imker	85								
Var: Imker (Intercept)	3.33								
Delta		0.00	0.12	0.24	0.46	0.89	0.97	1.13	1.14
Weight		0.17	0.16	0.15	0.13	0.11	0.10	0.09	0.09

***p < 0.001, **p < 0.01, *p < 0.05

Appendix D List of food plants found in stored pollen

Pollen type sorted by pollen most frequently found. Total of 65 different pollen types, of which 22 occur only once. And 31 less than 5 times.

Pollen type	found in # samples	% of samples	Max %	Average % when present
Brassicaceae	127	49,80	100	42,36
Trifolium_ALL	120	47,06	100	29,59
Lotus	68	26,67	85	25,44
Rosaceae	59	23,14	95	25,42
Calluna	55	21,57	100	32,82
Asteraceae	52	20,39	35	10,10
Hedera	43	16,86	100	46,98
Caryophyllaceae	39	15,29	60	12,18
Fagopyrum_type	37	14,51	95	21,76
Phacelia	31	12,16	80	25,81
Rubus	29	11,37	45	18,28
Ranunculaceae	23	9,02	75	16,09
Impatiens	22	8,63	100	33,64
Lamiaceae	22	8,63	45	13,18
Zea	22	8,63	70	8,18
Taraxacum_type	19	7,45	40	10,00
Liliaceae	18	7,06	100	46,39
Hypericum_type	15	5,88	70	27,00
Cirsium	13	5,10	15	5,81
Ligustrum	11	4,31	25	7,73
Rhamnus_type	11	4,31	95	21,36
Lythrum	10	3,92	45	13,00
Heracleum	9	3,53	10	6,67
Chenopodiaceae	8	3,14	40	14,38
Melilotus	8	3,14	30	17,50
Aesculus	6	2,35	50	14,17
Campanulaceae	6	2,35	30	9,17
Cornus	6	2,35	30	10,00
Fabaceae	6	2,35	35	17,50
Lysimachia_type	6	2,35	55	27,50
Medicago	6	2,35	80	45,00
Skimmia	6	2,35	35	12,50
Centaurea	5	1,96	5	5,00
Poaceae	5	1,96	70	19,00
Potentilla	4	1,57	70	33,75
Scrophulariaceae	4	1,57	15	11,25
Vicia	4	1,57	10	6,25
Asparagus	3	1,18	95	61,67
Lamium	3	1,18	45	31,67
Verbena	3	1,18	95	60,00

Pollen type	found in # samples	% of samples	Max %	Average % when present
Viburnum	3	1,18	45	23,33
Centaurea_cyanus	2	0,78	20	12,50
Daucus	2	0,78	5	5,00
Asparagus_	1	0,39	20	20,00
Asteraceaee	1	0,39	5	5,00
Atropa_type	1	0,39	5	5,00
Berberis	1	0,39	5	5,00
Centaurea_	1	0,39	5	5,00
Erica	1	0,39	5	5,00
Eupatorium	1	0,39	10	10,00
Fragaria	1	0,39	5	5,00
Ilex	1	0,39	5	5,00
Lathyrus	1	0,39	5	5,00
Nuphar	1	0,39	5	5,00
Nymphoides,type	1	0,39	5	5,00
Persicaria	1	0,39	5	5,00
Phlox	1	0,39	10	10,00
Potentilla_	1	0,39	30	30,00
Reseda,type	1	0,39	30	30,00
Salvia	1	0,39	5	5,00
Scrophularia_type	1	0,39	35	35,00
Symphoricarpus	1	0,39	5	5,00
Tagetes	1	0,39	5	5,00
Tanacetum	1	0,39	5	5,00
Veronica_type	1	0,39	10	10,00

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

Chemical residue /metabolite	LOQ (µg/kg) 2015
6-Chloronicotinic acid	10
Abamectin	10
Acetamiprid	0,5
Bendiocarb	1
Bifenazate	1
Chlorpyrifos	1
Clothianidin	2
Coumaphos	2
Cyfluthrin-beta	1
Cypermethrin-alpha	2
Deltamethrin	1
Dimethoate	1
DMA	25
DMF	5
DMPF	5
Emamectin benzoate	2
Esfenvalerate	1
Fipronil	0,5
Fipronil carboxamide	0,5
Fipronil desulfinyl	0,5
Fipronil sulfide	0,5
Fipronil sulfone	0,5
Fluvalinate-tau	2
Imidacloprid	0,5
Imidacloprid 5-hydroxy	5
Imidacloprid olefin	5
Imidacloprid urea	0,5
Imidacloprid, desnitro	0,5
Imidacloprid, desnitro olefin	0,5
Indoxacarb	2
Omethoate	1
Permethrin	0,5
Pyridaben	1
Thiacloprid	1
Thiamethoxam	2
Triflumizole	1

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.