Final Report Honeybee Surveillance Program the Netherlands 2014-2018

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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 Netherlands Ministry of Agriculture, Nature and Food Quality 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 which ran from 2014-2018.

The results of the winter mortality questionnaire are robust and representative. A random sample of approximately 500 beekeepers has been questioned about colony survival in their operation each year, apart from the first year when the procedure was slightly different. In 2018 it has been a coordinated effort in collaboration with the Netherlands Beekeeping Association (NBV) and Imkers Nederland. The results of the surveillance study are also robust and representative, as they are based on a large-scale stratified random sample from across the Netherlands.

The duration of the program has been four years and this report summarizes findings for all years. 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 Landbouw, Natuur en Voedselkwaliteit 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) en ImkersNederland. 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 omvat de resultaten van alle vier de jaren.

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1. Summary of results 2014-2018

1.1 Executive Summary

1 Commissioned by the Dutch ministry of Agriculture, Nature and Food Quality, the Honeybee Surveillance Program assessed honeybee winter mortality in the Netherlands between 2014-2018 and aimed to unravel the factors explaining winter colony losses. To achieve this, two studies were 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 analysed in the lab. This final report describes the results of the fourth year, 2017-2018, but the main focus is on the final results of the four year study.

2 **National-level colony survival**. The Honeybee Mortality Monitor reveals that winter survival in 2017-2018 was 84.3% (15.7% of colonies died) and 55% of beekeepers suffered no loss at all. While higher survival is always preferred, this figure is close to the 'normal' variation of around 5-15% winter mortality. Survival has now been above 80% for the last six years and above 85% for five of the last six years. The number of managed honeybee colonies in the Netherlands is estimated to be between 71 and 81 thousand.

3 **Apiary-level mortality**: High winter survival in apiaries is correlated with low *Varroa* mite levels and absence of ABPV virus in the apiary. The surrounding landscape shows a complex interaction with survival. Good beekeeping practice, e.g. control of parasites and diseases, will further improve colony survival and seems the single best way to do so.

4 **Colony-level mortality:** A large number of variables each contribute just a little bit to explain colony winter mortality and are often correlated with each other making it difficult to separate their roles. A few factors seem to have slightly more importance, but no single factor emerges as the main driver of colony winter loss. Colonies containing more *Varroa* mites and placed in highly fragmented landscape with substantial amounts of maize crops have lower survival probability. When stored pollen contained more *Asteraceae*, survival was also slightly lower. Finally, chemical residues of neonicotinoids and other compounds were detected in 30% of colonies, their presence is not correlated with winter mortality, except for dimethoate which was rarely detected, but when detected had a strong negative effect on colony survival.

5 **Main messages:**

- **Honeybee winter loss was lower than 20% in the last 6 years, but still above 10% in most years, while the majority of beekeepers do not lose colonies in winter.**
- **The factors underlying honeybee colony loss in colonies managed by beekeepers in our Dutch landscapes, are many, are variable in space and time, and are likely to interact.**
- **Improving beekeeping practice (e.g. treatment of pests and diseases) seems the best way to improve honeybee survival.**
- **Factors linked to beekeeping practice (e.g. pests and diseases) emerge as the most consistent factor determining colony winter mortality.**
- **Chemical residues, e.g. insecticides and acaricides, were detected in 30% of samples, but their presence is not correlated with colony winter mortality, except for dimethoate which is rarely found, but when it is found, it has a negative effect.**

1.2 Nederlandse samenvatting

1 Het Nederlandse Ministerie van Landbouw, Natuur en Voedselkwaliteit gaf opdracht tot het honingbijen surveillance programma, om de wintersterfte onder Nederlandse honingbijen (2014-2018) en de oorzaken die de wintersterfte kunnen verklaren te bestuderen. 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 4^{e} jaar, 2017-2018, maar de focus ligt op de eindrapportage van de 4-jarige studie.

2 **Bijensterfte in Nederland**. De Wintersterfte monitor laat zien dat de overleving van bijenvolken in Nederland in 2017-2018 hoog was, namelijk 84.3% (15.7% van de volken ging dood) en dat 55% van de imkers geen sterfte had. Lagere wintersterfte is altijd beter, maar de huidige sterfte ligt dicht bij de als normaal geziene variatie (rond de 5-15%). Overleving is de laatste zes jaar altijd hoger geweest dan 80% en in vijf van de zes jaar hoger dan 85%. Op basis van de monitor kunnen we een schatting maken van het aantal bijenvolken in Nederland; dat ligt tussen de 71 en 81 duizend.

3 **Sterfte per bijenstand.** De overlevingskans is hoger in bijenstanden met lagere Varroa infectieniveaus en bij afwezigheid van ABPV virus in de bijenstand. Het omringende landschap heeft een complexe relatie met het overlevingspercentage in een bijenstand. Verbetering van de imkerpraktijk, o.a. Varroa- en ziektebestrijding, lijkt de beste manier om overleving te verhogen.

4 **Sterfte per bijenvolk.** Een groot aantal factoren lijkt elk een heel kleine bijdrage te leveren aan wintersterfte. Veel van deze factoren zijn gecorreleerd, waardoor hun bijdrage niet goed te scheiden is. Enkele factoren verklaren een iets groter deel van de wintersterfte, maar niet één factor kan aangewezen worden als de belangrijkste. Volken met meer Varroamijten en geplaatst in zeer gefragmenteerde landschappen met veel mais hebben een kleinere overlevingskans. Bij volken met veel *Asteraceae* stuifmeel was overleving ook iets lager. Tenslotte werden er in 30% van de volken residuen van chemische middelen aangetroffen. De aanwezigheid daarvan had geen invloed op de wintersterfte, behalve wanneer er dimethoaat aangetroffen werd (niet vaak het geval) dan was de sterfte flink hoger.

5 **Belangrijkste boodschappen:**

- **De wintersterfte onder Nederlandse honingbijen was lager dan 20% in de laatste 6 jaar, maar meestal boven de 10%; de meerderheid van de imkers had geen wintersterfte.**
- **Wintersterfte onder Nederlandse honingbijen is gecorreleerd aan verschillende factoren die variëren in ruimte en tijd en vaak interacteren.**
- **Verbetering van de imkerpraktijken (o.a. varroa- en ziektebestrijding) lijkt de beste manier om overleving te verhogen.**
- **Factoren die gerelateerd zijn aan de imkerijpraktijk, zoals controle van ziekten en plagen, komen consequent boven als link met wintersterfte.**
- **Residuen van chemische middelen (o.a. insecticides of acaricides) werden in 30% van de monsters gevonden, maar hun aanwezigheid is niet gecorreleerd met wintersterfte, behalve voor dimethoaat. Dit wordt zelden gevonden, maar bij vondst heeft het wel een negatief effect.**

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 Secretary of State for Economic Affairs, 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.
It was concluded that such a program requires an integrated approach towards honeybee health and a substantial investment. The Dutch government 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 and Bram Cornelissen (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, was provided by the Ministry of Agriculture, Nature and Food Quality (51%) with Nefyto as co-financer (49%). The program started in 2014 and lasted four years.

2.1 Main objective of the surveillance program

The main objective of this program is to study patterns in and causes of winter mortality of honeybees in the Netherlands. To this end, we determined colony winter loss each year for four years using a randomized survey and we correlated various potential drivers of winter loss to colony fate. These factors include exposure to agro-chemicals, bee diseases and pests, use of pollen sources, landscape configuration and beekeeping practices (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 annual CoLoSS colony loss questionnaires that estimate winter mortality in many countries, initiatives like the Epilobee project that has addressed bee diseases and pests in a single year across Europe, activities of EFSA. The present research program is more complete (more possible drivers of loss are 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.

2- We use standardized protocols, most of which are applied in other projects and all of which have been validated before (see for details the CoLoSS beebooks on standard methods; http://www.coloss.org/beebook/) . In some cases 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. Results have been and will be disseminated through their established communication channels with the Dutch beekeepers.

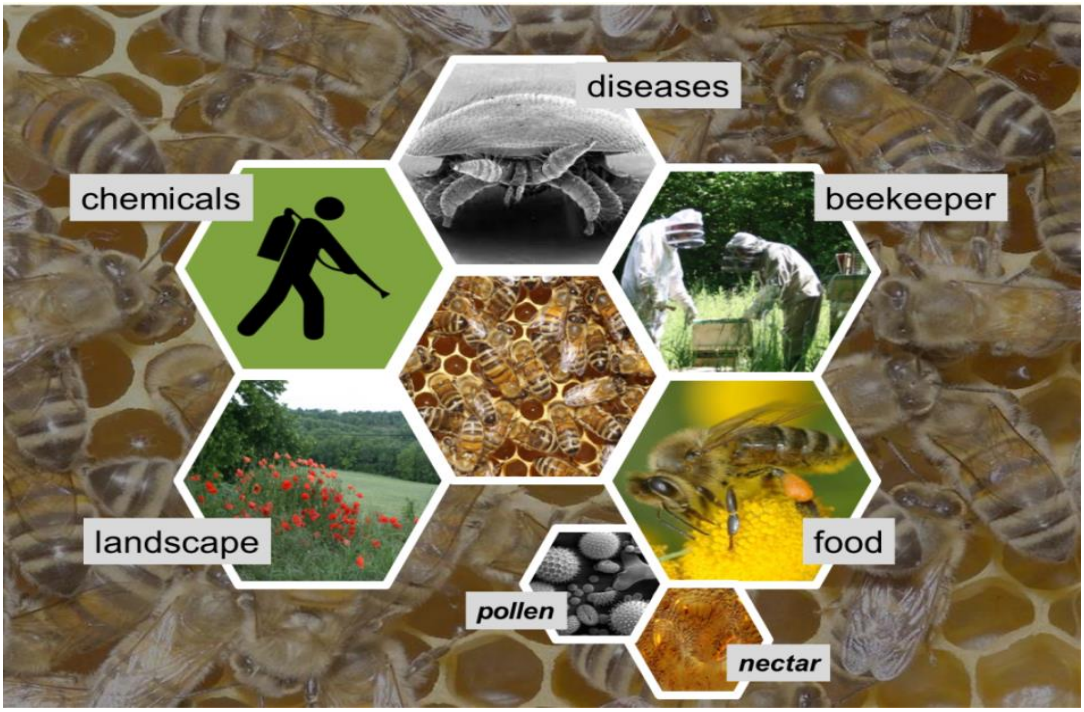


Figure 1. Overview of the main risk factors for honeybee colony survival that will be addressed in the surveillance program.

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 colonies. 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. This year also the new beekeepers association Imkers Nederland participated. 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 own bee colonies and many beekeepers did not respond to our request by e-mail, we started with a larger selection of 2000 beekeepers from NBV and 200 from Imkers Nederland. The differences in numbers per association are linked to their representative total number of members. In 2018, after approaching 2200 beekeepers, we got responses from 780 on which this year's results are based. These beekeepers all filled in the survey online using a Google form questionnaire.

The Honeybee Surveillance Study is set-up specifically for this program and consists of a random sampling of colonies in apiaries from around the Netherlands. Samples of bees, honey and pollen are taken by beekeepers themselves after which the consortium uses the samples 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 to conduct the field survey and collect samples. This method did not render the best results. Therefore, in the following years beekeepers were 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 apiary. Only a subset of the samples, up to 400 per year, could be analysed (due to limited funds), but all have been stored for future analyses.

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

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

3 Results

3.1 Honeybee Survival Monitor 2017/2018

3.1.1. Results from Honeybee survival Monitor

This year a large number of 780 beekeepers participated in the survival monitor. Both because we selected more beekeepers in the first place to ensure sufficient participants and because the newly merged association Imkers Nederland (former ANI and ABTB) also contributed. The 763 usable surveys (beekeepers that reported to have more hives after winter than before the winter were assessed as unreliable and were taken out of analysis) had a survival rate of 84.3%, therefore winter mortality was 15.7%. In total 5591 hives were prepared for winter and 4696 remained alive until April 2018 (table 1). However a large number of beekeepers 417 (55%) did not have any winter mortality at all.

Table 1. Winter survival of honey bee colonies in the Netherlands 2005-2018

Winter	Number of beekeepers	Number of colonies (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 NBV random sample
2016-2017	470	3479	85.7	14.3	HB-Surv NBV random sample
2017-2018	**763**	**5591**	**84.3**	**15.7**	**HB-Surv NBV/Imkers NL random sample**

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

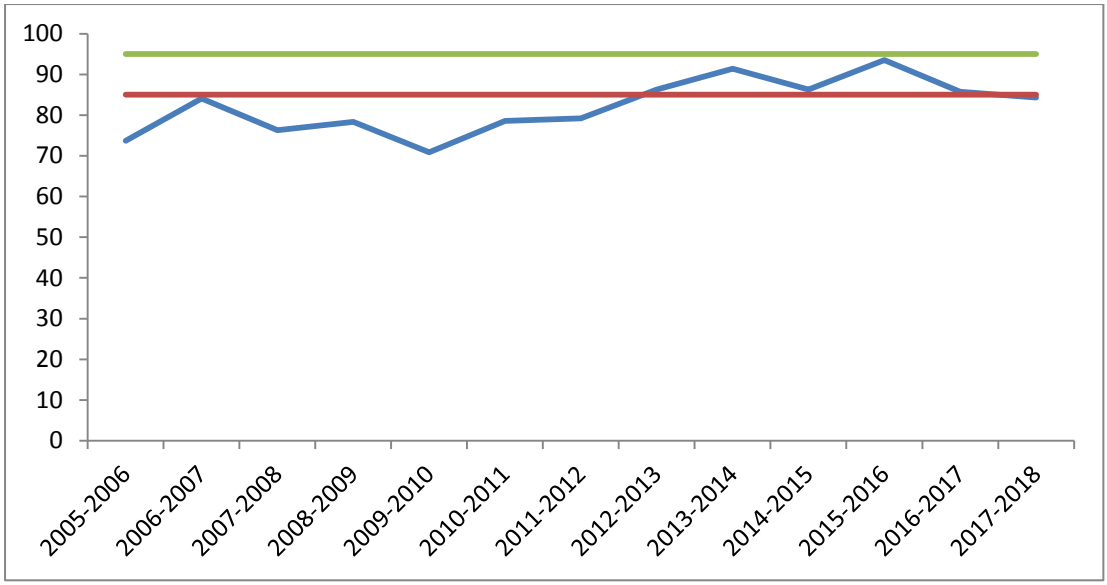


Figure 3. Winter survival 2005-2018. Added are two lines that indicate survival between 85% (red) and 95% (green) that are regarded as accepted.

The 15.7% mortality is a little bit higher than in the previous year(s), but as can be seen in figure 3, the annual winter mortality has not returned to the high percentage of more than 20% that was customary between 2005 and 2012. A mortality of 15.7% is, however, higher than the beekeeping community would want and on the high margin of what is thought to be a 'normal and manageable winter mortality level' which ideally should be below 10%. The percentage mortality that we report here is higher than that reported in an early spring quickscan by the beekeeper organization (9.3%). The main reason for this are that (1) the quickscan is non-random and failures are less likely reported than successes, (2) the quickscan had been performed earlier in the year , possibly excluding colonies that die in the last cold spell of winter.

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

The Netherlands needs to submit an estimate of the 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 two main beekeeping associations, the NBV and Imkers Nederland (formerly two separate associations: 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 2017-2018 were received from 763 beekeepers[1]. In total these beekeepers had 5591[2] hives in late autumn 2017. The average number of hives was 7.3 across all beekeepers[3] with a few large beekeepers and many with fewer hives. A total of 8292 beekeepers[7] is registered with one of the two beekeeping associations[4-5]. The total number of hives of these beekeepers is about 60761 (beekeepers * 7.3 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 and also the number of double memberships among the beekeepers. Both are unknown. We calculated the population of Dutch bee hives for degrees of registration between 70 and 95%[11-16]. The estimate increases from 60 thousand at complete registration to 86 thousand at 70% registration.

In conclusion: there were at least 60761 managed bee hives in the Netherlands in late Autumn 2017. This is certainly an underestimate due to incomplete registration. The best estimate may be between 71,000 and 81,000 bee hives present in the Netherlands in 2017.

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 2017**		**763**
2	Total number of hives going into winter		5591
3	Average number of hives per beekeeper		7.3
4	Number of beekeepers on NBV list (minus new members)		7092
5	Members of ImkersNederland		1200
6	Total number of beekeeper members		8292
7	Members without bees (percentage last year)		9.6%
8	Number of hives in associations (beekeepers * average hives per beekeeper)		60760
9	Estimated percentage of beekeepers member of one of the two associations		Estimated total number of hives
10		95%	63959
11		90%	67512
12		85%	71483
13		80%	75951
14		75%	81015
15		70%	86801

3.2 Honeybee Surveillance Study 2017-2018

3.2.1. Set-up of the field campaign

The field campaign is based on a random selection of beekeepers (more specifically single apiaries from beekeepers) from across the Netherlands. The participating beekeepers are asked to take their own samples, based on instructions outlined in 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 a single 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 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 for 2017/2018 was as follows:

1- Hive number 1 and 2 per apiary was selected for analysis in Autumn 2017. 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 2018, 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, at least a pair of colonies 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 colonies survived or all died, we did not analyse a third one. Third colonies were only analysed for selected apiaries to arrive at matched pairs of dead-alive colonies for as many apiaries as possible.
4- The samples of the third colony for the selected apiaries are distributed to the partners for analysis in April 2018. After that all data have been analysed in the respective laboratories, data has been integrated and analysed by Naturalis.

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 four 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 collected in autumn (n=296) were analysed for the presence and quantity of the parasite *Varroa destructor* and for the presences of 4 pathogens associated with winter mortality:

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

The average number of Varroa mites was 3 mites per 100 bees, while 35% (n=102) of the samples did not contain Varroa.. DWV was found in 76% of the colonies (table 3). This indicates that even in colonies in which no Varroa mites have been detected at the end of the summer Varroa mites must have been present previously or still were present at very low numbers as DWV is largely transmitted by Varroa-mites. *Nosema ceranae* and ABPV virus were both found a little over 10%, while *Nosema apis* was only found in 1% of the colonies.

Table 3. Presence of various pests and pathogens in honey bee samples in 2014 (n=91), 2015 (n=331), 2016 (n=314) and 2017 (296).

Pest/Disease	2014	2015	2016	2017
Varroa present (%samples)	73%	63%	68%	65%
Varroa mites / 100 bees	7	3	5	3
Nosema ceranae	89%	59%	22%	12%
Nosema apis	0%	0.6%	1%	1%
DWV virus	98%	93%	96%	76%
ABPV virus	0%	1%	9%	13%

Pollen sources used by colonies

In the 265 samples of beebread that were analysed, 68 different pollen types were found. The pollen types were counted only when they contributed 5% or more to the sample (see table 4 for 10 most recorded pollen types and appendix C for the full list). On average 5.4 different types of pollen were found per sample, ranging from 2 to 11 types. Note that not all pollen types indicate the presence of 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 colonies in late 2017. Pollen types that occur in at least 10% of the samples, for the complete list see Appendix C. Note that pollen of *Trifolium* (clover) can sometimes be assigned to a single species *Trifolium pratense*, but often not. In the latter case it is included in the *Trifolium* spp. category. That category includes other clover species, the hybrid clovers often used in agriculture and flower strips and probable some of the red clover.

Species	found in # samples	% of total	min	max	ave. When present
Brassicaceae	134	50.6	0	100	31.8
Trifolium spp.	105	39.6	0	100	27.9
Asteraceae	92	34.7	0	95	19.7
Rosaceae	86	32.5	0	85	22.0
Castanea	58	21.9	0	85	32.9
Phacelia	58	21.9	0	65	18.4
Trifolium pratense	58	21.9	0	85	25.5
Impatiens	51	19.2	0	100	38.1
Hedera	49	18.5	0	100	41.8
Calluna	43	16.2	0	95	33.6
Caryophyllaceae	34	12.8	0	50	11.9
Ranunculaceae (Clematis)	31	11.7	0	80	17.4
Parthenocissus	28	10.6	0	35	10.2

Chemical residues detected in honey

Stored honey samples from August 2017 were analysed 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 D). 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 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, including humans. Note that the 'honey samples' may not always refer to honey collected by the bees themselves. The stored reserves in August is the fuel that the colony is 'given' by the beekeeper. This may be honey actually stored by the bees, if beekeepers have not extracted it from the hive. It may, however, also be sugar or other honey substitute provided by the beekeeper after honey extraction. For our research, the importance is in the stored food reserves that the bees use through winter and in the chemical residues that may be present therein.

Of the 110 chemical compounds we aimed to detect, only fourteen compound were encountered in the samples from August 2017 and mostly at low frequency and concentration (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 in these samples.

Neonicotinoids (imidacloprid, thiacloprid, acetamiprid, thiamethoxam or clothianidin) were found in 39 hives (11.0%) of which 26 (7.5%) above LOQ, i.e. at a quantifiable dose. 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.

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.

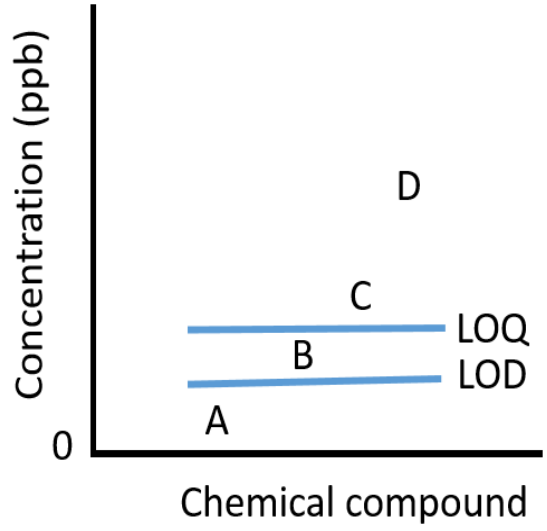


Table 5. Chemical residues present above LOQ level in samples of 2014 (90 hives), 2015 (327), 2016 (342), 2017 (294). Neonicotinoids are indicated with *, Acaricides used by beekeepers with ^ and fungicides with #. Given are percentage of hives in which each residue has been found above the level of quantification - LOQ (see box 1). ~During the study a change of analysis of residues was chosen; several residues were added after 2015 and due to high cost and low number of positive tests Fluvalinate-tau was taken out after 2015.

Chemical residue	2014	2015	2016	2017
Acetamiprid *	2.2%	2.8%	3.5%	3.4%
Amitraz ^ (banned)	8.9%	2.1%	1.8%	0.7%
Boscalid #	*Not tested~*	*Not tested*	4.1%	2.7%
Carbendazim #	*Not tested*	*Not tested*	0.6%	0.3%
Chlorfenvinphos	*Not tested*	*Not tested*	0.6%	0.3%
Coumaphos ^ (banned)	1.1%	2.4%	0.0%	1.0%
Dimethoate	0.0%	0.9%	0.3%	0.7%
Fluvalinate-tau ^	0.0%	0.9%	*Not tested*	*Not tested*
Fluopyram #	*Not tested*	*Not tested*	0.9%	1.4%
Imidacloprid * (banned for use in open cultivation)	6.7%	2.8%	0.3%	1.0%
Permethrin	0.0%	0.3%	0.0%	0.0%
Tebuconazole #	*Not tested*	*Not tested*	1.5%	0.0%
Thiacloprid *	2.2%	9.8%	8.2%	7.5%
Thiamethoxam/Clothianidin *	0.0%	0.9%	0.6%	0.0%
Neonicotinoids total *	**7.7%**	**15.0%**	**11.3%**	**11.6%**
Acaricides total ^	**7.7%**	**5.2%**	**8.1%**	**1.7%**

Landscapes in which bees forage

Landscapes determine in part the health of bee colonies. Not only do landscapes provide pollen and nectar sources (pollen sources are assessed in this study through pollen analysis, nectar sources are not assessed as stored winter food is often sugar), colonies that are exposed to mass-flowering crops and wild plants are also exposed to unhealthy conditions in the landscapes they forage in (e.g. agro-chemicals, pollution, drought and water shortage). To assess the importance of landscape factors, that cannot be related to bee diseases or pollen sources that were assessed separately, we use landscape factors as a proxy for the other aspects of landscape and habitat that may influence honeybee health.

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 (2017 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 colonies (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 in number of land use classes 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.

Table 6. Chemical residues encountered in 294 honey samples: presence, concentrations and LD50 for honeybees. Samples are scored as 'absent' (column 2; indicating nothing was found), LOD = 'detected but <LOQ' (column 3; very small quantity detected, but not sufficient to quantify, i.e. below LOQ). Several compounds can be detected as the compound itself or its metabolites, their values are recalculated generally according to standard residue definitions. Names of compounds referring to sets of chemicals are indicated with superscript numbers and are: 1 Amitraz (Amitraz + DMA + DMF + DMPF), 2 dimethoate (dimethoate + omethoate) both with independent definitions in Regulation (EC) No1107/2009), 3 Imidacloprid (Imidacloprid + Imidacloprid_5-Hydroxy + Imidacloprid_olefin + Imidacloprid_desnitro + Imidacloprid_desnitro_olefin + Imidacloprid_urea+ 6-Chloronicotinic_acid; note that the metabolites are not regulated). EcoTox database values are from: http://www.ipmcenters.org/ecotox/. Values in this database are in ug per bee, (with an individual bee weighing of 100 milligrams). The values in the last column are multiplied by 10.000 to be comparable to the detected concentrations in the previous columns.

Compound	absent	>LOD	>LOQ	average conc. (µg/kg) if present	maximum concentration found	LD50 (µg / kg in 48h tests) USDA EcoTox database
Acetamiprid	280	14	10	3.8	19	810000
Amitraz[1]	281	13	2	17.1	25	10000000
Coumaphos	264	30	3	3.2	3.6	
Dimethoate[2]	292	2	2	1.9	2.3	5600
Imidacloprid[3]	291	3	3	1.0	1.6	380
Thiacloprid	257	37	22	6.9	55	1794000
Boscalid	286	8	8	14.3	92	16600000
Carbendazim	293	1	1	4.4	4.4	5000000
Chlorfenvinphos	292	2	1	1.4	1.4	
Epoxiconazole	293	1	1	4.2	4.2	
Fluopyram	290	4	4	4.6	9.9	1023000
Metazachlor	293	1	1	6.7	6.7	
Pyridate	293	1	0	only >LOD		
Spinosyn	293	1	0	only >LOD		
Teflubenzuron	291	3	3	2.8	3.5	

In addition, we created a separate data layer called 'Natural habitat' 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 that we constructed, we refer to it as 'Crop area', 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, 2016 and 2017. Annual variation in the values for different landscape factors is small, indicating that we have sampled very similar landscapes in each year, a reflection of the random sampling protocol we apply in selecting the locations.

Landscape factor	2014 landscapes average (range)	2015 landscapes average (range)	2016 landscapes average (range)	2017 landscapes average (range)
Number of land use classes	9.1 (3-15)	9.4 (4-14)	9.6 (3-17)	9.1 (3-15)
% Bee Forage	20.7 (0-72)	18.0 (0-68)	15.4 (0-70)	15.8 (0-98)
% Natural habitat	10.7 (0-50)	8.6 (0-57)	9.1 (0-68)	9.3 (0-98)
% Crop area	29.1 (0-92)	30.1 (0-91)	29.4 (0-80)	28.6 (0-90)
% Maize cultivation	5.9 (0-29)	5.6 (0-32)	6.7 (0-31)	3.9 (0-24)

3.2.3. Integrated analysis results from Honeybee Surveillance Study 2017-2018

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 colony)]

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 colonies in apiary; Q2: survival/mortality of the single colony) 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 colonies surviving within an apiary (Q1) or to the probability for a single colony to survive (Q2).

<u>Q1 for 2017-2018: Is the percentage of survival at apiary level related to specific explanatory variables?</u>

Here we try to explain the % of winter survival (reverse of mortality) in the 2017-2018 season using land use, disease and size of the apiary. Factors that were tested in the model are given in table 8. A total of 122 apiaries could be included in this analysis.

<u>Result</u>: A few factors contributed to the percentage of colony survival in apiaries and some variation could be explained by the factors taken into account. There is one best model (Best 1 in table 9), the

factors appearing in that model contribute to survival, whereas the factors present only in the LU Full model do not contribute to explain winter survival at apiary level.
Factors negatively correlated to survival at apiary level are (in order of importance): The presence of ABPV virus and *Nosema ceranae*, the maximum percentage of *Varroa* mites found in an apiary, and the amount of maize grown close to the apiary. The number of land use classes had a positive influence, with a high number of classes correlated to slightly higher survival (for specific relationships see figure 4).

Other factors play a negligible role (the amount of food and crops in 3 km range; i.e. low coefficients in table). They may of course have been responsible for the mortality of individual colonies or low survival at some apiaries, but are not consistently contributing to mortality across the country's apiaries (table 9).

Conclusion: Higher survival in 2017-2018 is linked to lower disease and mite prevalence and thus to better beekeeping practices. The landscape in which the bees forage also has an impact on survival with more heterogeneous landscapes (at 1km scale) leading to slightly higher survival.

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 *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)	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 analysed this at two levels: 1000m and 3000m radius)	YES	YES
% nature	Area of (semi-)natural habitats around the apiary (we analysed 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.	YES	YES
% cropped area	Area of cropland, all crops summed, around the apiary (we analysed 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 analysed 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 analysed the dominant pollen types separately.	NO	YES

Table 9. Factors related to the survival percentage of colonies in an apiary. Values indicate the estimates from the model with standard error in parentheses. The final model is Best 1, which is better than the full model, 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.

Q1 LU Models 2017		
	LU Full	**Best 1**
(Intercept)	1.90 (0.44)	1.65 (0.28)
Land Use classes in 1km	**0.15** (0.04)	**0.12** (0.04)
Maize area in 1km	-0.03 (0.02)	-0.03 (0.02)
Forage in 1km	-0.00 (0.00)	-0.00 (0.00)
Land Use classes in 3km	-0.04 (0.04)	
Natural area in 3km	-0.00 (0.00)	
Crop area in 3km	-0.00 (0.00)	-0.00 (0.00)
Varroa mites / 100 bees	**-0.05** (0.01)	**-0.05** (0.01)
Nosema ceranae presence	**-0.68** (0.23)	**-0.65** (0.23)
DWV presence	0.16 (0.20)	
ABPV presence	**-0.56** (0.20)	**-0.54** (0.20)
Number of hives in apiary	-0.01 (0.00)	
AIC	526.72	523.36
BIC	560.37	545.79
Log Likelihood	-251.36	-253.68
Deviance	318.26	322.90
Number of apiaries	122	122

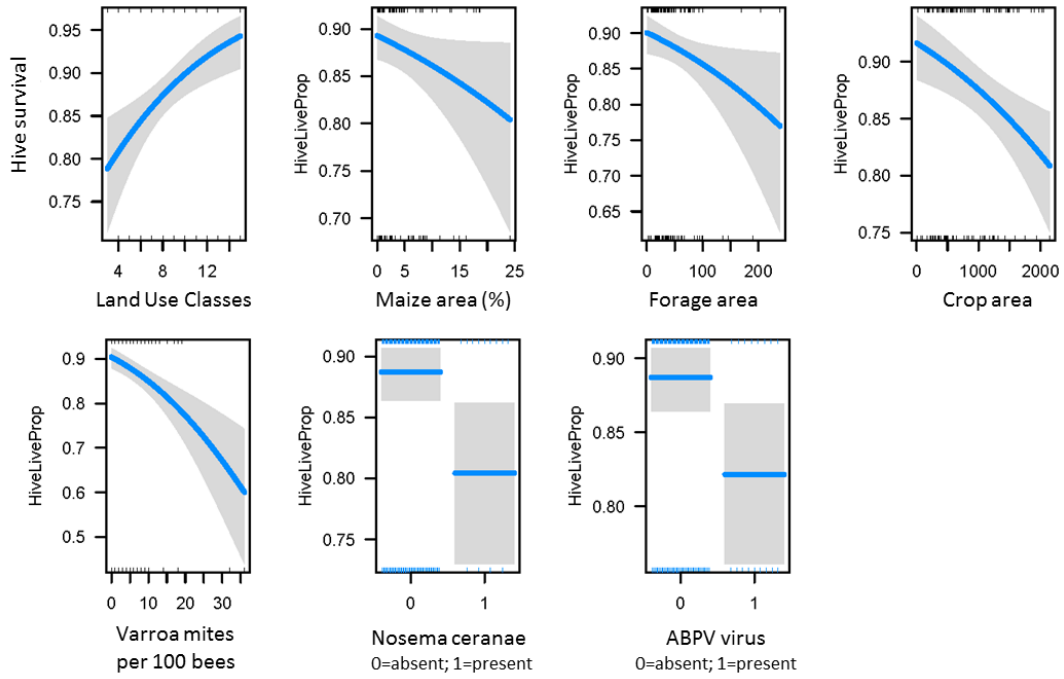


Figure 4. Relationships of factors affecting survival percentage at apiary level in season 2017-2018 (question Q1).

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 B). Step two analysed the final model using all the relevant variables resulting from the step 1 models.

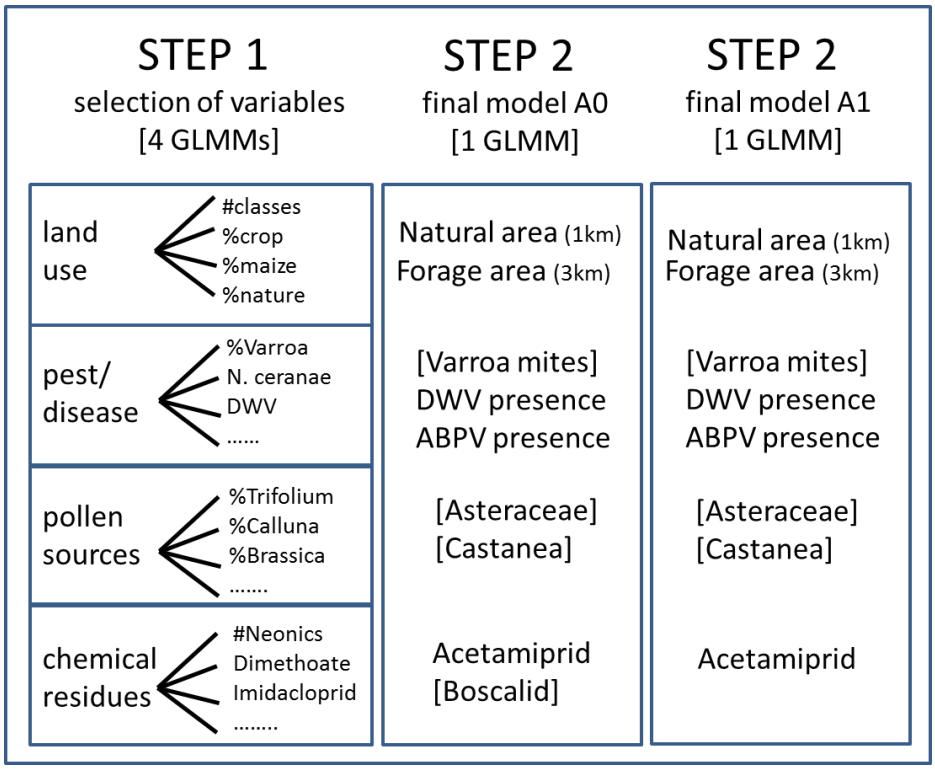


Figure 5. Schematic overview of analysis for question 2. STEP 1 selects the main variables within each of the four variable categories (boxes on the left) using GLMM models. STEP 2 uses the variables selected in STEP 1 (indicated in the two other boxes) in a final GLMM model [factors in [] were included in the full model, but were not included in the final 'best' 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 each 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.

Result: The best model explaining winter survival contains a number of variables with differing impact and results very similar for the Q2A0 and Q2A1 models. In other words, there were no differences in outcome whether we include chemical traces (Q2A1) or only those detected at quantifiable amounts (Q2A0). A few factors play an important role (Tables 10 and 11; figures 6 and 7).

ABPV levels in single hives could partially explain mortality over the winter of 2017-2018 with presence of ABPV having a negative correlation with survival. The incidence of *Varroa* mites in single hives in autumn did not have a negative correlation (not retained in the best model), and the presence of DWV had a small positive effect on survival.
Of the chemical compounds encountered only Acetamiprid was included in the STEP 2 model and retained in the best model. It was encountered in a few samples only and had a marginal positive relation to colony survival, with a lot of variation (see figures 6 and 7).
The amount of natural area had a strong negative effect on colony survival, whereas the amount of forage area had a small positive effect. Natural areas can consist of bee friendly habitats (e.g. chalk grasslands), but often consist of habitat that are only marginally beneficial to honeybees (coniferous forest, reed and march land or meadows managed for meadowbirds all of which can be largely devoid of pollen/nectar flowers) and are probably worse than cities, villages and public spaces.
Two pollen types were selected for the STEP 2 model (Asteraceae and Castanea pollen) but both are not included in the best model, indicating that they did not contribute to explaining colony survival in this study.

Conclusion: Only a small part of the mortality of bee colonies in the winter of 2017-2018 could be explained by the main factors that were analysed. More natural area and more ABPV virus correlated to lower survival and more forage area in the wider landscape had a positive effect. Other factors, including residues of chemical, had no or only marginal effect on colony survival in the winter of 2017-2018.

Table 10. Summary of factors related to the survival (where presence of chemicals is included only when they were above quantifiable amounts (LOQ).

Q2 A0 Final overall models		
	Q2A0 Full Model STEP 2	**Q2A0 best model STEP 2**
(Intercept)	1.88 (0.54)	1.74 (0.44)
Natural area in 1km	**-0.71(0.34)**	**-0.73 (0.33)**
Forage area in 3km	**0.80 (0.45)**	**0.85 (0.45)**
Acetamiprid	7.99 (24.76)	28.58 (1130298)
Boscalid	-1.14 (1.17)	
Asteraceae	-0.32 (0.20)	
Castanea	-0.24 (0.21)	
Varroa mites / 100 bees	-0.05 (0.04)	
DWV present	0.72 (0.52)	0.81 (0.50)
ABPV present	**-1.62 (0.66)**	**-1.84 (0.63)**
AIC	187.39	184.71
BIC	224.52	208.34
Log Likelihood	-82.69	-85.36
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	0.77	1.16

Table 11. Summary of factors related to colony survival in Q2A1 models (where presence of chemicals is included (LOD), not their quantifiable amounts (LOQ)

Q2 A1 Final overall models		
	Q2A1 Full model STEP 2	**Q2A1 Best model STEP 2**
(Intercept)	1.68 (0.53)	1.02 (0.59)
Natural area at 1km	**-0.63 (0.33)**	**-0.01 (0.01)**
Forage area at 3km	0.72 (0.43)	0.00 (0.00)
Acetamiprid	25.3 (1024)	13.9 (418)
Asteraceae	-0.30 (0.20)	
Castanea	-0.22 (0.21)	
Varroa mites / 100 bees	-0.24 (0.21)	
DWV present	0.70 (0.52)	0.75 (0.52)
ABPV present	**-1.72** (0.66)	**-1.88** (0.68)
AIC	185.50	183.62
BIC	219.25	207.25
Log Likelihood	-82.75	-84.81
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	0.75	1.05

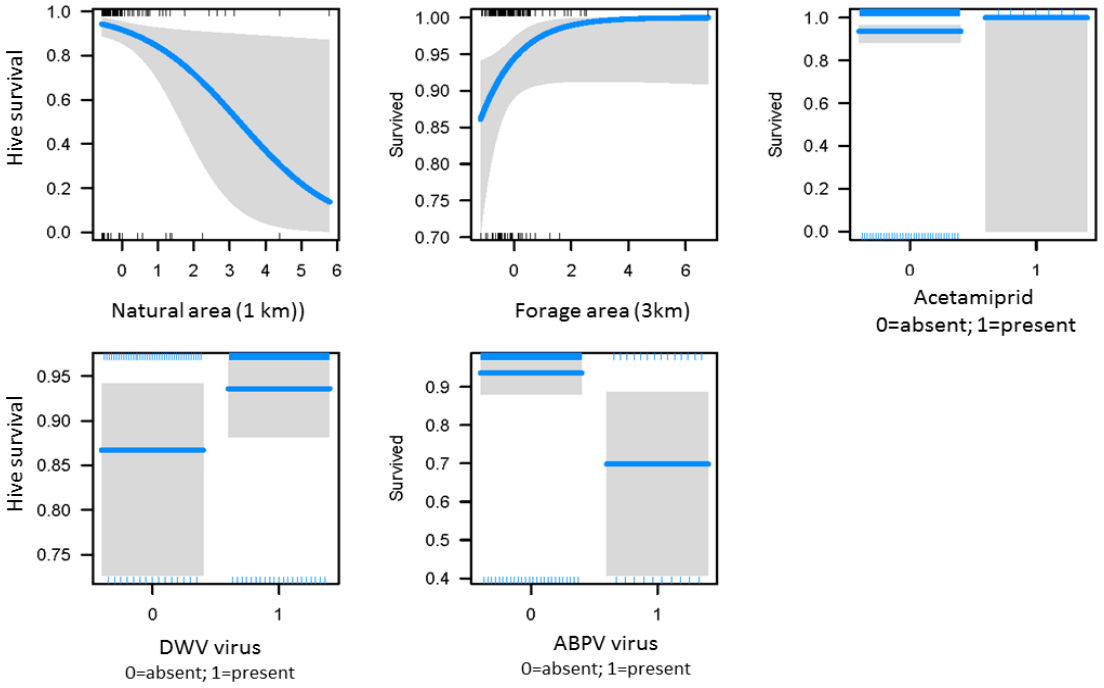


Figure 6. Relationships of factors affecting survival percentage of individual colonies in season 2017-2018 only considering those chemicals that have been detected at quantifiable amounts (>LOQ) (question Q2A0).

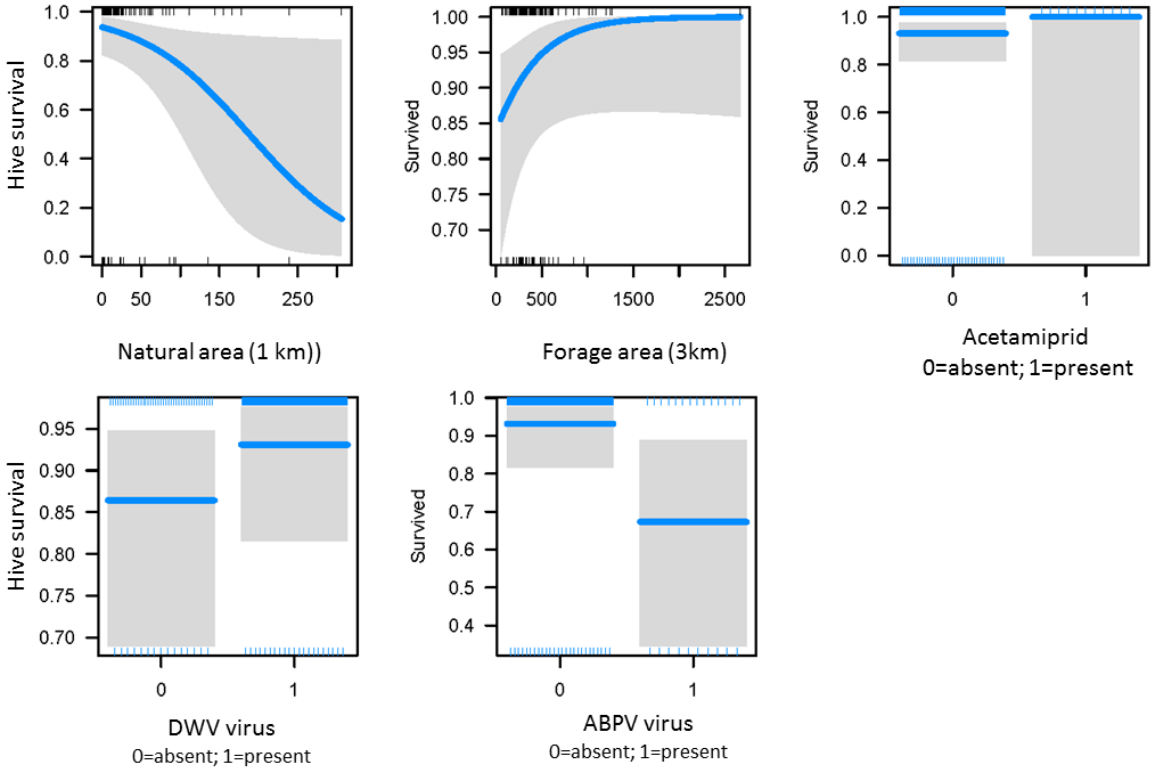


Figure 7. Relationships of factors affecting survival percentage of individual colonies in season 2017-2018 considering all chemicals that have been detected (>LOD) (question Q2A0). Note that results are very similar to the Q2A0 models. This is a result of chemical compounds playing no significant role in colony survival.

4. Comparison across four years of study: Results and Discussion

With this report, the fourth and final year of the study has been conducted and we can compile the results for the whole period of four years. First, we compare findings for individual factors, second we compare findings of the integrated model.

4.1. Single factor comparison

Parasites and pathogens

Over four years the most important observation is that presence of *Nosema ceranae* varies from 89% in 2014 to 22% in 2016 and only 12% in 2017. Presence of Varroa is very high and quite consistent over the years. *Nosema apis* is consistently low, whereas ABPV varies from 0% in 2014 to 13% in 2017. While beekeepers are well aware of the problem of the Varroa mite and its associated viruses, our study shows that Varroa mites are indeed present in most colonies at an average density of 3-7 mites per 100 bees depending on the year (see table 3 above). Even when Varroa mites are absent (or present at a very low density), DWV is omnipresent. DWV is transmitted by Varroa mites and its omnipresence indicates that virtually all colonies have or have had Varroa mites or their associated viruses. The rise in ABPV virus over the years is interesting and may need special attention.

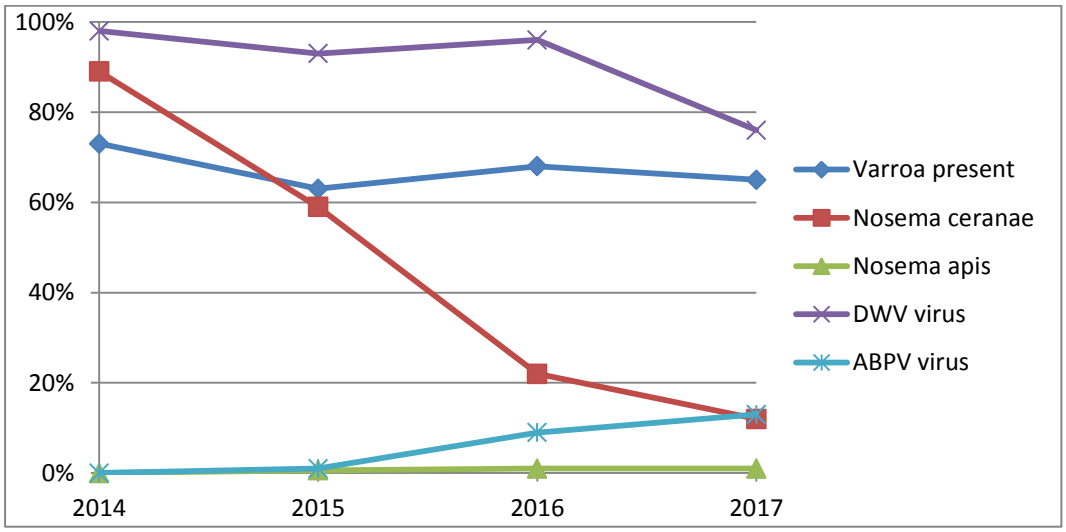


Figure 8. Presence of various pests and diseases in bee samples in 2014 (n=91), 2015 (n=331), 2016 (n=314) and 2017 (296).

Pollen sources used by colonies

A comparison over all four 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 colonies in all years. Some differences can be explained by differences in sampling period. In 2014 Hedera was found in more than 50% of the colonies, in the two years after that it was 16.7 resp. 8.5 percent, which can be explained by the earlier sampling (compared to flowering period) in later years.

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	% of total 2017	Average % over 4 years
Brassicaceae	mustards, rapeseed	33.3	49.8	53.3	50.6	46.8
Trifolium	clovers	25.6	47.1	42.3	39.6	38.7
Hedera	ivy	52.6	16.7	8.5	18.5	24.1
Calluna	heather	15.4	21.6	30.1	16.2	20.8
Asteraceae	dandelion family	3.8	20.4	32	34.7	22.7
Rosaceae	rose family	10.3	23.1	21	32.5	21.7
Lotus	birds foot trefoil	11.5	26.7	0.3	1.1	9.9
Caryophyllaceae	ragged robin and relatives	8.9	15.3	9.1	12.8	11.5
Impatiens	Himalayan balsam and relatives	10.3	8.6	13.5	19.2	12.9
Phacelia	phacelia	11.5	12.1	8.2	21.9	13.4
Castanea	chestnut	0	0	25.4	21.9	11.8
Fabaceae	legumes (various spp)	2.6	2.4	16.9	8.7	7.7
Zea	maize	0	8.6	11.9	1.9	5.6
Heracleum	hogweed	3.8	3.5	10	9.1	6.6
Fagopyrum	buckwheat type	1.3	14.5	0.6	0	4.1
Cornus	dogwood	0	2.4	13.8	1.1	4.3
Hypericum	St. John's wort and relatives	3.8	5.9	4.1	4.2	4.5
Rubus spp.	bramble	0	11.4	1.9	3	4.1

Chemical residues detected in honey

The majority of the honey samples (70%) did not contain any trace of any chemical compound that we assessed and above the very low levels of detection we assessed them at (see appendix D for complete list and LODs of 2017 and Appendix E for summary of all chemicals detected). Across all samples (1051 samples assessed in total), quantifiable neonicotinoid traces were found in 12%, and in most of these cases a single neonicotinoid compound was detected. Of the neonicotinoids, thiacloprid was present most often (and had also the highest presence of any chemical compound) and seems to have increased since the start of the study. Imidacloprid showed a steady decline (figure 9). Quantifiable amounts of Acaricides (mostly the illegal compounds Amitraz and Coumaphos) were detected in 6% of the samples. Other compounds were detected regularly, e.g. the systemic insecticide acetamiprid, and the fungicides boscalid and fluopyram (see table 5).

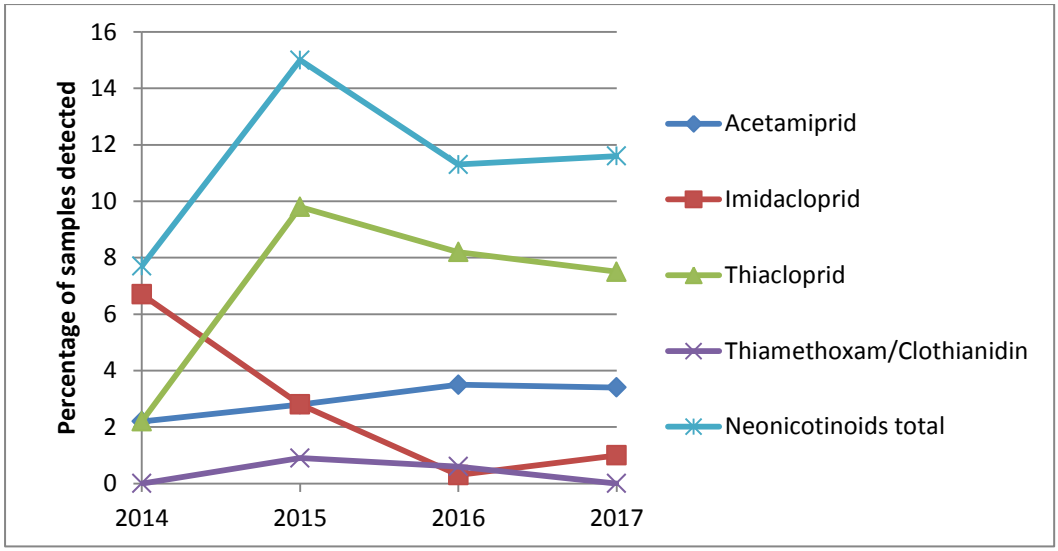


Figure 9. Presence of Neonicotinoid pesticide residues in bee samples. Note that despite the ban on imidacloprid for several cultivations, the presence of neonicotinoids has not decreased. Imidacloprid has been found rarely in honey samples for the last two years, but Thiacloprid has increased strongly and acetamiprid considerably over the period of our study.

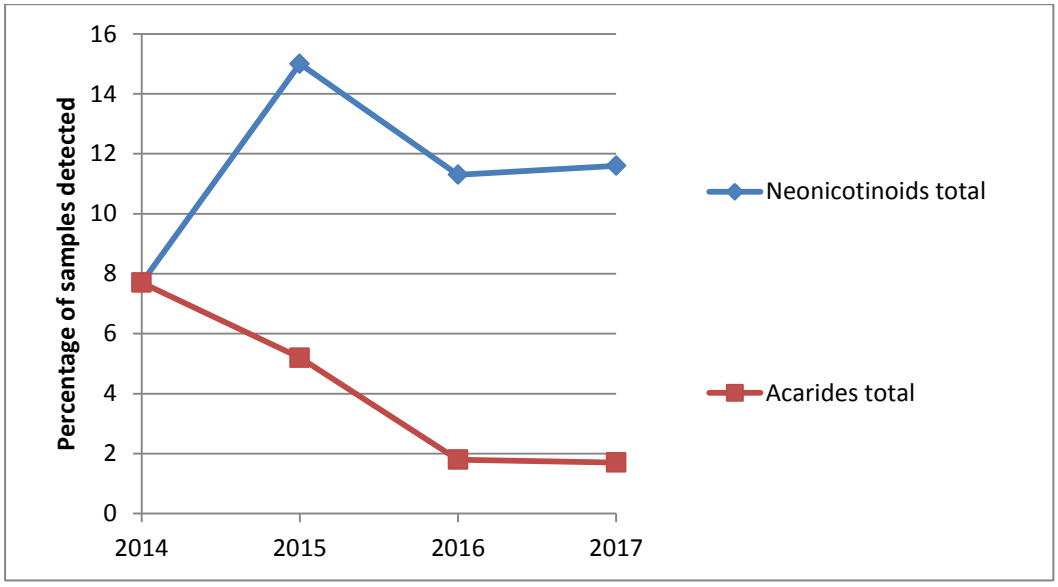


Figure 10. Presence of Neonicotinoid pesticides and Acaricides detected in honey samples (only samples were concentration above LOQ was detected). Every year we detected (illegal) acaricides in several samples (e.g. Amitraz, Coumaphos). It is most likely that these derive from beekeeping practices (probably used as Varroa treatment) and not from the wider landscape.

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 the change. Our sample is large and representative of the Dutch landscape and very similar across the four years (table 7; see also appendix F for sampling sites). This was the aim of our survey and the selection approach has thus been successful. The small differences observed between years are a result of the sampling sites themselves and not a result of changes in the Dutch landscape itself.

4.2. Integrated analysis results from Honeybee Surveillance Study 2014-2018

In this study we aim at explaining honeybee winter survival and mortality by analysing various factors that have been indicated previously to be responsible for colony 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 number of colonies that dies is large and the list of factors that we analyse seems to be able to explain just a small part of colony loss. Real world surveys 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.

When we consider **survival at apiary level (Q1)**, we see (table 13) that disease presence has a negative effect (most notably maximum Varroa levels and ABPV virus presence in the apiary) and that landscape factors show a complex interaction with survival. More maize grown close to the apiary is slightly positive, but more crop area close by and a landscape full of maize has a negative effect on survival. On the other hand, more forage habitat nearby decreases survival slightly, but a forage rich landscape is positive for survival.

When we consider **survival of individual colonies** (Q2), we also take into account the pollen sources stored, the bee diseases and chemicals detected in that specific colony. The main factors we analyse can explain only a small part of the colony loss. This indicates that there are many potential influences on honeybee colonies next to the ones we measure. For example, every beekeeper uses slightly different methods, queen age and quality varies a lot, queens can die or can be replaced all factors that are very difficult to capture. Finally, interactions between different factors may be of importance. By building up the dataset across the years we have reached a critical mass of data (>1000 colonies assessed) to obtain the best, broadest, most detailed picture of the factors influencing honeybee winter mortality in the Netherlands (see Appendix G for spatial distribution of sampling sites across the four years).

We assess survival factors in two steps explained above (see 3.2.3, table 8 and figure 5), because the total number of potential factors is too large to include all together. In step 1 we identify the most important specific factors from within the different factor groups. In addition, based on the attention in the media on the influence on pesticides on bee mortality, we report here analyses using the presence of any chemical trace in the samples (Q2A1 models; see 3.2.3). The following main factors are included in the integrated model: land use factors: number of land use classes in 3km area around the colony, area of maize both in 1 km and 3 km around the colony; chemicals: imidacloprid or dimethoate presence; pollen sources: percentage of Asteraceae or *Trifolium*; disease and pests: incidence of Varroa mites and presence of *Nosema ceranae* or ABPV virus. Other factors did not pass the selection at step 1 (for factor specific models see appendix B).

The analysis shows the following (table 14 and figure 12): there is no difference in survival across the years. Survival is much lower when the incidence of Varroa is high, when maize presence is high in the landscape or the landscape is highly fragmented (more land use classes within 3km area), when Asteraceae pollen is present or when dimethoate is detected (which is the case in very few colonies).

The area of maize close to the colonies (<1km) was positively correlated to survival, whereas presence of imidacloprid was correlated slightly positively.

Table 13. Factors related to the survival percentage of colonies in an apiary. Values indicate the estimates from the model with standard error in parentheses. The final model is Best 1, which is better than the full model, 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.

Q1 LU-Virus Models 2015-2018		
	LU Full model	**Best model 1**
(Intercept)	2.04 (0.24)	1.93 (0.16)
Number of land-use classes in 1km	-0.04 (0.07)	
Maize area in 1km	**0.22 (0.09)**	**0.23 (0.08)**
Forage area in 1km	-0.24 (0.14)	-0.12 (0.07)
Nature area in 1km	0.15 (0.14)	
Crop area in 1km	**-0.12 (0.10)**	**-0.20 (0.05)**
Number of land-use classes in 3km	-0.05 (0.06)	
Maize area in 3km	**-0.11 (0.09)**	**-0.16 (0.08)**
Forage area in 3km	**0.46 (0.18)**	**0.30 (0.13)**
Nature area in 3km	**-0.44 (0.19)**	**-0.27 (0.12)**
Crop area in 3km	-0.06 (0.10)	
Varroa mites /100 bees	**-0.17 (0.04)**	**-0.17 (0.04)**
Nosema ceranae present	-0.14 (0.14)	
DWV present	-0.03 (0.17)	
ABPV present	**-0.52 (0.15)**	**-0.49 (0.15)**
AIC	1538.42	1530.47
BIC	1602.17	1570.31
Log Likelihood	-753.21	-755.23
Num. obs.	397	397
Num. groups: Year	4	4
Var: Year Intercept)	0.10	0.09

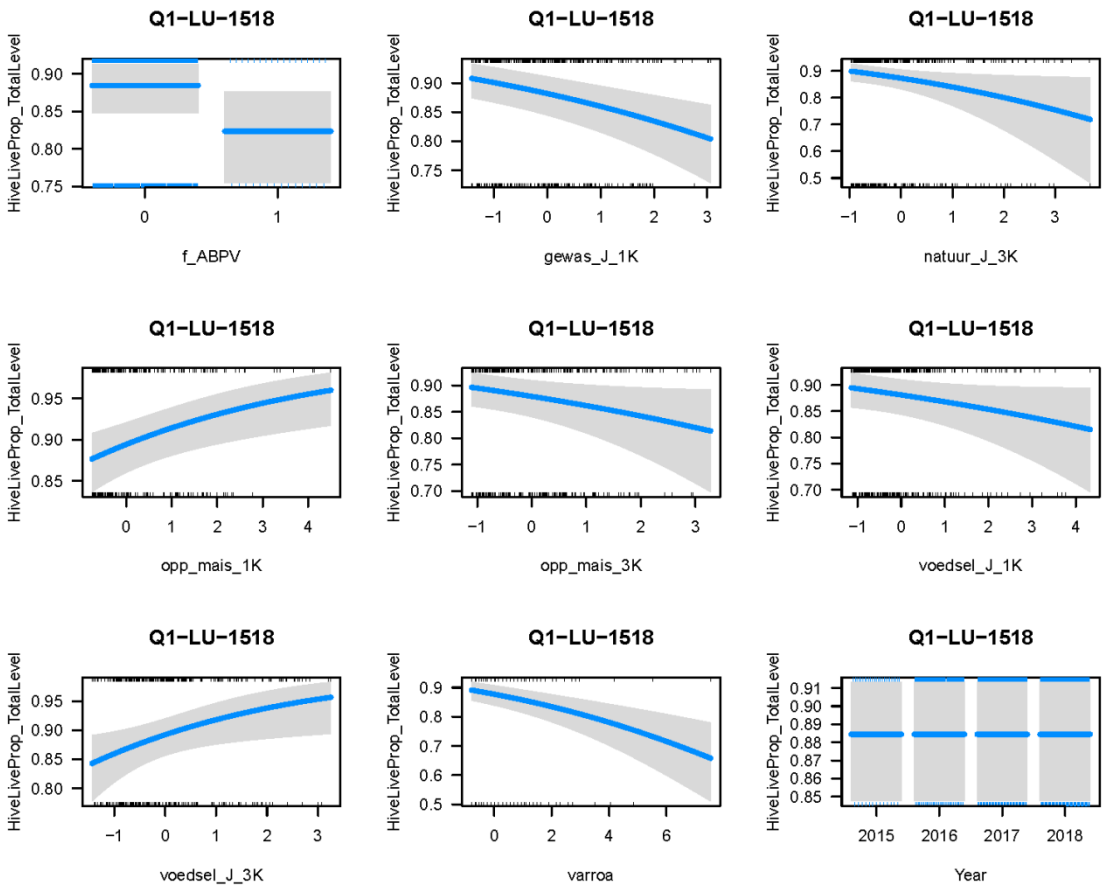


Figure 11. Relationships of factors affecting survival percentage at apiary level for the whole study (Q1). There is no difference in survival across the years (see figure lower right). Survival is much lower when the maximum level of Varroa in the apiary is high and when ABPV is present. Several landscape factors are slightly negative for bee survival (all graphs declining) with only amount of forage in the landscape and amount of maize grown close to the hives being slightly positive.

Table 14. Factors related to the survival percentage of individual colonies. Values indicate the estimates from the model with standard error in parentheses. The final model is Best 1, which is better than the full model, 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. In bold are the factors showing most impact (see also figure 12).

Q2 A1 Final overall models 1518		
	Q2A1 full model	**Q2A1 best model**
(Intercept)	2.05 (0.24)	4.60 (0.84)
Number of land-use classes in 1km	**-0.38 (0.15)**	**-0.16 (0.06)**
Maize area in 1km	**0.42 (0.20)**	**0.02 (0.01)**
Maize area in 3km	-0.34 (0.20)	-0.00 (0.00)
Imidacloprid	8.5 (35.1)	254.4 ($1.9x10^7$)
Dimethoaat	-1.38 (0.74)	-1.26 (0.71)
Asteraceae	**-0.26 (0.11)**	**-2.42 (0.88)**
Trifolium	-0.15 (0.11)	
Varroa mites / per 100 bees	**-0.32 (0.10)**	**-0.05 (0.02)**
Nosema ceranae present	0.27 (0.26)	
ABPV present	-0.41 (0.44)	
AIC	620.71	618.60
BIC	680.71	664.76
Log Likelihood	-297.35	-299.30
Num. obs.	747	747
Num. groups: Year:Imker	373	373
Num. groups: Imker	328	328
Var: Year:Imker (Intercept)	0.91	0.93
Var: Imker (Intercept)	0.00	0.00

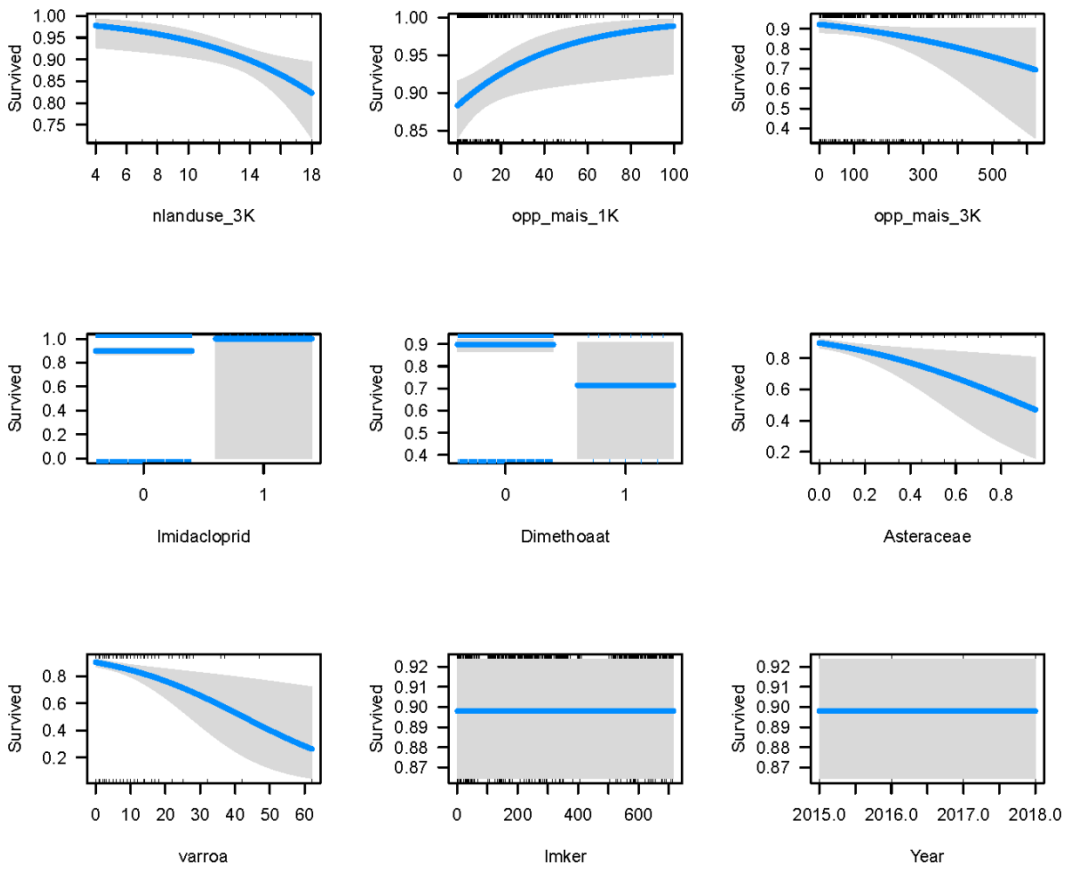


Figure 12. Relationships of factors affecting survival percentage of individual colonies for the whole study including any trace of any chemical observed (Q2A1). There is no difference in survival across the years (see figure lower right). Survival is much lower when the incidence of Varroa is high, when more maize is present in the landscape or the landscape is highly fragmented (3km area), when Asteraceae pollen is present or when dimethoate is detected. Positive effect on survival has the area of maize close to the hives (<1km), whereas presence of imidacloprid has a slight effect

5. Conclusions & Remarks

Conclusions: honeybee winter survival monitor 2017-2018

1 **Winter survival was 84.3% in 2017-2018** (15.7% of colonies died). While higher survival is always preferred, this figure is almost in line with the normal variation of around 5-15% winter mortality. Survival has now been above 80% for the last six years and above 85% for five of the last six years.

2 **Most beekeepers do not lose colonies in the winter** (unknown% in 2014-15; 64% in 2015-16; 53% in 2016-17 and 55% in 2017-18)

3 **Dutch beekeepers managed at least 71-81,000 colonies in 2017**.

Conclusions: factors correlated to honeybee winter survival 2014-2018

4 **Factors linked to beekeeping practice emerge as the most consistent factor determining colony winter survival.** Colony survival in apiaries is lower when *Varroa* mite numbers are high and when ABPV virus is present. Better control of parasites and diseases will further improve colony survival.

5 **A large number of variables each contribute just a little to explain why a honeybee colony survives or dies.** A few factors seem to have slightly more importance, but no single factor comes out as the main driver of colony winter loss. Colonies containing more Varroa mites and placed in highly fragmented landscape with substantial amounts of maize crops have lower survival probability. When stored pollen contained more Asteraceae, survival was also slightly lower.

6 **Chemical residues of neonicotinoids and other compounds were detected in 30% of samples, but their presence is not correlated with winter mortality**, except for the rarely detected chemical dimethoate the presence of which correlated strongly with colony mortality.

7 **Most other factors we studied** (pollen sources, landscape features) **also played small or no role in colony survival**. Many factors are correlated with each other and each year a different subset is related to winter loss (see Appendix F). 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.**

8 Impact of the five main factors that have been analysed can be summarized as follows [Note that for interpretation of all findings in this study, as in other studies, it is important to stress 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 also keeps associated viruses (DWV and ABPV) low. Factors linked to beekeeping practice come out as the most consistent factor determining colony winter survival. Training and education of beekeepers is of great importance when honeybee health and survival is to be improved.

Pests and diseases: Varroa and its associated viruses (e.g. DWV and ABPV) are omnipresent in Dutch honeybee hives. *Nosema ceranae* seems to be decreasing each year (see Appendix H).

Chemical residues: In this study we assessed whether the presence of chemical residues in stored winter food was correlated to colony winter survival. We did not study the effect of such compounds on the survival of individual bees exposed to these chemicals. Of the more than 100 chemical

compounds we screened for, including all neonicotinoids, several were detected in stored winter food in autumn (see Appendix H for spatial distribution on the most frequently found chemicals). Three of these substances are used by beekeepers for Varroa-control. Systemic insecticides, e.g. neonicotinoids, continue to be present in honey samples despite the severe limitations on the use of some of them in open cultivation. Samples positive for thiacloprid and to a lesser extent acetamiprid increased, whereas samples positive for imidacloprid decreased over the four years. Other chemical residues were rarely found and when present were not related to colony mortality, except for dimethoate, which has a strong link to colony mortality.

Pollen sources: Each year slightly different relationships turn up in the final models, but the four year study only reveals a slight negative trend in survival when Asteraceae pollen is present in large amounts. In individual years a similar correlation was found with presence of clover pollen. Both plant groups are good pollen sources, but a high amount stored in the hive may indicate an overreliance of these single pollen sources or lack of diversity of pollen sources (although no correlation with number of pollen sources was observed). Further study is needed to reveal what is behind this finding.

Landscape conditions: Highly diverse, fragmented, landscapes are linked to lower colony 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). The number of land use classes is strongly correlated to the area used for agricultural crops. In the most fragmented landscapes (with substantial crop area) colony survival was slightly lower. In addition, the relation with specific landscape variables is rather complex with maize at landscape scale being negative, but slightly positive when occurring close to the hive.

Remarks and suggestions for further research

1. **A randomized survey as an annual monitor of honeybee winter survival can be easily organized by Dutch beekeeper associations and Bijen@wur and requires limited external funds.** This is possible based on (1) the online forms and procedure developed by our consortium; (2) the experience from the last three years; and (3) the willingness of before-mentioned parties.
2. **Beekeepers should refrain from using illegal chemical substances for disease and pest control.** Among the chemicals we detected in winter food stored in bee hives, illegal acaricides, such as coumaphos and amitraz were regularly encountered.
3. **The role of the forage sources and surrounding landscape is complex and warrants additional study.** Analysis of the spring samples taken from selected colonies may give, together with the already analysed autumn samples, a more complete insight in the relationship between landscapes and colony survival.
4. **The clear patterns in disease prevalence in our sampling** (ABPV increasing, *Nosema ceranae* decreasing) **may need further study.** Given that pests and disease are prominent among the factors linked to colony loss, understanding the relationship better would be useful as would training of beekeepers in diagnosis of such diseases.
5. **Beekeeping in the Netherlands is largely a hobby and beekeeping associations should be responsible for education and training on bee management, diseases and other threats, and for (legal) solutions for disease treatment.** Government-imposed measures and control are less likely to render good results.

6. Appendices

A Winter mortality survey based on CoLoSS questionnaire

B Overview of results from GLMM analyses surveillance study

C List of food plants found in stored pollen

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

E Summary of residues detected in winter food / honey samples

F Overview of the factors important for honeybee winter survival over 4 years

G Overview of sampling points used in the four years of surveillance

H Spatial occurrence of different factors across the Netherlands over 4 years

Appendix A

Winter mortality Survey based on CoLoSS questionnaire

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 enquête in te vullen!

*Vereist

Imker gegevens

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.

1. **Naam imker**

2. **Adres bijenstand (zo precies mogelijk)** *

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

Korte/Lange enquete?

Wij bieden u de mogelijkheid te kiezen voor een korte enquête (hier kunt u alleen de uitwinteringsgegevens doorgeven) of een meer uitgebreide enquête, waarin ook naar andere aspecten gevraagd wordt (zoals varroa bestrijding etc.)

De korte enquête vraagt slechts naar de uitwintering,

De langere enquête bestaat uit een aantal extra vragen en duurt ongeveer 5 minuten.

4. **Wilt u de korte of iets langere enquete invullen?** *
   Markeer slechts één ovaal.

   ◯ Kort - alleen de vraag over de uitwintering *Ga naar vraag 16.*
   ◯ Langer - extra vragen over onder andere 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

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.

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
   - [ ] Winterbehandeling

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: _____

Najaarsdracht

De najaarsdracht is de laatste dracht voor de inwintering. Afhankelijk van de imkermethode wordt deze najaarsdracht 1) gebruikt als wintervoer en wordt er dus niet geslingerd; 2) wordt de najaarshoning deels gebruik als wintervoer en aangevuld met suikeroplossing om voldoende wintervoer aan de volken te geven en 3) de najaarshoning wordt geheel geslingerd en de volken worden 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: _____

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

_____

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

_____

We publiceren de resultaten van deze enquête op 15 april

Houdt hiervoor de website en de nieuwsbrief van de NBV in de gaten!
(http://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-international/Over-Plant-Research-International/Organisatie/Biointeracties-Plantgezondheid/Bijen/Surveillanceprogramma-Honingbijen.htm

Stop met het invullen van dit formulier.

In- en uitwinteringscijfers

16. **Hoeveel volken heeft u ingewinterd in 2016?** *

_____

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 B

Overview of results from GLMM analyses surveillance study

Full model for Q1

Q1 LU Models 2017		
	LU Full	**Best 1**
(Intercept)	**1.90 (0.44)**	**1.65 (0.28)**
Number of land-use classes in 1km	**0.15 (0.04)**	**0.12 (0.04)**
Maize area in 1km	-0.03 (0.02)	-0.03 (0.02)
Forage area in 1km	-0.00 (0.00)	-0.00 (0.00)
Number of land-use classes in 3km	-0.04 (0.04)	
Nature area in 3km	-0.00 (0.00)	
Crop area in 3km	**-0.00 (0.00)**	**-0.00 (0.00)**
Varroa mites / 100 bees	**-0.05 (0.01)**	**-0.05 (0.01)**
Nosema ceranae present	**-0.68 (0.23)**	**-0.65 (0.23)**
DWV present	0.16 (0.20)	
ABPV present	**-0.56 (0.20)**	**-0.54 (0.20)**
Hives IN	-0.01 (0.00)	
AIC	526.72	523.36
BIC	560.37	545.79
Log Likelihood	-251.36	-253.68
Deviance	318.26	322.90
Num. obs.	122	122

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

Q2 A0 LU Models		
	LU Full	**Best 1**
(Intercept)	2.19 (0.48)	2.27 (0.52)
Number of land-use classes in 1km	0.15 (0.37)	
Maize area in 1km	-0.12 (0.27)	
Nature area in 1km	-0.65 (0.34)	-0.66 (0.36)
Number of land-use classes in 3km	-0.51 (0.36)	
Forage area in 3km	0.72 (0.42)	0.67 (0.41)
Crop area in 3km	0.24 (0.31)	
AIC	199.63	194.46
BIC	226.63	207.96
Log Likelihood	-91.81	-93.23
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.46	1.91

Q2 A0 Virus Models		
	Virus Full	**Best 1**
(Intercept)	**1.74 (0.54)**	**1.85 (0.54)**
Varroa mites / 100 bees	-0.06 (0.04)	-0.06 (0.04)
Nosema ceranae present	0.99 (0.92)	
DWV present	1.02 (0.55)	0.99 (0.54)
ABPV present	**-1.49 (0.65)**	**-1.49 (0.65)**
AIC	187.87	187.23
BIC	208.12	204.11
Log Likelihood	-87.93	-88.62
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.41	1.42

Q2 A0 Pollen		
	Pollen Full	**Best 1**
(Intercept)	**2.30 (0.54)**	**2.35 (0.57)**
Number of pollen	-0.23 (0.26)	
Asteraceae	-0.50 (0.27)	-0.40 (0.23)
Brassicaceae	-0.27 (0.33)	
Calluna	0.14 (0.39)	
Castanea	-0.46 (0.26)	-0.33 (0.23)
Cornus	-0.06 (0.22)	
Fabaceae	-0.23 (0.24)	
Heracleum	-0.30 (0.23)	
Impatiens	-0.32 (0.29)	
Rosaceae	-0.24 (0.25)	
Trifolium	-0.00 (0.29)	
Zea	-0.06 (0.25)	
AIC	208.88	194.01
BIC	256.13	207.51
Log Likelihood	-90.44	-93.00
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.85	2.25

Q2 A0 Chemicals Models		
	Chemicals Full	**Best 1**
(Intercept)	**2.24 (0.28)**	**2.28 (0.27)**
Acetamiprid present	26.04 (304026.72)	25.15 (261852.11)
Thiacloprid present	1.70 (1.76)	
Boscalid present	-2.48 (1.81)	-1.14 (1.18)
AIC	195.67	195.20
BIC	212.55	208.70
Log Likelihood	-92.84	-93.60
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	2.23	2.18

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

Q2 A1 LU Models		
	LU Full	**Best 1**
(Intercept)	**2.19 (0.48)**	**2.27 (0.52)**
Number of land-uses classes in 1km	0.15 (0.37)	
Maize area in 1km	-0.12 (0.27)	
Nature area in 1km	-0.65 (0.34)	-0.66 (0.36)
Number of land-uses classes in 3km	-0.51 (0.36)	
Forage area in 3km	0.72 (0.42)	0.67 (0.41)
Crop area in 3km	0.24 (0.31)	
AIC	199.63	194.46
BIC	226.63	207.96
Log Likelihood	-91.81	-93.23
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.46	1.91

Q2 A1 Virus Models		
	Virus Full	**Best 1**
(Intercept)	**1.54 (0.52)**	**1.64 (0.52)**
Varroa mites / 100 bees	-0.30 (0.21)	-0.31 (0.21)
Nosema ceranae present	0.99 (0.92)	
DWV present	1.02 (0.55)	0.99 (0.54)
ABPV present	**-1.49 (0.65)**	**-1.49 (0.65)**
AIC	187.87	187.23
BIC	208.12	204.11
Log Likelihood	-87.93	-88.62
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.41	1.42

Q2 A1 Pollen Models		
	Pollen Full	**Best 1**
(Intercept)	**2.30 (0.54)**	**2.35 (0.57)**
Number of pollen	-0.23 (0.26)	
Asteraceae	-0.50 (0.27)	-0.40 (0.23)
Brassicaceae	-0.27 (0.33)	
Calluna	0.14 (0.39)	
Castanea	-0.46 (0.26)	-0.33 (0.23)
Cornus	-0.06 (0.22)	
Fabaceae	-0.23 (0.24)	
Heracleum	-0.30 (0.23)	
Impatiens	-0.32 (0.29)	
Rosaceae	-0.24 (0.25)	
Trifolium	-0.00 (0.29)	
Zea	-0.06 (0.25)	
AIC	208.88	194.01
BIC	256.13	207.51
Log Likelihood	-90.44	-93.00
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.85	2.25

Q2 A1 Chemicals Models		
	Chemicals Full	**Best 1**
(Intercept)	**2.14 (0.27)**	**2.17 (0.26)**
Acetamiprid present	26.99 (521821.81)	26.15 (391123.39)
Thiacloprid present	0.73 (0.99)	
Boscalid present	-1.76 (1.48)	
AIC	195.38	192.86
BIC	212.26	202.99
Log Likelihood	-92.69	-93.43
Num. obs.	216	216
Num. groups: Imker.x	116	116
Var: Imker.x (Intercept)	1.98	1.98

Appendix C

List of food plants found in stored pollen

Pollen type sorted by pollen most frequently found in 2017. Total of 68 different pollen types were found.

Species	found in # samples	% of total	min	max	ave. When present
Brassicaceae	134	50.6	0	100	31.8
Trifolium	105	39.6	0	100	27.9
Asteraceae	92	34.7	0	95	19.7
Rosaceae	86	32.5	0	85	22.0
Castanea	58	21.9	0	85	32.9
Phacelia	58	21.9	0	65	18.4
Trifolium pratense	58	21.9	0	85	25.5
Impatiens	51	19.2	0	100	38.1
Hedera	49	18.5	0	100	41.8
Calluna	43	16.2	0	95	33.6
Caryophyllaceae	34	12.8	0	50	11.9
Ranunculaceae (Clematis)	31	11.7	0	80	17.4
Parthenocissus	28	10.6	0	35	10.2
Heracleum	24	9.1	0	15	6.0
Taraxacum	24	9.1	0	35	9.4
Fabaceae	23	8.7	0	85	30.7
Aesculus	21	7.9	0	65	15.7
Centaurea jacea	18	6.8	0	25	10.0
Ligustrum	15	5.7	0	20	8.7
Persicaria	14	5.3	0	10	5.7
Amaryllidaceae	13	4.9	0	35	16.2
Lythrum	13	4.9	0	20	7.7
Origanum	13	4.9	0	20	8.8
Hypericum	11	4.2	0	20	10.9
Centaurea cyanus	10	3.8	0	15	8.5
Chenopodium	9	3.4	0	40	10.6
Cirsium	8	3.0	0	10	6.3
Rubus	8	3.0	0	50	20.0
Ranunculaceae - Papaver	7	2.6	0	70	31.4
Melilotus	6	2.3	0	45	15.8
Pisum	6	2.3	0	10	8.3
Spiraea	6	2.3	0	60	26.7
Tropaelum majus	6	2.3	0	15	8.3
Apiaceae	5	1.9	0	5	5.0

Eryngium	5	1.9	0	95	33.0
Zea	5	1.9	0	10	6.0
Asparagus	4	1.5	0	65	36.3
Epilobium	4	1.5	0	10	6.3
Foeniculum	4	1.5	0	20	8.8
Robinia	4	1.5	0	80	30.0
Salvia	4	1.5	0	20	12.5
Aegopodium	3	1.1	0	10	6.7
Cornus	3	1.1	0	5	5.0
Lotus	3	1.1	0	5	5.0
Medicago	3	1.1	0	25	13.3
Poacceae	3	1.1	0	15	13.3
Polygonum	3	1.1	0	25	13.3
Symphoricarpus	3	1.1	0	15	8.3
Acer	2	0.8	0	30	17.5
Campanulaceae	2	0.8	0	10	7.5
Echium	2	0.8	0	15	10.0
Filipendula	2	0.8	0	10	10.0
Quercus	2	0.8	0	15	12.5
sporen	2	0.8	0	15	10.0
Borago	1	0.4	0	5	5.0
Cytisus-type	1	0.4	0	10	10.0
Datura	1	0.4	0	5	5.0
Helianthus-type	1	0.4	0	10	10.0
Ilex	1	0.4	0	5	5.0
Jasione	1	0.4	0	5	5.0
Mahonia	1	0.4	0	15	15.0
Nymphaea	1	0.4	0	5	5.0
Nymphoides	1	0.4	0	5	5.0
Oreganum	1	0.4	0	5	5.0
Reseda (?)	1	0.4	0	45	45.0
Rhamnus	1	0.4	0	5	5.0
Solanum	1	0.4	0	50	50.0
Vitis	1	0.4	0	85	85.0

Appendix D

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.

Component	LOQ (µg/kg) 2017	Component	LOQ (µg/kg) 2017
6-Chloronicotinic_acid	10	Fluopyram	1
Abamectin	10	Fluquinconazole	1
Acetamiprid	0.5	Flusilazole	1
Aldicarb sulfon	5	Fluvalinate tau	2
Azamethifos	1	Haloxyfop-methyl	5
Bendiocarb	1	Imidacloprid	0.5
Bifenazate	1	Imidacloprid_5-Hydroxy	5
Bifenthrin	1	Imidacloprid_desnitro	0.5
Bixafen	1	Imidacloprid_desnitro	0.5
Boscalid	1	Imidacloprid_olefin	5
Carbaryl	1	Imidacloprid_urea	0.5
Carbendazim	1	Indoxacarb	2
Chlorfenvinphos	1	Ioxynil	1
Chlorpyriphos	1	Malathion	1
Chlorpyriphos-methyl	5	Metaflumizone	1
Clothianidin	2	Metazachlor	1
Coumaphos	2	Methidathion	5
Cyfluthrin-Beta	1	Methomyl	1
Cypermethrin	5	Novaluron	5
Cyproconazole	1	Omethoate	1
Deltametrin	5	Paraoxon-methyl	1
Diazinon	1	Pendimethalin	5
Dichlorprop	5	Permethrin	5
Dichlorvos	5	Phorate	1
Dimethoate	1	Phorate sulfon	1
Disulfoton-sulfone	1	Phorate sulfoxide	1
Disulfoton-sulfoxide	5	Phorate-O sulfoxide	1
DMA	25	Phosmet	1
DMF	5	Phoxim	1
DMPF	5	Pirimiphos-methyl	1
Edifenphos	1	Prochloraz	1
Emamectin	2	Profenofos	1
Epoxiconazole	1	Propetamphos	1
Esfenvalerate	1	Propiconazole	5

Ethiofencarb	1	Propoxur	1
Ethiofencarb sulfon	1	Prothioconazole-desthio	1
Ethiofencarb sulfoxide	1	Pyrazophos	1
Etofenprox	1	Pyridaben	1
Famoxadone	5	Pyridate	1
Fenpropidin	5	Rotenone	1
Fenpropimorph	1	Spinosyn A	1
Fensulfothion	1	Spinosyn D	5
Fensulfothion-O sulfon	1	Spiroxamine	1
Fensulfothion-O	1	Tebuconazole	1
Fensulfothion-sulfon	1	Teflubenzuron	1
Fenthion	1	Tepraloxydim	5
Fenthion sulfon	1	Tetrachlorfenvinphos	5
Fenthion sulfoxide	1	Tetraconazole	5
Fenthion-O sulfon	1	Thiabendazole	1
Fenthion-O sulfoxide	1	Thiabendazole	5
Fipronil	0.5	Thiacloprid	1
Fipronil-carboxamide	0.5	Thiamethoxam	2
Fipronil-desulfinyl	0.5	Thiophanate-methyl	1
Fipronil-sulfide	0.5	Triazophos	1
Fipronil-sulfone	0.5	Triflumizole	1
Fluazifop-P-butyl	1		

Appendix E

List of chemical residues detected in honey samples in each of the four years. Given are the percentages of samples in which traces were found (all samples >LOD; columns 3-6) and in which quantifiable amounts were found (only samples >LOQ; columns 7-10). The final two columns indicate the percentage of traces (all) and quantifiable (>LOQ) samples in the whole study.

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.

Use type	Chemical compound	% of samples with traces				samples with concentration > LOQ				% samples (1052)	
		2015	2016	2017	2018	2015	2016	2017	2018	all	>LOQ
Acaricide	Amitraz	7.8	7.3	2.6	4.4	5.6	2.1	1.8	0.7	5.0	1.9
Acaricide	Coumaphos	1.1	14.7	5.9	10.2	1.1	2.4	0.0	1.0	9.4	1.1
Acaricide	Fluvalinate tau	0.0	2.1	0.0	0.0	0.0	0.9	0.0	0.0	0.7	0.3
Fungicide	Boscalid	0.0	0.0	5.9	2.7	0.0	0.0	4.1	2.7	2.7	2.1
Fungicide	Carbendazim	0.0	0.0	0.6	0.3	0.0	0.0	0.3	0.3	0.3	0.2
Fungicide	Epoxiconazole	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.3	0.1	0.1
Fungicide	Fluopyram	0.0	0.0	0.9	1.4	0.0	0.0	0.6	1.4	0.7	0.6
Fungicide	Tebuconazole	0.0	0.0	1.5	0.0	0.0	0.0	0.6	0.0	0.5	0.2
Herbicide	Metazachlor	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.3	0.1	0.1
Herbicide	Pyridate	0.0	0.0	0.0	0.3	0.0	0.0	0.0		0.1	0.0
Insecticide	Chlorfenvinphos	0.0	0.0	0.6	0.3	0.0	0.0	0.6	0.3	0.3	0.3
Insecticide	Dimethoate	1.1	2.8	0.3	0.7	1.1	0.9	0.3	0.7	1.2	0.7
Insecticide	Permethrin	0.0	0.3	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.1
Insecticide	Spinosyn A/D	0.0	0.0	0.0	0.3	0.0	0.0	0.0		0.1	0.0
Insecticide	Teflubenzuron	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.3	0.3
Neonic	Acetamiprid	2.2	8.3	3.8	4.8	2.2	2.8	3.8	3.4	5.3	3.2
Neonic	Imidacloprid	6.7	2.8	0.3	1.0	6.7	2.8	0.3	1.0	1.8	1.8
Neonic	Thiacloprid	2.2	37.6	8.2	12.6	2.2	9.8	4.4	7.5	18.1	6.7
Neonic	Thiamethoxam /Clothianidin	0.0	3.1	0.6	0.0	0.0	0.9	0.0	0.0	1.1	0.3

Appendix F

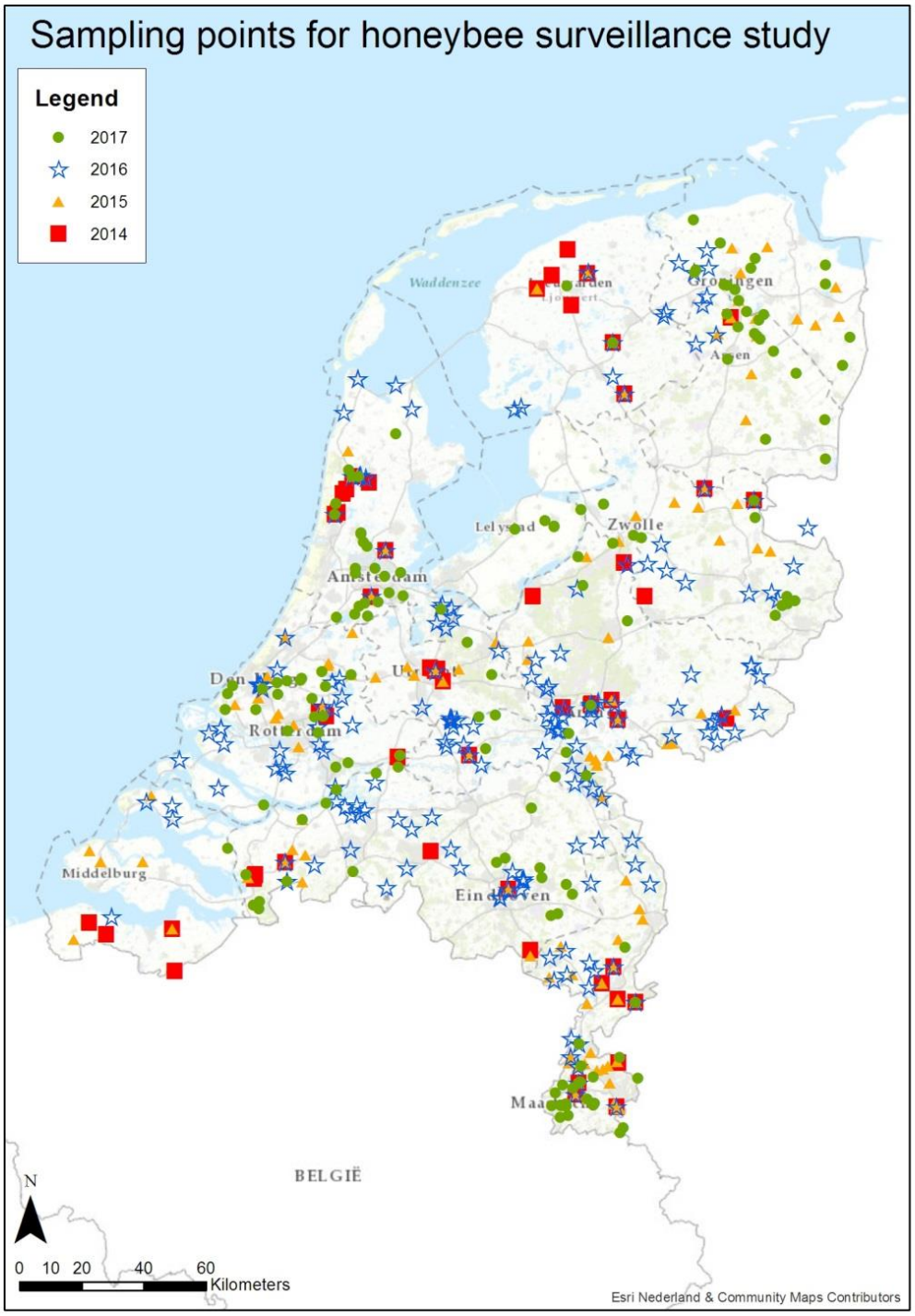
Overview of the factors important for honeybee winter survival for each of the four years of the honeybee surveillance study and the overall model for Questions 1 (Q1 model) and 2 (Q2A1 model). POS=important positive correlation with survival, pos= positive correlation in some models, [pos] small positive correlation. NEG=important negative correlation with survival, neg= negative correlation in some models, [neg] small negative correlation.

Q1 model	2015	2016	2017	2018	Overall
Hives IN		POS			
ABPV			NEG	NEG	NEG
DWV					[neg]
Nosema ceranae				NEG	[neg]
Varroa Max%	NEG	NEG		NEG	NEG
Crop area 1km			neg		NEG
Crop area 3km				NEG	[neg]
Forage 3km			pos		POS
Forage area 1km				NEG	NEG
Land use classes 1km		NEG		POS	[neg]
Land use classes 3km	NEG		NEG		[neg]
Maize area 1km		POS		NEG	POS
Maize area 3km					NEG
Nature area 1km	NEG		neg		[pos]
Nature area 3km	POS				NEG

Q2 A1 model	2015	2016	2017	2018	Overall
Acetamiprid				POS	
Boscalid			neg		
Dimethoate		NEG			NEG
Imidacloprid					POS
Tebuconazole			NEG		
ABPV				NEG	[neg]
DWV				POS	
Nosema ceranae			POS		[pos]
Varroa%	NEG				NEG
Crop area 1km	POS				
Forage 3km				POS	
Land Use Classes [1km]	neg	NEG	NEG		NEG
Maize area 1km					POS
Maize area 3km					NEG
Nature area 1km				NEG	
Nature area 3km	neg				
Asteraceae					NEG
Brassicaceae			POS		
Heracleum			pos		
Impatiens	neg		POS		
Trifolium	NEG	NEG			[neg]

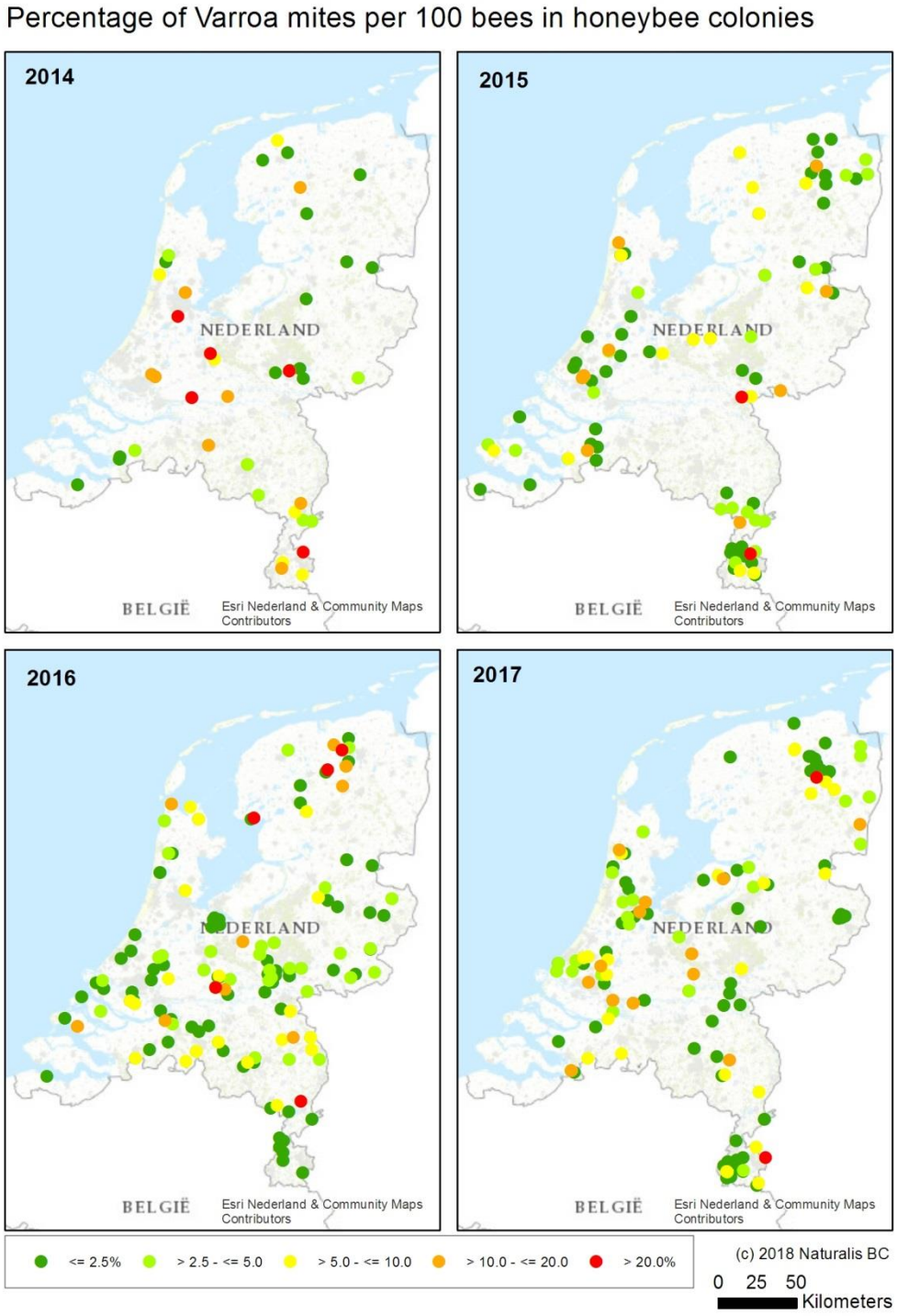
Appendix G

Overview of sampling points used in the honeybee surveillance study in each of the four years of survey.

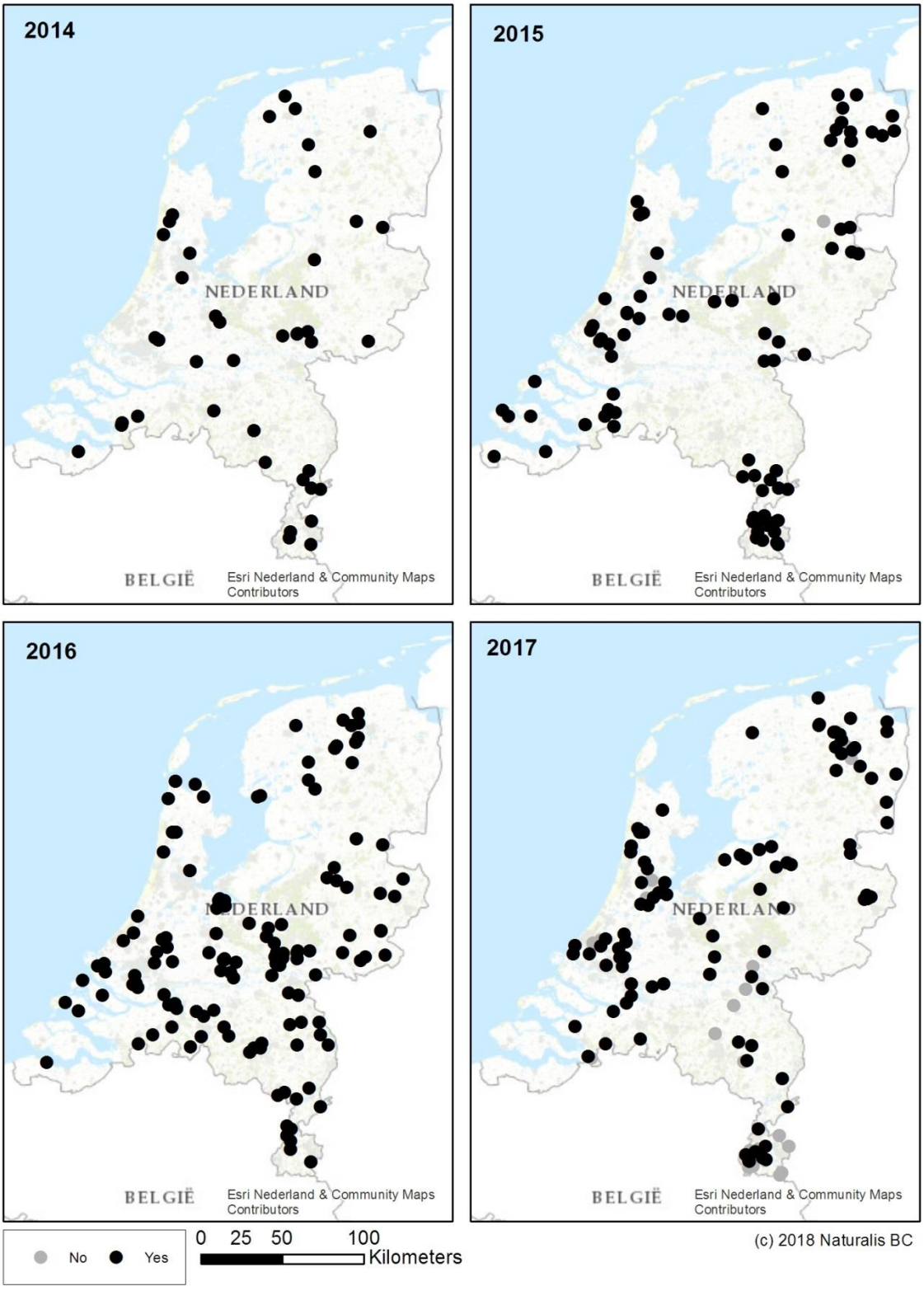


Appendix H

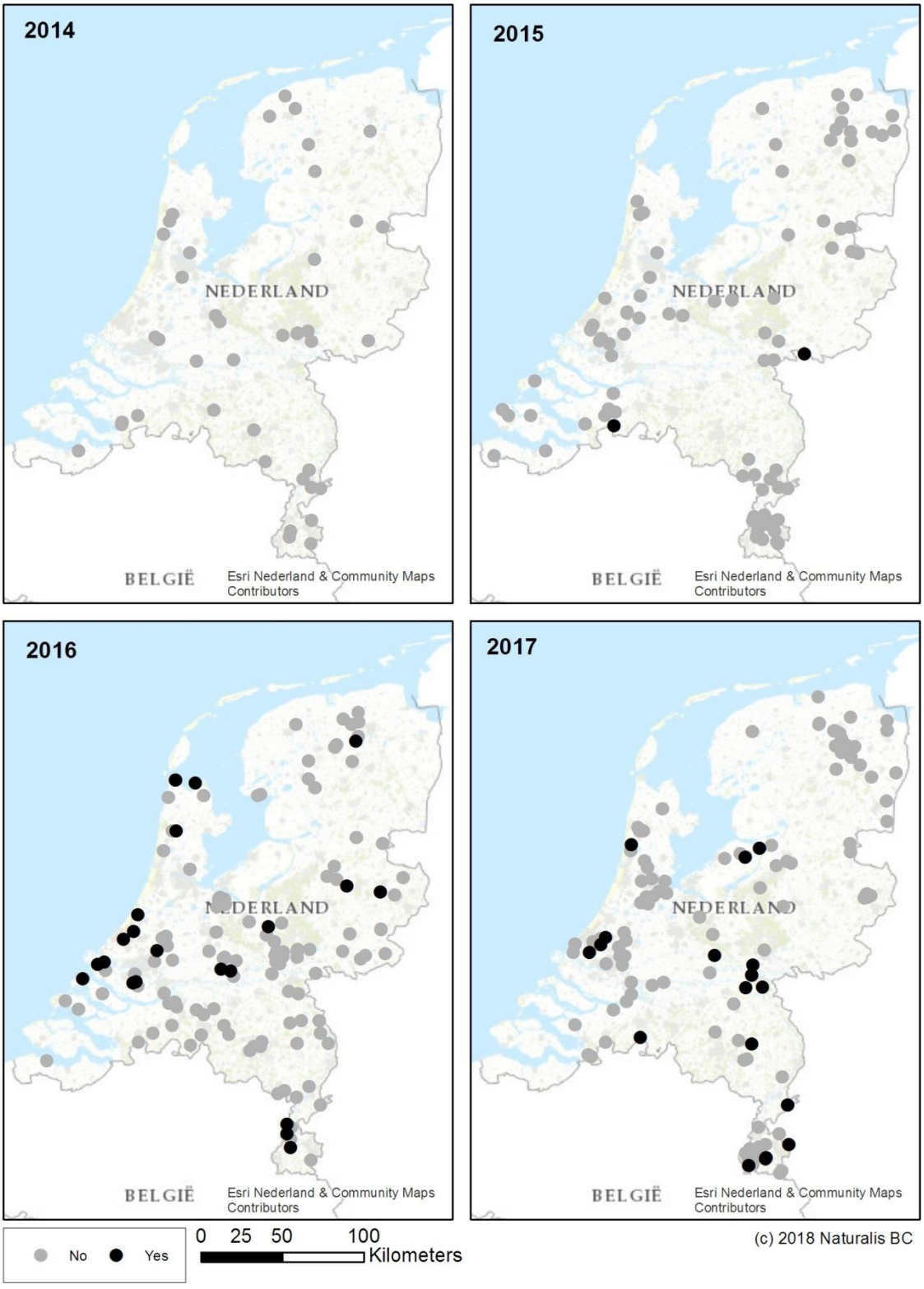
Spatial occurrence of different factors and variables across the Netherlands and across the four years of study. For chemical residues only samples with residue concentration above Level of Quantification for that chemical compound is indicated as presence.



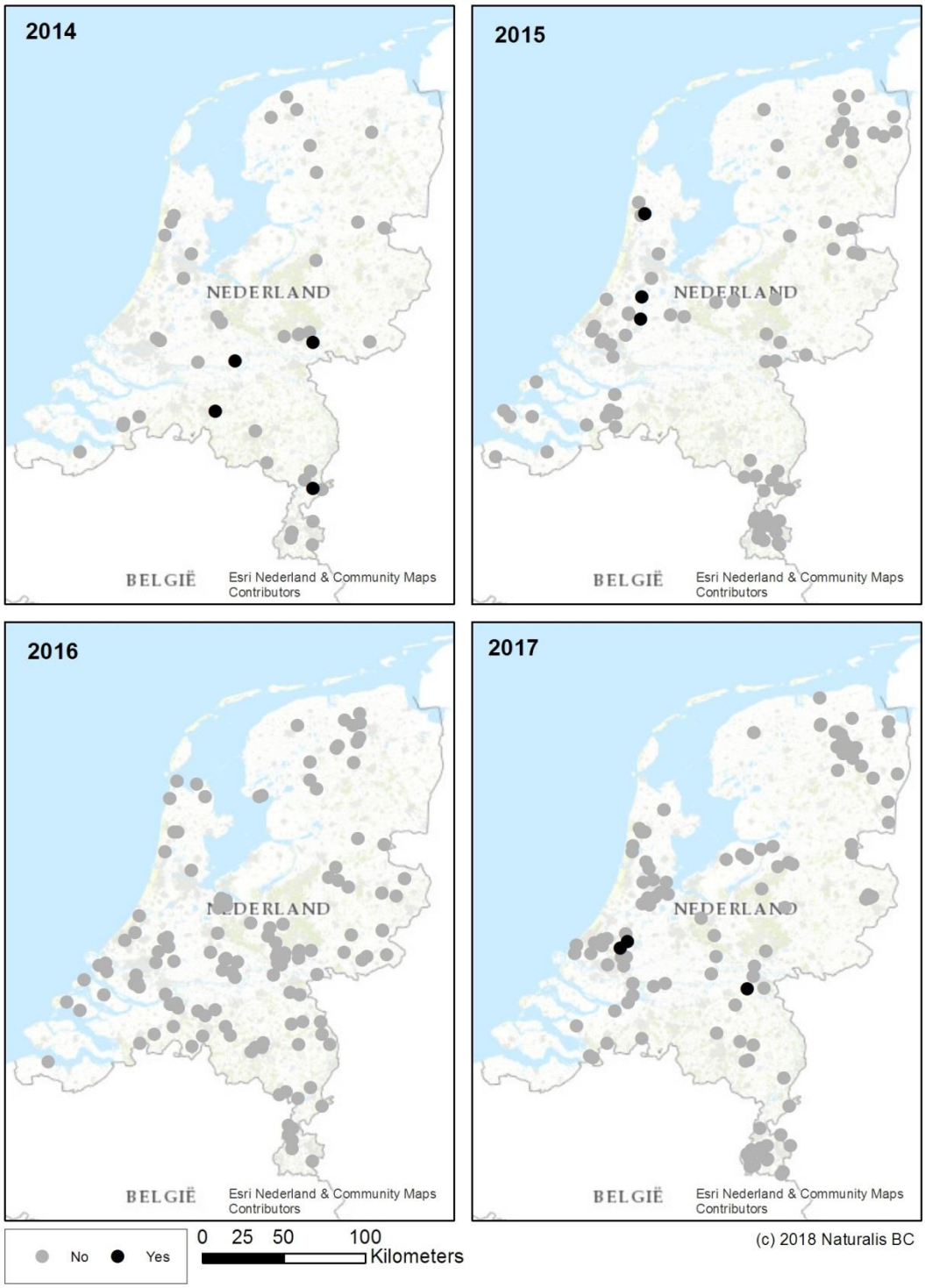
Presence of DWV in honeybees colonies



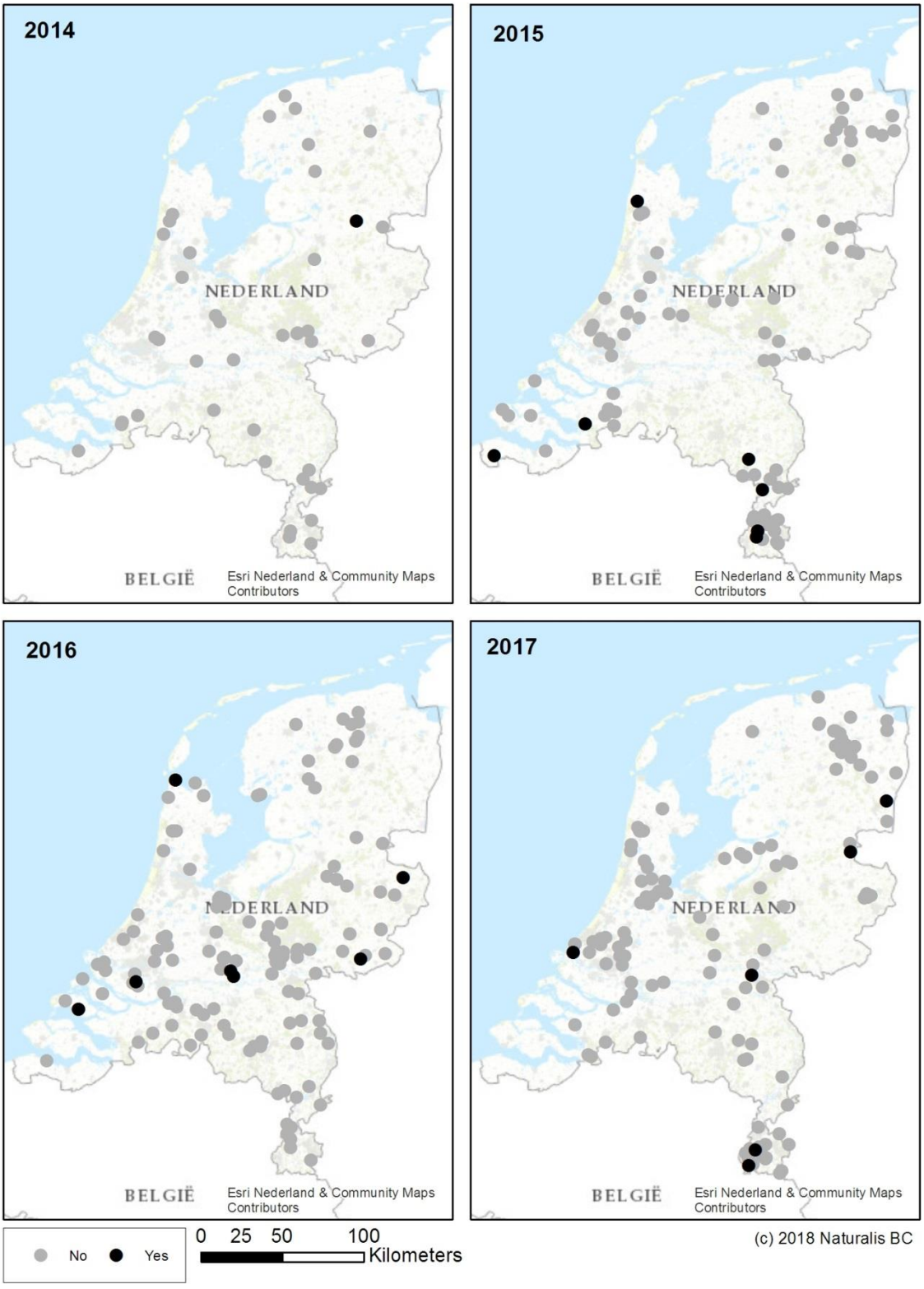
Presence of ABPV in honeybees colonies



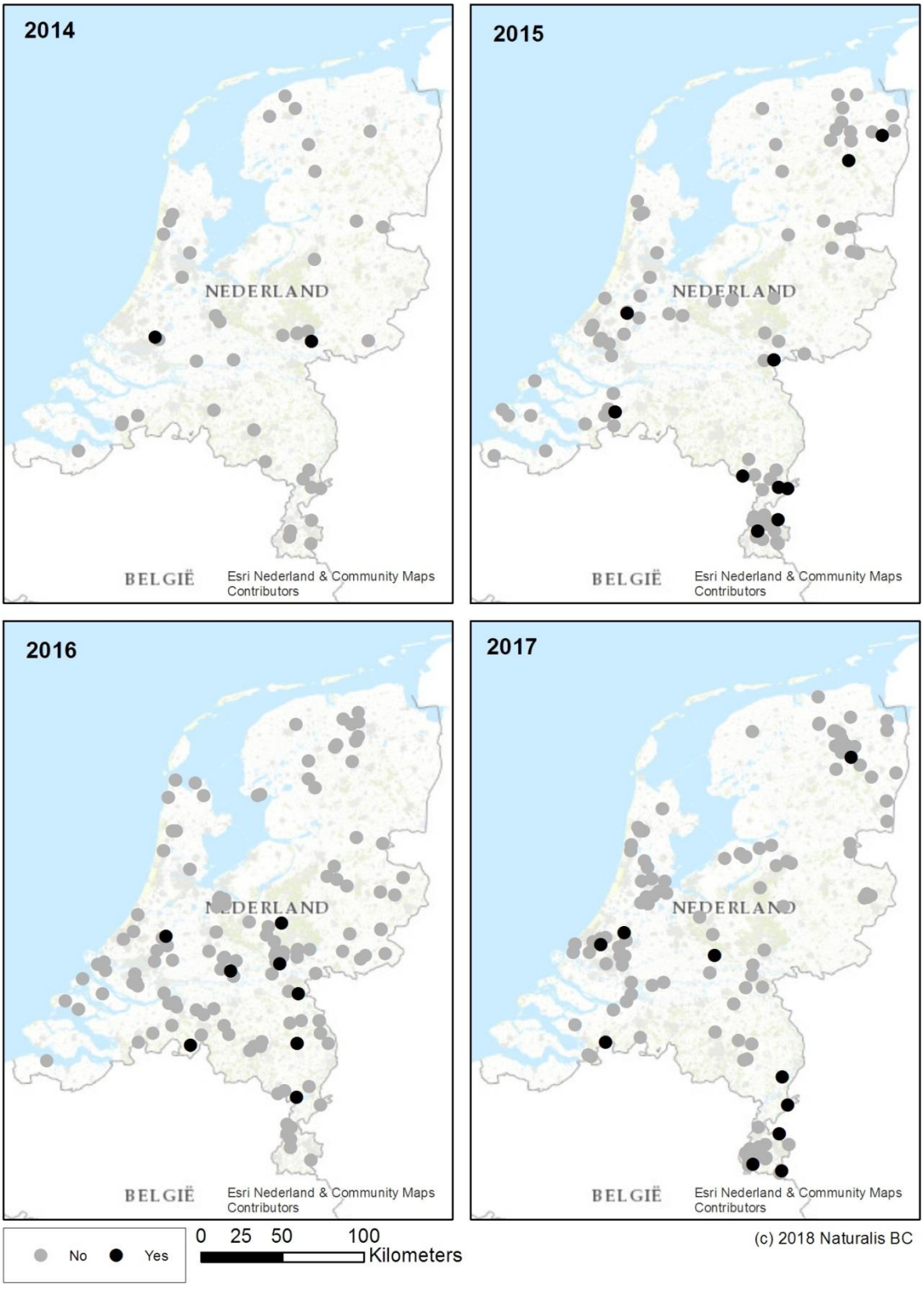
Presence of Imidacloprid in stored winter food of honeybee colonies



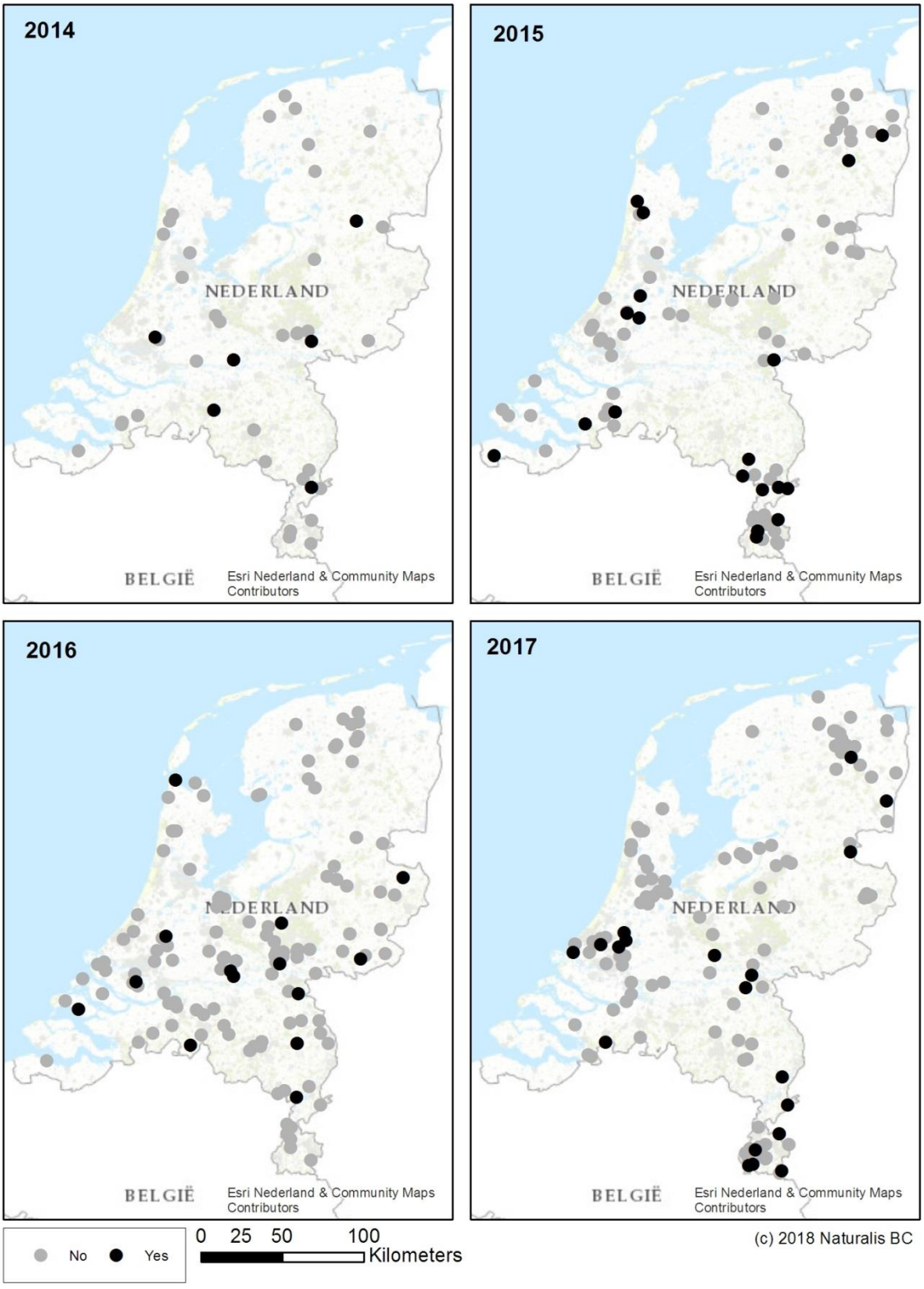
Presence of Acetamiprid in stored winter food of honeybee colonies



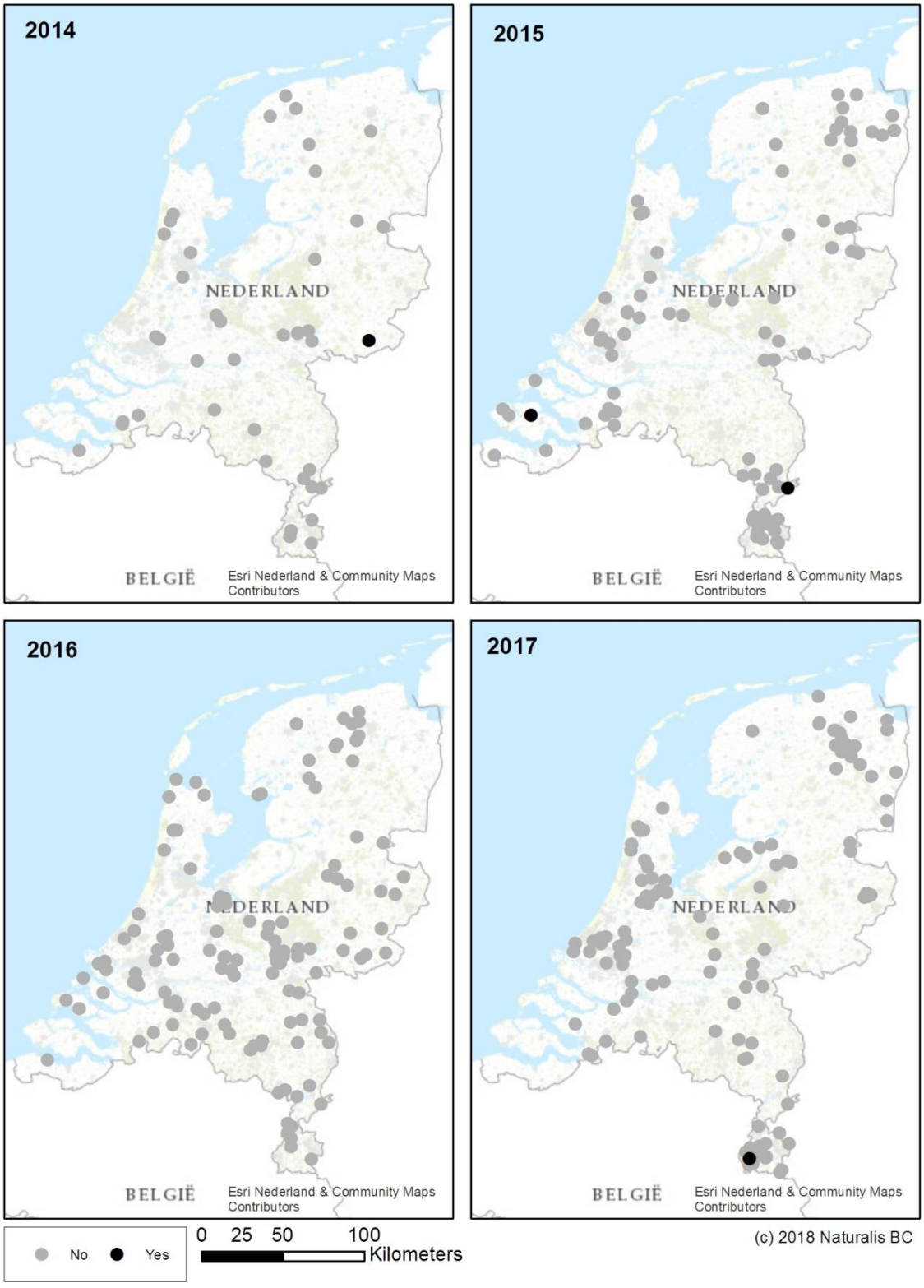
Presence of Thiacloprid in stored winter food of honeybee colonies



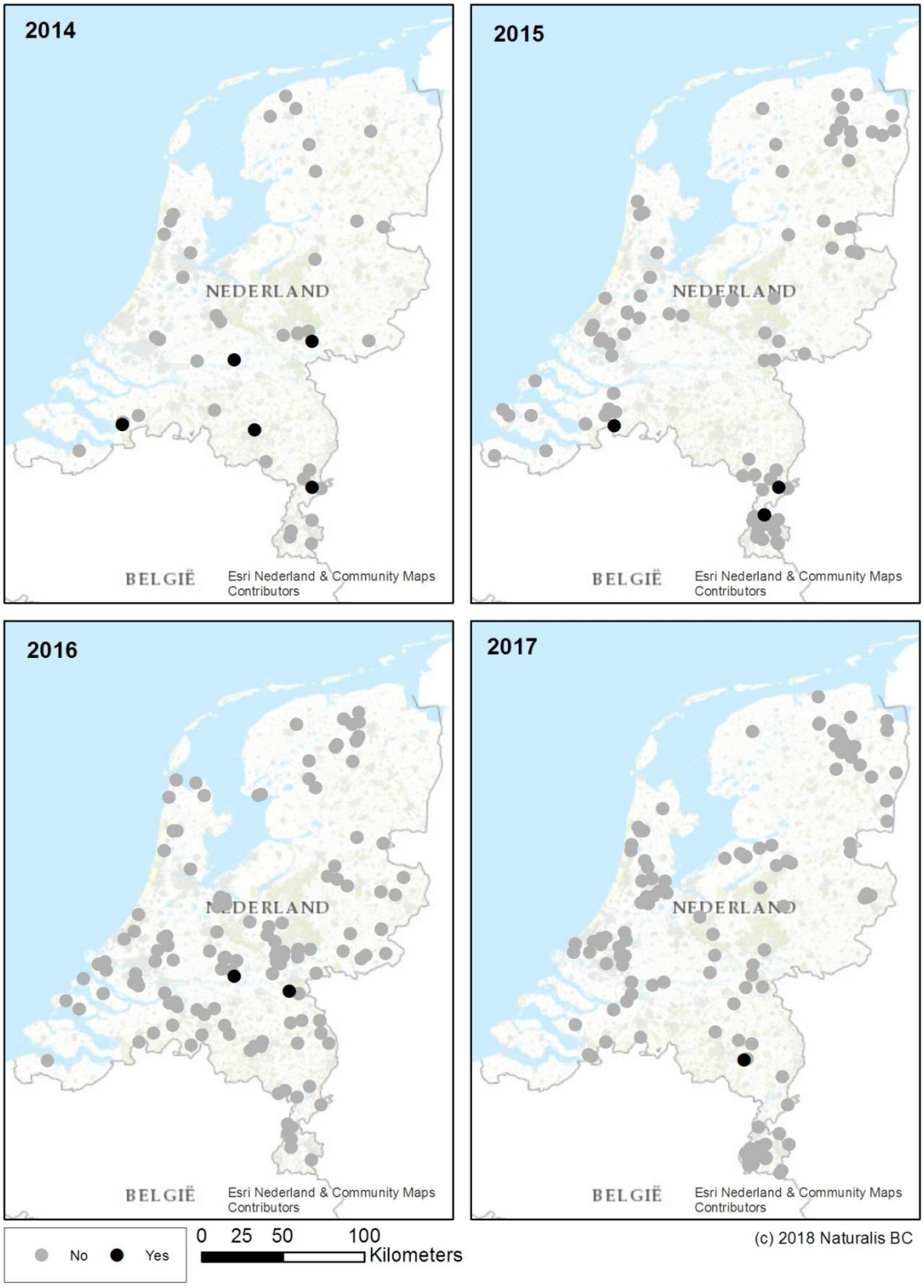
Presence of neonicotinoids in stored winter food of honeybee colonies



Presence of Dimethoate in stored winter food of honeybee colonies



Presence of Amitraz in stored winter food of honeybee colonies



Presence of Coumaphos in stored winter food of honeybee colonies