esa

ECOSPHERE

Single and interactive effects of *Varroa destructor*, *Nosema* spp., and imidacloprid on honey bee colonies (*Apis mellifera*)

Coby van Dooremalen,† Bram Cornelissen, Chula Poleij-Hok-Ahin, and Tjeerd Blacquière

Bees@wur, Wageningen Plant Research, Wageningen University and Research, Droevendaalsesteeg 1, Wageningen 6708 PB The Netherlands

Citation: van Dooremalen, C., B. Cornelissen, C. Poleij-Hok-Ahin, and T. Blacquière. 2018. Single and interactive effects of *Varroa destructor*, *Nosema* spp., and imidacloprid on honey bee colonies (*Apis mellifera*). Ecosphere 9(8):e02378. 10.1002/ecs2.2378

Abstract. High losses of honey bee colonies in recent decades are of great societal and economical concern and experienced as a sign of the vulnerability of the environment, including the service of crop pollination, and of the beekeeping sector. There is no single cause for the colony losses, but many contributing stressors may act in concert. *Varroa destructor* infestation is acknowledged as an important cause of these losses. The roles of infestation by *Nosema ceranae* or exposure to insecticides are controversial. Interactions between exposure to pesticides and *V. destructor* or *Nosema* spp. have previously been implicated. In two years of field experiments, we studied the effects of and possible interactions between the stressors *V. destructor* infestation, *Nosema* spp. infestation, and chronic sublethal exposure to a field-realistic dose of the insecticide imidacloprid on the performance and survival of honey bee colonies. Colonies highly infested by *V. destructor* were 13% smaller in size and were 59.1 times more likely to die than colonies infested with low levels of *V. destructor*. Infestation with high levels of *Nosema* sp. led to 2% decrease in size and 1.4 times higher likelihood to die compared to colonies with low levels of *Nosema* sp. No effects of chronic sublethal exposure to imidacloprid on colony size or survival were found in this study. Exposure to *V. destructor* and imidacloprid led to a slightly higher fraction of bees infested with *Nosema* sp., but in contrast to the expectations, no resulting interactions were found for colony size or survival. Colonies as a superorganism may well be able to compensate at the colony level for sublethal negative effects of stressors on their individuals. In our experimental study under field-realistic exposure to stressors, *V. destructor* was by far the most lethal one for honey bee colonies.

Key words: colony losses; colony size; colony survival; field-realistic exposure; pesticides; stressor.

Received 6 July 2018; accepted 9 July 2018. Corresponding Editor: Debra P. C. Peters.

† **E-mail:** coby.vandooremalen@wur.nl

Introduction

High losses of honey bee colonies in recent decades are a great societal and economical concern and experienced as a sign of the vulnerability of the environment, including the service of crop pollination (Klein et al. 2007, Aizen and Harder 2009, Potts et al. 2010*a*), and of the beekeeping sector (Potts et al. 2010*b*). There is no single cause for the colony losses, but many contributing stressors may act in concert (Potts et al. 2010*a*, VanEngelsdorp and Meixner 2010, Goulson et al. 2015). Exposure to the parasitic mite *Varroa destructor*, and connected diseases, is generally seen as one of the important stressors in honey bee colonies (Genersch et al. 2010, Le Conte et al. 2010, Rosenkranz et al. 2010). *Varroa destructor* is currently present in all habitable continents except Australia and in almost all *Apis mellifera* colonies (Genersch et al. 2010, Guzman-Novoa et al. 2010, Neumann and Carreck 2010, Rosenkranz et al. 2010, Nazzi and Le Conte

2016). The damage at colony level includes lower reproductive capacity and increased colony mortality (Genersch et al. 2010, Rosenkranz et al. 2010, Van Dooremalen et al. 2012, Nazzi and Le Conte 2016). The roles of neonicotinoid insecticides (Blacquière et al. 2012, Godfray et al. 2015, Pisa et al. 2015, Tsvetkov et al. 2017, Woodcock et al. 2017) or the endoparasite *Nosema ceranae* (Fries et al. 2013, Higes et al. 2013, Goblirsch 2018) in causing high colony losses remain debated. Recent studies show it is unlikely that field-realistic, sublethal doses of neonicotinoid insecticides (Dively et al. 2015, Rundlöf et al. 2015, Woodcock et al. 2017) or infestations with *N. ceranae* (Fernández et al. 2012, Botías et al. 2013, Higes et al. 2013, Van der Zee et al. 2015) are the sole cause of colony losses. The question remains to what extent a neonicotinoid insecticide and *N. ceranae* exposure negatively affect honey bees in relation to the effect of *V. destructor* and whether these stressors interact. This question is of particular importance to be answered at the colony level.

Implications of interacting stressors on honey bee colony health are generally studied by monitoring studies (Genersch et al. 2010, Francis et al. 2013, Meixner et al. 2014, Van der Zee et al. 2015, Tsvetkov et al. 2017) or in response to parasite treatments (Little et al. 2015). However, experimental exposure to combined stressors at (partly) a colony level is still relatively rare (Alaux et al. 2011, Mariani et al. 2012, Pettis et al. 2012, Blanken et al. 2015).

Blanken et al. (2015) showed that the combined exposure of colonies to a field-realistic dose of imidacloprid on top of the effect of the single stressor *V. destructor* reduced the flight performance of these colony's pollen foragers. Foraging is a crucial task that supplies a colony with necessary nutrients and energy. Reduced food inflow due to reduced flight performance may lead to increased demand for additional, and thus younger, foragers (Johnson 2010), and/or the reduction of the brood nest size (Mattila and Otis 2007). Perry et al. (2015) found by modeling that precocious foraging may cause forager loss and ultimately may lead to colony collapse. Either by bee loss or by a reduced amount of brood, the colony size is expected to become smaller or grow slower when these effects take place.

Here, we studied the interactive effects on colonies exposed to *V. destructor*, *Nosema* spp., and imidacloprid in a $2 \times 2 \times 2$ experimental design and all at field-realistic exposures (against control groups). The neurotoxic pesticide imidacloprid belongs to a group of chemicals called neonicotinoids and is used to fend off pest insect mainly of the order Hemiptera (aphids, whiteflies, and planthoppers) and Coleoptera (beetles) (Nauen and Denholm 2005). Small traces of neonicotinoids were found in bee collected pollen, nectar and bee products, but these residues were almost always below the acute and chronic toxicity levels (Blacquière et al. 2012). We therefore used a chronic, sublethal dose of the neonicotinoid insecticide imidacloprid in sugar syrup to simulate field-realistic insecticide colony exposure via nectar (against a control of clean sugar syrup). Previously, *Nosema* spp. did not seem to affect colony losses (Francis et al. 2013), and is therefore also expected to be a sublethal stressor. In The Netherlands, *Nosema* spp. consists mostly of *N. ceranae*. *Nosema ceranae* was present in 60–100% of the colonies, depending on the season (Van der Zee 2010, Van der Zee et al. 2015, Biesmeijer 2017). *Nosema apis* occurred only in 0.6% of the colonies ($N = 370$) in 2015 (Biesmeijer 2017). *Varroa destructor* is currently present in almost all *A. mellifera* colonies (Genersch et al. 2010, Guzman-Novoa et al. 2010, Le Conte et al. 2010, Neumann and Carreck 2010, Rosenkranz et al. 2010, Nazzi and Le Conte 2016). The damage at colony level includes lower reproductive capacity and colony mortality (Genersch et al. 2010, Neumann and Carreck 2010, Rosenkranz et al. 2010, Van Dooremalen et al. 2012, Nazzi and Le Conte 2016). As *V. destructor* and *N. ceranae* are both present in almost all Dutch colonies, we used the natural infestations with these parasites as our "high (+) exposure" level and compared these exposures to "low (−) exposure" levels by treating control groups of colonies against these parasites.

Whereas in Blanken et al. (2015) the flight performance of individual bees from exposed colonies was investigated, in this study we investigated the effect of imidacloprid and *V. destructor* on the colonies themselves, adding also the factor *Nosema* spp. The colonies used in Blanken et al. (2015) were part of the present study, in which we investigated the relative and

interactive effects on colony size over time and colony survival. We hypothesize that the interactive effect of imidacloprid and *V. destructor* on the flight performance of foragers will result in smaller colonies. This effect will be larger, when a third stressor (*Nosema* spp.) is allowed to develop.

Methods

Bees and setup

At an apiary of Wageningen UR (51°59′32.35″ N, 5°39′46.81″ E), The Netherlands, eight randomly assigned groups in a 2 × 2 × 2 experimental design were field-realistically exposed to *Varroa destructor*, *Nosema* spp., and imidacloprid (against control groups). The groups consisted of 10 colonies each and were kept in one or two wooden 10-frame story hives (type: simplex). The experiment was conducted between May 2013 and April 2014 (from here on further referred to as 2013) and was repeated between May 2014 and April 2015 (2014). All colonies from 2013 were removed and replaced by completely new colonies in 2014 for the second-year trial. Compared to the average weather in The Netherlands, the summer 2013 was hot and dry, the autumn 2013 was wet, and the winter 2014 was warm and sunny. The summer 2014 was warm, but wet; the autumn 2014 was very warm, dry, and sunny; and the winter 2015 was also warm and wet (Dutch weather website KNMI, visited on 21 November 2017).

The honey bee colonies (Dutch mixed breed *Apis mellifera* spp.) originated from a professional beekeeping company (Inbuzz v.o.f.) and were by visual inspection free from disease at the start of the experiment and supplied with a young (0–1 yr), strong and healthy laying queen. At the setup, each colony was given five frames (2013) or four frames (2014) of bees, one frame open brood (to facilitate oxalic acid treatment in the control group), and a similar amount of beebread (approximately one frame). The apiary was located on the organic test farm of Wageningen University and Research and was also close to two towns (<2 km). At the apiary, the orientation of the differently colored flight entrances of colonies alternated, facing southeast or southwest to reduce drifting between colonies. We also placed the colonies at least 1 m apart and gave them different entrance colors and small flight openings (respectively for recognition and defense).

Treatments

In both years, half of the colonies (randomly selected) were treated against *V. destructor* to create colonies with a relatively low *V. destructor* infestation (*V*−), while the other half was not treated to create colonies with a relatively high *V. destructor* infestation (*V*+). Varroa treatment consisted of an oxalic acid spray application (30 g/L oxalic acid dihydrate, 3%) prior to the start of experiment (May/June). For the bees, this dermal application corresponds to circa 175 μg/bee, while the no-observed-adverse-effect level is 212.5 μg/bee (Rademacher et al. 2017), The colonies that were treated had no capped brood during the application (Cornelissen et al. 2013).The same colonies were additionally treated using Apistan (tau-fluvalinate 824 mg/strip; Vita Europe Ltd., Basingstoke, UK) to reduce mite levels in September. Two Apistan strips per fully occupied 10-frame story were applied after we stopped feeding imidacloprid. Only 10% of the compound is released over an 8-week period (Johnson et al. 2010), exposing the bees to 3.0% of the LD50 on a daily basis (6.75 μg/bee). In 2014, samples of mites from our colonies were tested at Rothamsted Research (UK) for tau-fluvalinate resistance (RR) using the method as described in Gonzalez-Cabrera et al. (2013). Out of a total 164 mites from 39 colonies (*V*− and *V*+, but mostly *V*+ as there were hardly any mites left to collect in the *V*− colonies) of our population, only in one colony, one mite (out of five mites tested from that colony) showed the RR gene. The population was therefore considered to be tau-fluvalinate sensitive. The *V*+ colonies were not treated in spring or autumn. In January, all (*V*− and *V*+) colonies that were still alive were treated to reduce mite levels using oxalic acid trickling. All seams of bees were trickled with 5 mL oxalic acid solution (35 g/L oxalic acid dihydrate in sugar syrup). Frames were not removed from the hive because of low ambient temperatures.

In both years, half of the colonies (randomly selected) were treated against *Nosema* spp. to create colonies with a relatively low *Nosema* spp. infestation (*N*−), while the other half was not treated to create colonies with a relatively high

Nosema spp. infestation (*N*+). To obtain a low infestation, we fed *N*− colonies 0.250 L sugar syrup containing 30 mg/L fumagillin active ingredient (a.i.) (Fumagilin-B, from Medivet, in sugar syrup 5.7 g/L), once a week for four weeks each in June and October. The application was performed prior to and after, but never coinciding with the imidacloprid treatments. The *N*+ colonies were not treated with fumagillin.

We exposed honey bees to imidacloprid by providing sugar syrup in glass jars with feeding holes in the lid, upside down on top of the inner cover (crown board). The supplied sugar is quickly distributed through the colony as is illustrated by Nixon and Ribbands (1952), by whom 76% of the foragers and 43–60% of all the bees were radioactive within 27 h after introduction of six sugar-labeled bees in a colony of 24,500 bees. Our colonies were exposed to imidacloprid 6 μg imidacloprid a.i. (99.4% pure grade; Bayer Crop Science, Monheim am Rhein) per L sugar syrup or plain sugar syrup (0 μg/L) from June through September (2013 week 25–38; 2014 week 29–39). During this period, none of the *V. destructor* or *N. ceranae* control treatments were carried out. The sugar syrup (with or without imidacloprid) was fed twice a week in volumes of 0.33 L per colony per feeding. The concentration of 0 μg/L (*I*−) is considered the control, and the 6 μg/L (*I*+) resembles a worst-case field-realistic concentration. Per feeding session, the bees in each *I*+ colony were exposed to ~2 μg imidacloprid a.i. (See discussion for a more extensive explanation on the exposure.) The exposure was corrected for the amount of sugar water not consumed by the colonies (when replacing the jars, leftovers were measured).

All sugar syrup used in the experiment was made by mixing tap water and granulated sugar (sucrose) in a 1:1 ratio (v/w), and the concentration ranged between 49% and 51% sugar. In addition to the sugar syrup, all colonies had continuous access to sugar dough (FondaBee, from Belgosuc), and in October, all colonies were prepared for winter by feeding them bulk sugar syrup (5–10 L) as much as each colony would take up. The colonies were regularly checked on the presence of eggs and larvae; in case of no eggs and/or larvae present, queen failure was assumed (after a double check 1 week later). A mated queen from a stock of small spare colonies was introduced. Introduction of a queen was attempted twice maximally per colony. Most queen failures occurred between June and October, when we were handling the colonies the most (to do also additional measurements, e.g., Blanken et al. 2015). When the second introduction failed, the colony was withdrawn from the experiment (from the first queenless period onward).

Measurement: colony size and survival

Every first week of the month between June (2014) or July (2013) and April of the next year, survival was checked and colony size was estimated. A colony, queenright with eggs, larvae, and workers, was determined to be "alive" (but see Treatments in the Methods section). High-resolution pictures were taken from the top of the hive (of both chambers in case of a two-story hive) using a DSLR camera (Nikon D90/D3100, Nikkor 18–55 mm). Using ImageJ (www.imageJ.nih.gov/ij), the area available and area occupied by the bees were measured. The fraction of bees (area occupied divided by the area available) was used to estimate the number of bees in the colony. We assumed that a fully covered 10-frame hive contains 16,830 bees, calculated by using a Dutch inner frame size of 34 × 19.8 cm and an average cover of 1.25 bees/cm^2 (Delaplane et al. 2013), and that a partly covered hive rates accordingly. We use these data to make a model that can be used to estimate colony size (number of bees) based on the picture from the top side of the hive. Colonies that had died during our experiment were either kept in the dataset ("0 bees") or excluded from the dataset ("missing value").

Delaplane et al. (2013) proposed to estimate colony size by taking photographs or visually estimating size based on sum of the single frames. However, taking apart the colonies on a monthly basis for 11 subsequent months would have been too invasive for the colonies: Taking out frames breaks the propolis envelope, part of the bees' immunity (Evans and Spivak 2010), increases costs for thermoregulation, especially when ambient temperature is low (Seeley and Visscher 1985), and increases the chance that queens stop laying or are accidentally killed by the researcher (which is particularly a problem in autumn and winter, when replacement of queens

is not possible). Additionally, it would take too long to photograph 80 hives (times 10–20 frames) while preventing partial underestimation of the number of bees, as records of bees on combs vary with time of day and bee foraging activity (Delaplane et al. 2013). Working simultaneously on different hives will result in bees robbing and defending against robbers, and would also lead to an underestimation of the number of bees. To test whether taking a picture from the top side of the hive yields similar results as estimating the number of bees based on the method of Delaplane et al. (2013), we simultaneously photographed the top sides in 20 hives (10 frames) and each side of all frames during the months July to October in 2012. We found that the colony size estimation based on the picture of the top side of the hive strongly relates to the estimation based on the sum of the single frames and does not differ between months (Fig. 1, linear mixed model [REML] with repeated measures for month and subject colony [covariance type "scaled identity"]; bees on frames, $F_{1,64} = 80.4$, $P < 0.001$; month, $F_{3,64} = 0.5$, $P = 0.68$; interaction bees on frames × month, $F_{3,64} = 1.7$, $P = 0.18$; $N = 148$).

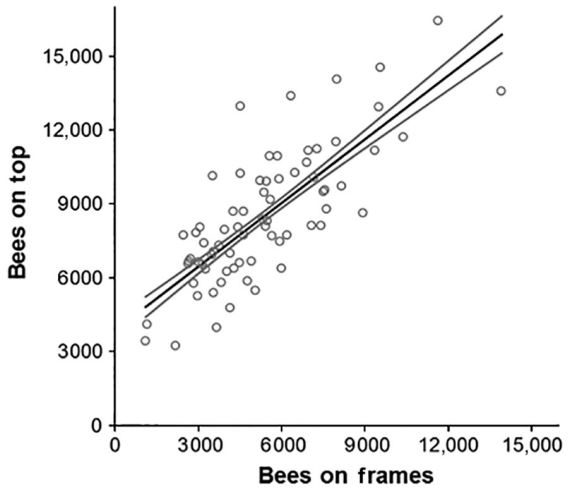

Fig. 1. Relation between the method for estimation of the number of bees based on a picture of the top side of the hive and the method for estimation of the number of bees based on the sum of the single frames (cf. Delaplane et al. 2013). Open dots represent the raw data, the black line presents the predicted values of the model (excluding month), and the gray lines present the standard error of mean of the predictions.

This relation shows that the method based on the picture from the top side estimated the colonies to be a little larger compared to the method using the sum of the frames. First, from the above we assume the full cylinder below the top coverage is filled with bees, while the bees may actually be clustered in a sphere. This results in a larger overestimation in smaller colonies. Differences in colony size may be slightly underestimated as small colonies may actually even be a little smaller. Second, but not mutually exclusive, we may have underestimated the number of bees using the sum of frames, as some bees took off when taking the hive apart (despite our effort to prevent it). In this paper, we used pictures from the top side of each hive to estimate the colony sizes between July 2013 and April 2015.

Measurement: validation of the treatments

At the same time when measuring colony size in our experiment, >100 bees were sampled for measuring *V. destructor* infestation (June–December). Each sample was weighed (total of all bees) and checked for *V. destructor* mites by shaking the bees in soapy water (dishwashing liquid) for 30 s and then thoroughly rinsed over a sieve with a mesh size to let the mites through and a mesh size to keep the mites in (Dietemann et al. 2013). The mites were collected and counted for each sample (per colony and month). The mite infestation was expressed as the number of mites per gram bees.

An additional 30 bees were sampled from the colonies to measure *Nosema* spp. infection. We sampled bees in July (after the first time treated with fumagillin), in October and November (before and after the second treatment). For each sample, all individual bees were used for microscopic examination of the midgut for spores (Fries et al. 2013). The fraction of bees infected with spores was determined. During the season, no clinical symptoms of *Nosema* spp. were observed.

For imidacloprid, we collected samples of each batch of sugar syrup that was spiked with imidacloprid. These samples were pooled per week (equal ratio). The research institute RIKILT Wageningen UR, The Netherlands, determined the imidacloprid concentrations (LOQ < 0.5 μg/kg) of the samples by the QuEChERS method (acetonitrile extraction and LC-MS × MS (Van

der Zee et al. 2015). Samples were kept at −20°C until analysis, which was performed in the winter following the collection.

Statistics

A survival analysis with a Cox regression model was used to test differences in colony survival between treatments (*V*, *N*, and *I*) over time. For this test, "time" was expressed by month. The Cox proportional hazards model used 942 uncensored cases and 715 censored cases.

A generalized linear model was used to estimate the likelihood to be dead in April, based on the number of mites per gram bee in the month September of the preceding year, using a binary distribution and logistic link function. Year and the interaction with year were not included in the model, as these terms were not significant when included and also because the random factor year in the model for infestation was not significant.

Several linear mixed models were used. Colony size (excluding or including "0" values for dead colonies), *V. destructor* infestation, and *Nosema* spp. infestation were analyzed as a function of the different treatments (*V*, *N*, and *I*) and month in a full factorial design. Non-significant interactions with the factor month were excluded from the final models, but only when removing those factors from the model lowered the Akaike information criterion (AIC) value. As measurements between months were not independent, month (within each year) was added as a repeated measure with colony as subject. The year the experiment took place was added as a random factor and was kept in the model, also when non-significant. Colony size (including dead colonies) was also analyzed as a function of *Month* and the different treatments in the model replaced with the binomial factor final status of the colony in April (dead or alive). Also here, month (within each year) was added as a repeated measure with colony as subject and year was added as a random factor.

For imidacloprid, some calculations were done first. Based on the weekly concentrations of imidacloprid and the standard amount of sugar syrup fed to the colonies—that we corrected for the amount that was non-consumed (non-consumed 2013 1.70%; 2014 0.02% of total dosed)—we calculated the daily exposure to imidacloprid (μg imidacloprid a.i.) for each colony in each month. This daily exposure was used in the model as a function of the treatment for *V. destructor* (*V*), *Nosema* spp. (*N*) and month in a full factorial design. Non-significant interactions with the factor month were excluded from the final models, but only removing those factors from the model lowered the AIC value. As measurements between months were assumed to be independent, no repeated measures were added to the model. The year the experiment took place was added as a random factor and was kept in the model (as it was part of the setup), also when non-significant. Based on the daily exposure and colony size, we estimated the daily exposure per bee. We did not measure colony size in June 2013, but we started already the feeding of imidacloprid in the last week of June. To estimate the exposure per bee during this week in June, we used colony size of July 2013 also in June (the exposure was the last week of June, and we measured colony size always at the beginning of the month, so at the beginning of July). The linear mixed model for the daily exposure per bee was similar to the previous linear mixed model, save that due to the dependency between months for colony size, month (within each year) was added as a repeated measure with colony as subject.

For all linear mixed models, the best fitting model was selected by choosing the covariance matrix for the repeated measures and the random factor with the lowest AIC index. Additionally, the model restricted maximum likelihood (REML) or maximum likelihood (ML) with the lowest AIC value was used. To test the main effects of the significant interactions, Sidak's pairwise comparisons were performed. When variables are natural-logarithm-transformed, this will be mentioned in the text. Assumptions for normality were met for all factors when normality was required. Means are estimated marginal means ± SE unless explained differently.

Results

Colony size

The colony size (if dead, then size value is a "missing value") did not differ between *I*+ (bees 9601 ± 739) and *I*− colonies (bees 9510 ± 739). *N*+ colonies were on average 2.5% smaller (bees 9434 ± 739) than *N*− colonies (9677 ± 739). *V*+

colonies were on average 11.4% smaller (bees 8975 ± 742) than *V*− colonies (10135 ± 739). This pattern changed with *Month*; until September, *V*+ colonies were 2–6% larger than *V*− colonies, but from September onward, *V*+ colonies were smaller and became increasingly smaller than *V*− colonies (Fig. 2; Table 1). There was no (random) effect of year. For the mean colony size for both experimental years and for all groups, see Appendix S1: Fig. S1.

When for the dead colonies a number "0" was included in the data, the effect of the stressor for *Varroa destructor* was even larger; *V*+ colonies were then on average 21% smaller than *V*− colonies (Table 1). *N*+ colonies were on average 3.5% smaller than *N*− colonies. Colony size did again not differ for *I*+ or *I*− colonies. For the mean colony size (including "0 bees" for dead colonies) for both experimental years and for all groups, see Appendix S1: Fig. S2.

When relating imidacloprid, *Nosema* spp., and *V. destructor* with the final status of the colony in April (dead or alive), the colony size in the summer before (June–September) was on average 3% larger in colonies that had died before or in April compared to colonies that survived (Table 1).

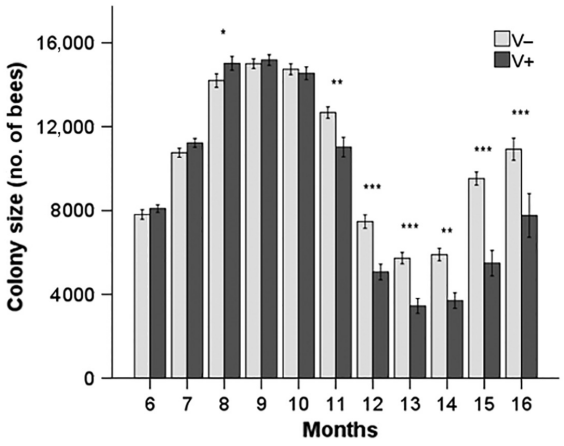

Fig. 2. Colony size (number of bees) as a function of the month of the year and whether the colonies were exposed to low numbers of *Varroa destructor* mites (*V*−) or high numbers of mites (*V*+). If colonies died during the experiment, the colony size in subsequent months was a missing value (all colony sizes shown are of surviving colonies). The symbols depict differences between groups ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Survival

V+ colonies were 59.1 times more likely to die than *V*− colonies (Table 2). *N*+ colonies were 1.4 times more likely to die than *N*− colonies, and therefore much less likely to die than *V*+ colonies. *I*+ colonies were not more (or less) likely to die due to exposure than *I*− colonies (Fig. 3), and thereby less likely to die than *V*+ or *N*+ colonies. Survival did not differ between the two experimental years ($W = 0.02$, $P = 0.89$), and this factor was left out of the analysis.

Varroa destructor *infestation*

Treating *V*− colonies against mites resulted in *V*+ colonies that had on average 8.6 times more mites per gram bees than *V*− colonies (Table 1, Fig. 4a). Differences in mite infestation levels occurred from August onward until we stopped measuring in December. In December, *V*+ colonies had 1.7 ± 0.1 mites/g bees, while *V*− colonies had 0.2 ± 0.1 mites/g bees. No effects on mite levels were shown by *Nosema* spp. or imidacloprid. For the mean number of *V. destructor* mites per gram bees for both experimental years and for all groups, see Appendix S1: Fig. S3. The number of mites/g bees in the month September predicted the likelihood to be dead in April after winter for both years (Wald $\chi^2 = 28.1$, df = 1, $P < 0.001$; Fig. 4b).

Nosema *spp. infection*

Treating *N*− colonies against *Nosema* spp. resulted in *N*+ colonies that had on average a 2.3 times larger fraction of bees within the colonies infected with spores than *N*− colonies. Differences were larger directly after the treatment application in the months July and November (Fig. 5a, Table 1). For the fraction of infected bees with *Nosema* spp., there was an interactive effect: *V*+*I*+ colonies had a higher fraction of infection with *Nosema* spp. compared to other colonies (Fig. 5b). The pattern differed somewhat between *N*+ and *N*− colonies (Appendix S1: Fig. S4a). For the mean fraction of infected bees for both experimental years and for all groups, see Appendix S1: Fig. S4b. Results for the fraction of colonies that was infected by *Nosema* spp. (where a non-infected colony had no bees positive for spores, and infected colony had at least one or more bees positive for spores, binomial data) were very similar to the fraction of bees

within the colonies infected with spores. These data are therefore not shown. In July in the *N*− colonies, 20% were infected with *Nosema* spp. on a colony level, whereas in October and November, approximately 57% and 58% of the colonies were infected. In the *N*+ colonies, between 71% and 73% of the colonies were infected in July, October, and November.

Table 1. (a) Results of the linear mixed models for effects of the independent variables and (b) model attributes on each dependent variable related to colony size or level of exposure.

	Colony size			Exposure			
Variable or attribute	Bees (−)	Bees (0)	Bees Jun–Sep	*Varroa destructor*	*Nosema* spp.	Imid	Imid/bee
(a) Independent variable							
Imid							
F	0.8	1.3	–	0.1	3.3	–	–
P	0.37	0.25	–	0.75	0.07	–	–
Nspp							
F	5.8	10.3	–	3.4	38.8	0.01	1.1
P	**0.02**	**<0.01**	–	0.07	**<0.001**	0.93	0.30
Vd							
F	71.8	320.7	–	288.6	2.8	0.7	2.1
P	**<0.001**	**<0.001**	–	**<0.001**	0.09	0.41	0.15
Imid × Nspp							
F	2.8	3.5	–	0.9	1.2	–	–
P	0.10	0.06	–	0.36	0.28	–	–
Imid × Vd							
F	0.1	0.2	–	0.6	6.1	–	–
P	0.82	0.63	–	0.44	**0.01**	–	–
Nspp × Vd							
F	2.9	1.1	–	0.9	0.4	0.01	0.8
P	0.09	0.29	–	0.34	0.54	0.91	0.39
Imid × Nspp × Vd							
F	1.1	1.0	–	2.3	4.3	–	–
P	0.30	0.31	–	0.14	**0.04**	–	–
Mo							
F	506.2	645.2	354.7	159.6	9.4	0.8	125.9
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.48	**<0.001**
Mo × Vd							
F	13.4	48.2	–	51.7	–	–	–
P	**<0.001**	**<0.001**	–	**<0.001**	–	–	–
Mo × Nspp							
F	–	–	–	–	7.8	–	2.9
P	–	–	–	–	**<0.001**	–	**0.05**
DiA							
F	–	–	4.9	–	–	–	–
P	–	–	**0.03**	–	–	–	–
Mo × DiA							
F	–	–	4.4	–	–	–	–
P	–	–	**<0.01**	–	–	–	–
Yr							
Wald Z	0.99	0.99	†	0.28	0.69	1.0	†
P	0.32	0.32	†	0.78	0.49	0.32	†
(b) Attributes							
Estimation method	ML	ML	ML	REML	REML	ML	ML
Transformation	None	None	None	LN+	LN+	None	LN+
Added value				0.01	0.01		0.00002

(Table 1. Continued.)

Variable or attribute	Colony size			Exposure			
	Bees (−)	Bees (0)	Bees Jun–Sep	*Varroa destructor*	*Nosema* spp.	Imid	Imid/bee
N	1474	1657	558	1020	471	280	278
AIC	27134	30649	9825	3213.1	1388.0	−916.2	−616.3
Rand Cov Type	SI	SI	CS	VC	VC	VC	VC
Rep Cov Type	DIAG	DIAG	UN	CS	DIAG		UN
Rep measures	Yr × Mo	Yr × Mo	Yr × Mo	Yr × Mo	Yr × Mo		Yr × Mo
Subject	Colony	Colony	Colony	Colony	Colony		Colony

Notes: Dependent variables in the model are, for colony size, the number of bees, where, after colonies died, the data in the subsequent months are missing values (Bees (−)) or entered as "size = 0" (Bees (0)). Colony size between June and September (Bees Jun–Sep) involved no dead colonies yet (size always >0). Dependent variables for the level of exposure are *Varroa destructor* (*V. destructor*, mites/gram bees), *Nosema* spp. (*Nosema* spp., fraction of bees positive for spores), imidacloprid (Imid, μg/day), and imidacloprid per bee (Imid/bee, estimated exposure $\mu g \cdot day^{-1} \cdot bee^{-1}$). For each independent variable in the model, the *F* and *P* value are given. Independent stressor variables are *V. destructor* (Vd), *Nosema* spp. (Nspp), and imidacloprid (Imid). Other independent model variables are month (Mo), dead in April (DiA), and year (Yr), where year was a random variable (Wald statistic and *P* value are given). Model attributes are the applied method of estimation (ML, maximum likelihood; REML, restricted maximum likelihood), whether we LN−transformed the dependent variable (LN + the added value), the sample size (*N*), the value of the Akaike information criteria (AIC), the repeated covariance type (Rep Cov Type) for the repeated measure and the random factor (Rand Cov Type; DIAG, diagonal; UN, unstructured; CS, compound symmetry; SI, scaled identity; VC, variance components), the repeated measures, and the subject of the repeated measures. Bold values show significant independent variables. An empty cell/endash shows that this independent variable was not included in the model.

† The random factor year was redundant, but kept in the model.

Table 2. Results of the survival analysis for the effects of the stressors *Varroa destructor* (*V*), *Nosema* spp. (*N*), and imidacloprid (*I*) on colony survival over time (Month).

Independent variable	β	SE	Wald	df	*P*	Exp(β)
I	−0.1	0.1	0.4	1	0.55	0.9
N	0.3	0.2	4.2	1	**0.04**	1.4
V	4.1	0.6	49.1	1	**<0.001**	59.1

Notes: The Cox proportional hazards model used 942 uncensored cases and 715 censored cases. For each factor in the model, the Wald value, df, and *P* value are given as well as the average rate at which colonies died (β ± SE). The hazard (exp(β)) is the number of times a colony in the "+ group" is more likely to die than a colony in the "− group." Bold values show significant independent variables.

Imidacloprid exposure

Each *I*+ colony was exposed in total to 52.7 ± 0.2 μg imidacloprid (a.i.) over a period of 13.5 weeks in 2013 or to 33.7 ± 0.01 μg imidacloprid (a.i.) over a period of 11 weeks in 2014. Not all colonies consumed the full amount of sugar syrup. In 2014, this occurred only once in one colony, and all other occurrences were in 2013 (45 occurrences in 25 colonies). The exposure was corrected for the non-consumption. Average daily exposure to imidacloprid did not differ between the experimental groups or between months (Table 1; Appendix S1: Fig. S5). Due to the increase in colony size during June–September, the daily imidacloprid exposure per average bee decreased with month, but did not differ between exposed treatments (Table 1, Fig. 6). The interaction between month and the factor *Nosema* spp. was caused by a lack of difference between June and July, only in the *N*+ group (all other months differed from each other and no differences occurred between the *N*− and *N*+ groups within months).

Discussion

Relative effects of the single factors

In this study, we investigated the relative and interactive effects on colony size and survival of colonies field-realistically exposed to *Varroa destructor*, *Nosema* spp., and dosed sublethal quantities of imidacloprid in sugar syrup. We found no interactive effects on colony size and survival, but found different impacts of the exposures to the single stressors:

1. The impact of *V. destructor* was large, as *V*+ colonies infested with *V. destructor* were on average 11% smaller in size and were 59.1 times more likely to die in winter than *V*− colonies that were treated against *V. destructor*.

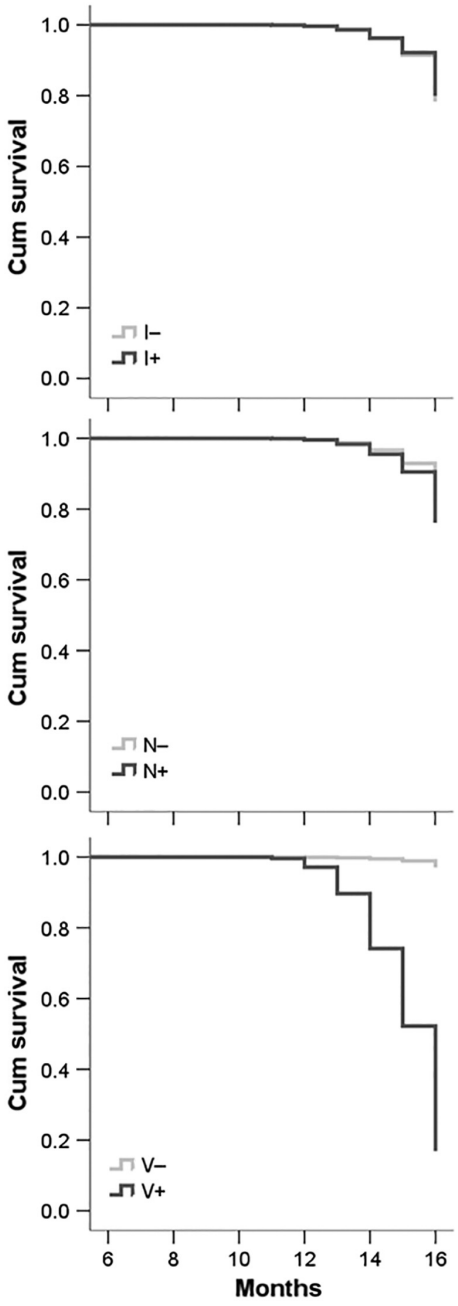

Fig. 3. Cumulative survival in response to imidacloprid (top panel), *Nosema* spp. (center panel), and *Varroa destructo*r (bottom panel) treatments and as a function of month of the year (where month >12 is the following year; e.g., month 13 is January). Survival was not reduced in response to imidacloprid, slightly reduced in response to *Nosema* spp., and strongly reduced in response to *V. destructor*.

2. The impact of *Nosema* spp. was relatively small (compared to the effect of *V. destructor)*, as *N*+ colonies infested with *Nosema* spp. were 2.5% smaller in size and were 1.4 times more likely to die than *N*− colonies that were treated against *Nosema* spp. It should be noted that even the N+ colonies were by no means *Nosema*-diseased colonies.
3. The impact of imidacloprid was negligible, as no effects of the chronic sublethal exposure to imidacloprid on colony size or survival were found in this study.

Colonies as a superorganism may well be able to compensate at the colony level for sublethal negative effects of stressors on their individuals. In our experimental study under field-realistic exposure to stressors, *V. destructor* was by far the most lethal one for honey bee colonies.

Hormesis

Surprisingly, colonies that had died in April were 3% larger during the preceding bee growing season (high food availability June–September). This result was due to a 2–6% larger colony size in *V*+ colonies between June and September. The increased colony size suggests that the colonies respond to increasing mite levels by investing energy and resources in additional brood and subsequently colony size, perhaps to compensate negative effects from stressors (mainly *V. destructor* in this study). It might be an example of hormesis: "a dose-response relationship for a single endpoint that is characterized by reversal of response between low and high doses of chemicals, biological molecules, physical stressors, or any other initiators of a response" (Kendig et al. 2010). Calabrese and Baldwin (2002) suggest that hormesis can be an overcompensation for repairing a disturbed homeostasis and therefore is an underlying adaptive mechanism, although it is debated whether this explanation should be part of the definition of hormesis. Hormesis has not been very often described in bees (only in relation to toxicology) and, as far as we know, never on a colony or superorganism level. Despite, or perhaps because of, their increased investment to "turn things right," most of our exposed colonies still failed to survive. Most likely, the stress level due to

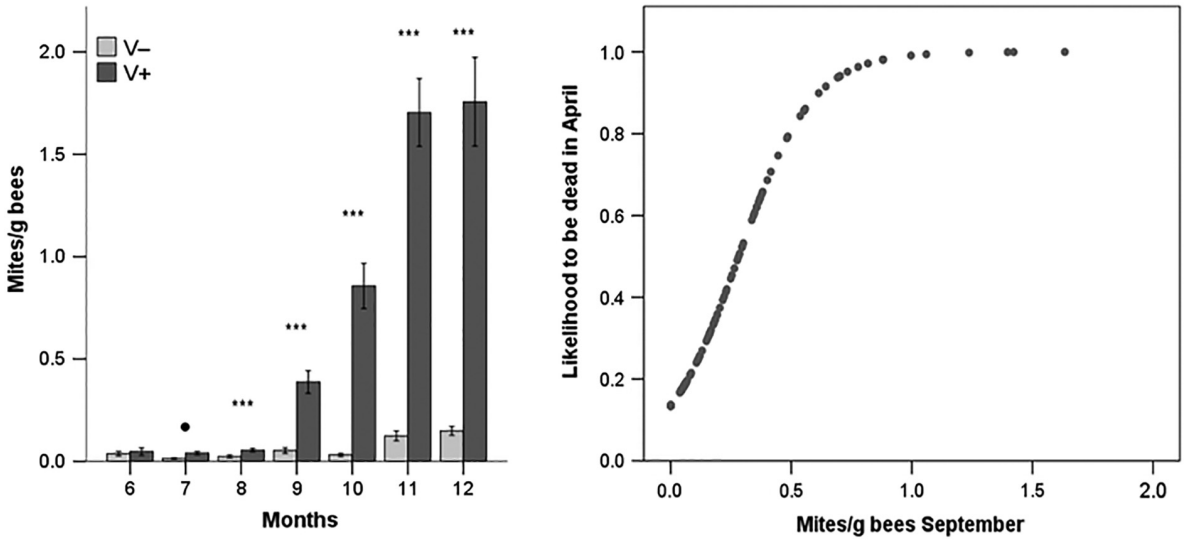

Fig. 4. Number of mites per gram bees as (left panel) a function of month and in response to *Varroa destructor*, and (right panel) the predictor in September for the likelihood to be dead in April. The symbols depict differences between groups (•$P = 0.05$; $^{***}P < 0.001$). In this manuscript, we assumed 8 bees per gram to calculate mite infestation percentages.

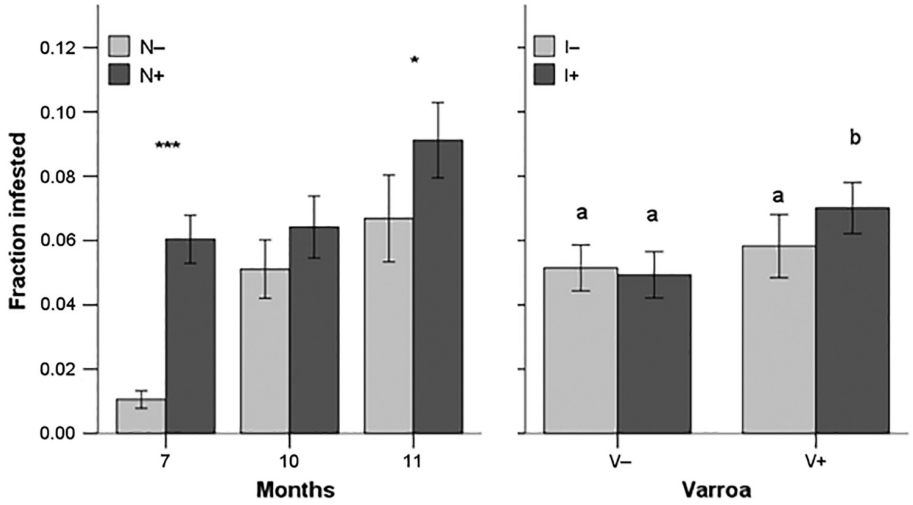

Fig. 5. Fraction of bees within a colony infested with *Nosema* spp. spores as a function of month and *Nosema* spp. treatment (left panel), and as a function of *Varroa destructor* and imidacloprid (right panel). The symbols depict differences between groups (ns $P > 0.05$; $^{*}P < 0.05$; $^{***}P < 0.001$). Different letters depict differences per group. Symbols and letters are based on post hoc test on LN-transformed data.

V. destructor (varroosis) was too high. Less likely, but not mutually exclusive, would be a reduction in size of the *V*− colonies (in contrast to larger *V*+ colonies) due to a postponed reaction to the oxalic acid treatment two months before the start of the experiment. Although a small batch of worker bees may have died directly after oxalic acid application and some of the remaining bees may have had a reduced lifespan (Rademacher et al. 2017), bees are well able to cope with such

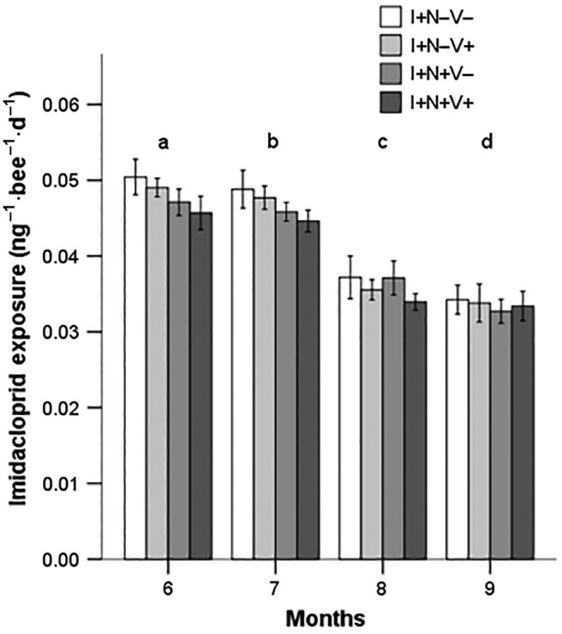

Fig. 6. Imidacloprid exposure per bee per day (ng) as a function of month and *Varroa destructor* and/or *Nosema* spp. treatment. The treatments were whether the colonies were exposed to low (*V*−) or high (*V*+) numbers of *V. destructor* mites, or low (*N*−) or high (*N*+) numbers of *Nosema* spp. In 2013, colonies were exposed during 1 week (1 measurement per colony, hence the lack or error bars) at the end of June until the first three weeks of September. Because we did not measure colony size in June 2013, we used the colony size in July to estimate the exposure per bee in June. In 2014, colonies were exposed during 2.5 weeks at the end of July until the end of September. Letters show differences between months (based on post hoc test on LN-transformed data).

accidental losses of workers (Johnson 2010, Perry et al. 2015), colony sizes were similar at the start of the experiment, and moreover, no such pattern was found for *N*− vs. *N*+ colonies after its Fumagilin-B application (which could potentially also cause bee mortality).

Single factor Varroa destructor

The devastating effect of *V. destructor* infestation on colony survival is well known. Fries et al. (2003) and Rosenkranz et al. (2006, as cited in Rosenkranz et al. 2010) found that untreated colonies which exceeded an infestation of 30% in the adult bees (eq. 30 mites/100 bees) during summer did not survive the following winter. Additionally, levels above 6% showed more than 10% losses (Genersch et al. 2010, Rosenkranz et al. 2010). In our study, *V. destructor* was even more lethal, as we found much lower thresholds. Colonies that exceeded an infestation rate of 17% in September (summer) did not survive the following winter, and a mite infestation on adult bees in September as low as 5% resulted already in 69% loss (assuming 1 g bees contains on average eight adult workers). This suggests that the likelihood to die at low infestation levels increased in the last decades, or lethalness of mites differs between locations. The average infestation by *V. destructor* of the colonies in our study was slightly lower than in the German monitoring, where German colonies showed an October infestation of 3.6% in surviving colonies and 14.8% in dead colonies in 2007 (Genersch et al. 2010), while our colonies showed an average October infestation of 1.5% in surviving colonies and 13.1% in dead colonies. This suggests infestation levels in general stayed relatively the same (although we seemed to be better at reducing mite levels).

Single factor Nosema *spp.*

The effect of *Nosema* spp. on colony size and survival was small, especially in relation to the much larger negative effect of the factor *V. destructor* in this experiment. This could be partly explained because we found control methods for *Nosema* spp. (2.3 times reduction of parasite level) are not as effective as methods for *V. destructor* (8.6 times reduction of parasites level). As a result, we may have underestimated the effect on colony size, because the *N*− may have had an even larger size if the treatment would have been more effective. However, because no colonies died in the *N*− group, survival is independent of the reduction in parasite load (less parasites could not have led to more survival). In our study, a field-realistic fraction of bees infected with *Nosema* spp. spores led to a 1.4 times higher likeliness to die compared to our treated control group, making *Nosema* spp. a much less deadly stressor compared to the factor *V. destructor*, which showed a 8 59.1 times higher likeliness to die when exposed to field-realistic levels of mites. Our data therefore support our hypothesis that *Nosema* spp. are mostly a

sublethal stressor and to some extent support the finding that *Nosema* spp. does not affect colony losses (Van der Zee et al. 2015). In our study, *Nosema* spp. did affect colony losses, but only in a minor number of cases.

In the *N*+ colonies, approximately 70% of the colonies was infected with *Nosema* spp. spores (at least one of the 30 tested bees in that colony was positive for spores), which was lower compared to the 80% of ($N = 86$) colonies that tested positive for either *Nosema ceranae*, *N. apis*, or both in Van der Zee et al. (2015), and again lower than the 87% of ($N = 681$) colonies infected during the two-year study in Spain by Botías et al. (2013).

Single factor imidacloprid

The role of neonicotinoid insecticides in causing high colony losses is highly debated (Blacquière et al. 2012, Godfray et al. 2015, Pisa et al. 2015, Rundlöf et al. 2015, Tsvetkov et al. 2017, Woodcock et al. 2017). Our findings support that it is unlikely that field-realistic, sublethal doses of neonicotinoid insecticides on a colony level (Dively et al. 2015, Blacquière and van der Steen 2017, Woodcock et al. 2017) are the sole cause of colony loss. Our study shows that honey bee colonies subjected to a worst-case chronic (12-week) field-realistic exposure did not show negative effects of this exposure on colony size or survival during, directly after exposure nor during the winter after exposure. Each bee in our (*I*+) colonies was (for 12 weeks) exposed daily to on average (±SE) 0.040 ± 0.0006 ng imidacloprid a.i. (Fig. 6), which is approximately 1% of the acute oral LD50 (=3.7 ng/bee). Our daily exposure per bee ranges between the bee exposure groups of 5 and 20 ng/bee in the study of Dively et al. (2015). The exposure period was similar. Our findings on colony size concur with Dively et al. (2015), but our survival was higher (in the exposed and control groups). Their more invasive colony strength monitoring may have led to additional stress and hence lower winter survival. Our data also support the findings of Faucon et al. (2005), who fed their bees 0.5 or 5 μg/L imidacloprid in sugar water (0.015 ng/bee per day and 0.15 ng/bee per day, respectively; Calabrese and Baldwin 2002), and who found no negative effects on colony level. Similar for Meikle et al. (2016), whose lowest exposure of 20 μg/L imidacloprid in sugar water (0.76 ng per bee per day as calculated by Blacquière and van der Steen [2017]) did not result in less bees in the colonies after six weeks of exposure. Overall, our findings for imidacloprid support that encountered high ranges of field doses relevant for seed-treated crops (5 μg/kg) had negligible effects on colony health and are unlikely a sole cause of colony losses. Detoxification by individual bees most likely plays a significant role in this process (Cresswell et al. 2014).

Our daily exposure per bee was much lower and longer than most other field studies (like in Wu-Smart and Spivak [2016]) (Blacquière and van der Steen 2017). But, how does our exposure relate to the field? Foragers are most likely to be the bees exposed to the highest amounts of pesticides (Thompson 2012), as they are the ones that have the most contact with the outside world, but they also are the bees that consume the most sugar water (Rortais et al. 2005). Over the whole exposure period, our colonies consisted on average of 13,000 bees. Let us assume one-third of the bees in the colonies were foragers that consumed half of the fed sugar water each week. The remaining bees and larvae were assumed to consume the other half of the sugar water. Feeding on 660 mL of sugar water per week contains 409 g of sugar, supplying each forager with 47 mg of sugar per week (4333 foragers dividing half of the sugar), while an average nectar forager normally consumes 225–900 mg and a pollen forager 73–110 mg sugar per week (Rortais et al. 2005). Nectar foragers are however more likely to experience the highest exposure to both sprayed and systemic seed and soil treatment compounds (Thompson 2012). Our supplied sugar therefore comprises of 5–21% of the sugar diet of an average nectar forager. To be exposed to field conditions, these nectar foragers should forage for 5–21% of their time on a field supplying nectar with the concentration we provided (5.9 ng/mL in 2013 or 4.6 ng/mL a.i. imidacloprid in 2014). However, the concentration of imidacloprid in nectar of sunflower or canola was found to be 1.9 ppb (ng/mL) (Schmuck et al. 2001), and field-realistic concentrations average 2 ng/g in nectar (Godfray et al. 2015). To gain a similar exposure as the concentration we fed the bees, nectar foragers should spend 15–62% (based on 2013 exposure) or 12–48% (based on exposure in 2014) of their time collecting food

from fields with a concentration, on average, of 2 ng/mL. As bees hardly ever forage on one crop only (Winston 1987), this exposure is field realistic. For pollen foragers, the exposure is up to 2 × field-realistic exposure, as bees can only spend maximally 100% of their time foraging on a field. Based on their sugar consumption, pollen foragers should spend 127–191% (based on 2013 exposure) or 99–149% (based on exposure in 2014) of their time collecting food from fields with a concentration, on average, of 2 ng/mL.

Interactive effects

Although previously exposures to neonicotinoids and *Nosema* spp. resulted in interactive effects on performance of survival of individual bees (Alaux et al. 2010, Pettis et al. 2013) (but see Goblirsch [2018] for an overview), we found no interactive effects of exposure to imidacloprid and *Nosema* spp. at the exposure and measurement levels in this field-realistic study. Blanken et al. (2015), who extracted pollen foragers from our exposed colonies, found that exposure to the combined stressors imidacloprid and *V. destructor* reduced the flight performance of the collected pollen foragers by ~24%. In contrast to our expectations, based on the findings by Blanken et al. (2015), we did not find interactive effects on colony size. These findings suggest that the negative impact due to interactive effects between imidacloprid on flight performance may be overcome at colony level, while this may be more difficult for the negative impact of *V. destructor*.

We did find one small interactive effect, where. *V+I+* colonies had a higher fraction of infection with *Nosema* spp. compared to other colonies, but the inconsistency of the patterns between the *N+* and *N−* colonies and years (not significant; Appendix S1: Fig. S4a, b) make it difficult to give a biological interpretation of the results. Mariani et al. (2012) found more spores in summer in *V+* colonies, but then for us it would have been more likely to have found an interaction between *V. destructor* and *Nosema* spp. or a main effect of *V. destructor* on the number of bees infected with spores (assuming more spores leads to more infected bees in a colony), which we did not find.

Social resilience

As a "superorganism," a honey bee colony can restore its function following a disturbance by redistributing the worker bees' division of labor (Johnson and Linksvayer 2010). This adaptive capacity is called social resilience (Sendova-Franks and Franks 1994) and enables the colony to maintain homeostasis (Schmickl and Crailsheim 2004). We suggest that bees also use this capacity to respond to chronic stress. Stressors such as imidacloprid impacting mostly foragers can be first counteracted by recruiting foragers at younger age (precocious foraging; Perry et al. 2015). If this does not restore homeostasis (e.g., many foragers are affected or the exposure continues for a longer period), nurse bees can reduce the number of larvae, thus decreasing the demand for foragers (Schmickl and Crailsheim 2002), but thereby also the colony size. A stressor like *V. destructor* however already affects the bees in the pupal stage (reducing their quality and/or quantity), resulting in a need for more nurse bees. The homeostasis can be first restored by increasing the brood nest size, which would lead to a larger colony, but maybe also to precocious foraging as more resources are required to rear this additional brood. If not successful, a reduction in the brood nest size would reduce the need for nurse bees, but in this case would not solve the problem as long as the stressor is not removed from the colony. This hypothesis is supported by our finding that the likelihood to die during winter was very high in *V+* colonies and that their size first increased (2–6%) compared to *V−* colonies, before the collapse. In light of our previous explanation, these finding agree with the model hypotheses of Perry et al. (2015) that precocious foraging may lead to an increased chance of colony collapse when the stress exposure of a colony exceeds the social resilience of a colony. In this study, we showed that it was the single stressor *V. destructor* causing the most harm to the colonies in our field experiment with field-realistic exposure to *V. destructor*, *Nosema* spp., and imidacloprid, and not the synergistic effects between the stressors (Thompson 2012).

Acknowledgments

This project was funded from 2013 to 2015 by the Ministry of Economic Affairs (EZ, currently LNV) of The Netherlands 2013–2014 (BO-20-003-003) and 2014–2015 (BO-20-003-024). The authors contributed to this study as follows: Coby van Dooremalen designed

the experiment, with input from Tjeerd Blacquière and Bram Cornelissen, and analyzed the data and wrote the manuscript; Chula Poleij-Hok Ahin and Bram Cornelissen did most of the fieldwork and collected the raw data; and all authors revised the manuscript. The authors declare that they have no competing interests, nor financial, ideological, and political conflict of interests. The authors thank students Lisa Blanken, Jolanda Tom, Janse Heijn, Maayke Broerse, Maria Meijer, Iris van Veen, and Wouter Meijer for helping with the field and laboratory work, students Jolanda Tom and Berend van der Ark for the primary analyses of the data, and Frank van Langevelde for supervising most of these students. Thanks go to Helen Goossen and Jolanda Tom for making the reference list. Inbuzz v.o.f. kindly provided the authors with additional queens upon request. Bayer Crop Science AG (Monheim am Rhein, Germany) kindly produced pure imidacloprid. Vita Europe Ltd kindly provided Apistan. The authors thank a member of the Dutch Board for the Authorisation of Plant Protection Products and Biocides for input on the method requirements for optimal risk assessments.

Literature Cited

Aizen, M. A., and L. D. Harder. 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Current Biology 19:915–918.

Alaux, C., C. Dantec, H. Parrinello, and Y. Le Conte. 2011. Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. BMC Genomics 12:496.

Alaux, C., F. Ducloz, D. Crauser, and Y. Le Conte. 2010. Diet effects on honeybee immunocompetence. Biology Letters. https://doi.org/10.1089/rsbl.2009.0986

Biesmeijer, J. C. 2017. Report Honeybee Surveillance Program the Netherlands 2016–2017. Naturalis Biodiversity Center. http://edepot.wur.nl/423719

Blacquière, T., G. Smagghe, C. A. van Gestel, and V. Mommaerts. 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21:973–992.

Blacquière, T., and J. J. van der Steen. 2017. Three years of banning neonicotinoid insecticides based on sub-lethal effects: Can we expect to see effects on bees? Pest Management Science 73:1299–1304.

Blanken, L. J., F. van Langevelde, and C. van Dooremalen. 2015. Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honeybees. Proceedings of the Royal Society B 282:20151738.

Botías, C., R. Martín-Hernández, L. Barrios, A. Meana, and M. Higes. 2013. *Nosema* spp. infection and its negative effects on honey bees (*Apis mellifera iberiensis*) at the colony level. Veterinary Research 44:25.

Calabrese, E. J., and L. A. Baldwin. 2002. Defining hormesis. Human & Experimental Toxicology 21:91–97.

Cornelissen, B., T. Blacquière, and J. J. M. van der Steen. 2013. Effectieve bestrijding van Varroa (Tweede, licht gewijzigde druk). Report, Plant Research International Wageningen. http://edepot.wur.nl/292797

Cresswell, J. E., F. X. L. Robert, H. Florance, and N. Smirnoff. 2014. Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (*Apis mellifera*) and bumblebees (*Bombus terrestris*). Pest Management Science 70:332–337.

Delaplane, K. S., A. Dag, R. G. Danka, B. M. Freitas, L. A. Garibaldi, R. M. Goodwin, and J. I. Hormaza. 2013. Standard methods for pollination research with *Apis mellifera*. Journal of Apicultural Research 52:1–28.

Dietemann, V., F. Nazzi, S. J. Martin, D. L. Anderson, B. Locke, K. S. Delaplane, Q. Qauquiez, C. Tannahill, E. Frey, and B. Zielgelmann. 2013. Standard methods for varroa research. Journal of Apicultural Research 52:1–54.

Dively, G. P., M. S. Embrey, A. Kamel, D. J. Hawthorne, and J. S. Pettis. 2015. Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PLoS ONE 10:e0118748.

Evans, D., and M. Spivak. 2010. Socialized medicine: individual and communal disease barriers in honey bees. Journal of Invertebrate Pathology 103: S62–S72.

Faucon, J. P., C. Aurières, P. Drajnudel, L. Mathieu, M. Ribière, A. C. Martel, S. Zeggane, M. P. Chauzat, and M. F. Aubert. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. Pest Management Science 61:111–125.

Fernández, J. M., F. Puerta, M. Cousinou, R. Dios-Palomares, F. Campano, and L. Redondo. 2012. Asymptomatic presence of *Nosema* spp. in Spanish commercial apiaries. Journal of Invertebrate Pathology 111:106–110.

Francis, R. M., S. L. Nielsen, and P. Kryger. 2013. Varroa-virus interaction in collapsing honey bee colonies. PLoS ONE 8:e57540.

Fries, I., M. P. Chauzat, Y. P. Chen, V. Doublet, E. Genersch, S. Gisder, M. Higes, D. P. McMahon, R. Martín-Hernández, and M. Natsopoulou. 2013. Standard methods for *Nosema* research. Journal of Apicultural Research 52:1–28.

Fries, I., H. Hansen, A. Imdorf, and P. Rosenkranz. 2003. Swarming in honey bees (*Apis mellifera*) and *Varroa destructor* population development in Sweden. Apidologie 34:389–397.

Genersch, E., W. von Der Ohe, H. Kaatz, A. Schroeder, C. Otten, R. Büchler, S. Berg, W. Ritter, W. Mühlen, and S. Gisder. 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41:332–352.

Goblirsch, M. 2018. *Nosema ceranae* disease of the honey bee (*Apis mellifera*). Apidologie 49:131–150.

Godfray, H. C. J., T. Blacquière, L. M. Field, R. S. Hails, S. G. Potts, N. E. Raine, A. J. Vanbergen, and A. R. McLean. 2015. A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proceedings of the Royal Society B 282:20151821.

Gonzalez-Cabrera, J., T. G. E. Davies, L. M. Field, P. J. Kennedy, and M. S. Williamson. 2013. An amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa destructor*. PLoS ONE 8:e82941.

Goulson, D., E. Nicholls, C. Botías, and E. L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347:1255957.

Guzman-Novoa, E., L. Eccles, Y. Calvete, J. McGowan, P. G. Kelly, and A. Correa-Benitez. 2010. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. Apidologie 41:443–450.

Higes, M., A. Juarranz, J. Dias-Almeida, S. Lucena, C. Botías, A. Meana, P. García-Palencia, and R. Martín-Hernández. 2013. Apoptosis in the pathogenesis of *Nosema ceranae* (Microsporidia: Nosematidae) in honey bees (*Apis mellifera*). Environmental Microbiology Reports 5:530–536.

Johnson, B. R. 2010. Division of labor in honeybees: form, function, and proximate mechanisms. Behavioral Ecology and Sociobiology 64:305–316.

Johnson, R. M., M. D. Ellis, C. A. Mullin, and M. Frazier. 2010. Pesticides and honey bee toxicity—USA. Apidologie 41:312–331.

Johnson, B. R., and T. A. Linksvayer. 2010. Deconstructing the superorganism: social physiology, groundplans, and sociogenomics. Quarterly Review of Biology 85:57–79.

Kendig, E. L., H. H. Le, and S. M. Belcher. 2010. Defining hormesis: evaluation of a complex concentration response phenomenon. International Journal of Toxicology 29:235–246.

Klein, A. M., B. E. Vaissiere, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274:303–313.

Le Conte, Y., M. Ellis, and W. Ritter. 2010. *Varroa* mites and honey bee health: Can *Varroa* explain part of the colony losses? Apidologie 41:353–363.

Little, C. M., D. Shutler, and G. R. Williams. 2015. Associations among *Nosema* spp. fungi, *Varroa destructor* mites, and chemical treatments in honey bees, *Apis mellifera*. Journal of Apicultural Research 54:1–8.

Mariani, F., M. Maggi, M. Porrini, S. Fuselli, G. Caraball, C. Brasesco, C. Barrios, J. Principal, and E. Martin. 2012. Parasitic interactions between *Nosema* spp. and *Varroa destructor* in *Apis mellifera* colonies. Zootecnia Tropical 30:81–90.

Mattila, H. R., and G. W. Otis. 2007. Dwindling pollen resources trigger the transition to broodless populations of long-lived honeybees each autumn. Ecological Entomology 32:496–505.

Meikle, W. G., J. J. Adamczyk, M. Weiss, A. Gregorc, D. R. Johnson, S. D. Stewart, J. Zawislak, J. M. Caroll, and G. M. Lorenz. 2016. Sublethal effects of imidacloprid on honey bee colony growth and activity at three sites in the US. PLoS ONE 11: e0168603.

Meixner, M. D., R. M. Francis, A. Gajda, P. Kryger, S. Andonov, A. Uzunov, G. Topolska, C. Costa, E. Amiri, and S. M. Berg. 2014. Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. Journal of Apicultural Research 53:215–229.

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Archives of Insect Biochemistry and Physiology 58:200–215.

Nazzi, F., and Y. Le Conte. 2016. Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. Annual Review of Entomology 61:417–432.

Neumann, P., and N. L. Carreck. 2010. Honey bee colony losses. Journal of Apicultural Research. https://doi.org/10.3896/IBRA.1.49.1.01

Nixon, H. L., and C. R. Ribbands. 1952. August Food transmission within the honeybee community. Proceedings of the Royal Society B 140: 43–50.

Perry, C. J., E. Søvik, M. R. Myerscough, and A. B. Barron. 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proceedings of the National Academy of Sciences of the United States of America 112:3427–3432.

Pettis, J. S., J. Johnson, and G. Dively. 2012. Pesticide exposure in honey bees results in increased levels

of the gut pathogen *Nosema*. Naturwissenschaften 99:153–158.

Pettis, J. S., E. M. Lichtenberg, M. Andree, J. Stitzinger, and R. Rose. 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. PLoS ONE 8: e70182.

Pisa, L. W., V. Amaral-Rogers, L. P. Belzunces, J. M. Bonmatin, C. A. Downs, D. Goulson, D. P. Kreutzweiser, C. Krupe, M. Liess, and M. McField. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research 22:68–102.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010*a*. Global pollinator declines: trends, impacts and drivers. Trends in Ecology Evolution 25:345–353.

Potts, S. G., S. P. Roberts, R. Dean, G. Marris, M. A. Brown, R. Jones, P. Nuemann, and J. Settele. 2010*b*. Declines of managed honey bees and beekeepers in Europe. Journal of Apicultural Research 49:15–22.

Rademacher, E., M. Harz, and S. Schneider. 2017. Effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae). Insects 8:84.

Rortais, A., G. Arnold, M. P. Halm, and F. Touffet-Briens. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36:71–83.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. 2010. Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology 103:S96–S119.

Rundlöf, M., G. K. Andersson, R. Bommarco, I. Fries, V. Hederström, L. Herbertsson, O. Jonsson, B. K. Klatt, T. R. Pedersen, and J. Yourstone. 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521:77.

Schmickl, T., and K. Crailsheim. 2002. How honeybees (*Apis mellifera L*) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. Behavioral Ecology and Sociobiology 51:415–425.

Schmickl, T., and K. Crailsheim. 2004. Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply. Apidologie 35:249–263.

Schmuck, R., R. Schöning, A. Stork, and O. Schramel. 2001. Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Management Science 57:225–238.

Seeley, T. D., and P. Visscher. 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecological Entomology 10:81–88.

Sendova-Franks, A. B., and N. R. Franks. 1994. Social resilience in individual worker ants and its role in division of labour. Proceedings of the Royal Society B 256:305–309.

Thompson, H. M. 2012. Interaction between pesticides and other factors in effects on bees. EFSA Supporting Publications 9: https://doi.org/10.2903/sp.efsa.2012.EN-340

Tsvetkov, N., O. Samson-Robert, K. Sood, H. S. Patel, D. A. Malena, P. H. Gajiwala, P. Maciukiezwicz, V. Fournier, and A. Zayed. 2017. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. Science 356:1395–1397.

Van der Zee, R. 2010. Colony losses in the Netherlands. Journal of Apicultural Research 49:121–123.

Van der Zee, R., A. Gray, L. Pisa, and T. de Rijk. 2015. An observational study of honey bee colony winter losses and their association with *Varroa destructor*, neonicotinoids and other risk factors. PLoS ONE 10:e0131611.

Van Dooremalen, C., L. Gerritsen, B. Cornelissen, J. J. van der Steen, F. van Langevelde, and T. Blacquière. 2012. Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. PLoS ONE 7:e36285.

VanEngelsdorp, D., and M. D. Meixner. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103:S80–S95.

Winston, M. L. 1987. The biology of the honey bee. Harvard University Press, Cambridge, Massachusetts, USA.

Woodcock, B. A., J. M. Bullock, R. F. Shore, M. S. Heard, M. G. Pereira, J. Redhead, H. Ridding, D. Dean, P. Henrys, and J. Peyton. 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356:1393–1395.

Wu-Smart, J., and M. Spivak. 2016. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. Scientific Reports 6:32108.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2378/full