Research in Veterinary Science 144 (2022) 1–10

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Compliance with recommended *Varroa destructor* treatment regimens improves the survival of honey bee colonies over winter

Julie Hernandez [a,b,f,*], Jan Hattendorf [c,g], Alexandre Aebi [a,d], Vincent Dietemann [b,e]

[a] *Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland*
[b] *Agroscope, Swiss Bee Research Centre, Bern, Switzerland*
[c] *Department of Public Health and Epidemiology, Swiss Tropical and Public Health Institute, Basel, Switzerland*
[d] *Institute of Anthropology, University of Neuchâtel, Neuchâtel, Switzerland*
[e] *Department of Ecology and Evolution, Biophore, UNIL-Sorge, University of Lausanne, Lausanne, Switzerland*
[f] *Interjurasienne Rural Foundation (FRI), Courtemelon, Switzerland*
[g] *University of Basel, Basel, Switzerland*

ARTICLE INFO

Keywords:
Apis mellifera
Pest control
Varroa destructor
Compliance
Colony mortality
Beekeeping management

ABSTRACT

The ectoparasitic mite *Varroa destructor* affects honey bee colony health and survival negatively, thus compelling beekeepers to treat their colonies every year. A broadly used mite control regimen is based on two organic molecules: formic and oxalic acids. To ensure optimal efficiency, several applications of these acids at pre-defined time points are recommended. These recommendations are mainly based on experiments conducted under controlled conditions. Studies evaluating the effectiveness under natural field conditions are lacking.

We enrolled 30 beekeepers in a longitudinal study in three cantons in Switzerland and monitored the management and health of their colonies for two years. We assessed compliance with mite control recommendations and measured *V. destructor* infestation rates, indexes of colony productivity (brood size and honey harvest), and colony mortality in 300 colonies.

We observed a 10-fold increased risk of colony death when beekeepers deviated slightly from the recommended treatment regimen compared to compliant beekeepers (odds ratio: 11.9, 95% CI: 2.6–55.2, $p = 0.002$). The risk of colony death increased 25-fold in apiaries with substantial deviations from the recommendations (odds ratio: 50.4, 95% CI: 9.7–262.5, $p < 0.0001$). The deviations led to increased levels of *V. destructor* infestation ahead of wintering, which was likely responsible for colony mortality. After communicating the apparent link between low compliance and poor colony survival at the end of the first year to the beekeepers, we observed better compliance and colony survival in the second year.

Our results highlight the positive impact of compliance with the recommended *V. destructor* treatment regimen on the health of honeybee colonies and the need to better communicate the consequences of deviating from the recommendations to improve compliance. Compliance also occasionally decreased, which hints at concept implementation constraints that could be identified and possibly addressed in detail with the help of social sciences to further promote honey bee health.

1. Introduction

During the last 15 years, increased colony mortality of the western honeybee, *Apis mellifera*, an economically important insect, has fostered intense research on the factors affecting its health (Steinhauer et al., 2018; Smith et al., 2013). The possible causes of colony losses identified include parasites and pathogens (Smith et al., 2013; vanEngelsdorp and Meixner, 2010). In particular, the invasive ectoparasitic mite *Varroa destructor* is regarded as the main biotic threat to *A. mellifera* of European origin (Guichard et al., 2020; Traynor et al., 2020). This mite functions as a vector of viruses (Berthoud et al., 2010; Conte et al., 2010), reducing the lifespan of adult honey bee workers (Dainat et al., 2012) and the ability of colonies to survive, especially over winter (Rosenkranz et al., 2010; Traynor et al., 2020). Without mite control, a colony is predicted to collapse within one to three years (Ritter, 1981; Korpela et al., 1992; Fries and Rosenkranz, 1996; Conte et al., 2007), compelling beekeepers

---

* Corresponding author at: Laboratory of Soil Biodiversity, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland.
E-mail address: julie.hernandez@unine.ch (J. Hernandez).

https://doi.org/10.1016/j.rvsc.2021.12.025
Received 22 July 2021; Received in revised form 16 December 2021; Accepted 29 December 2021

to apply effective mite control yearly to maintain their stocks and productivity (Rosenkranz et al., 2010). The implementation of control measures is aimed at reducing the *V. destructor* infestation levels of the so-called "winter bees", which are long-lived individuals who are thought essential to ensure colony survival over winter (van Dooremalen et al., 2012).

Several recent studies have shown that beekeepers can reduce winter colony mortality by applying varroacidal treatments (Oberreiter and Brodschneider, 2020; Jacques et al., 2017; Haber et al., 2019; Giacobino et al., 2015; Giacobino et al., 2016; Giacobino et al., 2017; Haber et al., 2019). However, colony losses remain excessive and fluctuate in an unpredictable manner (Charrière and Neumann, 2010; Oberreiter and Brodschneider, 2020; Brodschneider et al., 2018; Gray et al., 2020). This observation is attributable to factors other than *V. destructor* (Smith et al., 2013) but can also be due to failures in mite control due to incorrect implementation, which is yet to be investigated systematically. Incorrect implementation is especially likely for the so-called "alternative control methods" because they rely on several applications of organic acids at particular times, which leaves a margin for deviations (Dietemann et al., 2012). Several applications are required to reach sufficient treatment efficacy, equivalent to that of the previously used products containing synthetic active compounds, such as pyrethroids (e.g., tau-fluvalinate and flumethrin) and phosphorothioates (e.g., coumaphos) (Rosenkranz et al., 2010). Their application at particular times during the beekeeping season is due to the dependency of efficacy on ambient factors, which affect the distribution of the active ingredients within the colony (Rosenkranz et al., 2010; Beyer et al., 2018). While this dependency is problematic to determine application time, it is an advantage because, when correctly used, these ingredients evaporate and do not accumulate in the hives (Imdorf et al., 1996; Bogdanov et al., 2002). This lack of residue accumulation ensures that the hive products remain uncontaminated (Rosenkranz et al., 2010) and decreases the risk of resistance development in mites. In fact, after several decades of use, the alternative methods have not led to the development of resistance in mites, whereas such resistance arose within a few years of synthetic product use (Elzen et al., 2000; Maggi et al., 2011; Milani, 1999). Because of these advantages, as well as their proven effectiveness when tested under controlled research conditions (Fries et al., 1991; Imdorf et al., 1996), organic acid-based concepts are recommended for controlling *V. destructor* in several countries :Switzerland (Imdorf et al., 2003), (Charrière et al., 1997), Austria (Oberreiter and Brodschneider, 2020; Brodschneider et al., 2019), Denmark, Sweden, Netherlands, Germany (Genersch et al., 2010; van der Steen and Vejsnæs, 2021). However, it is not clear to what extent deviations from the recommended treatment regimen contribute to the recurrent winter colony losses recorded in these countries (Gray et al., 2020).

Previous studies aimed at determining the role of *V. destructor* control in the maintenance of colony health were of short duration (Giacobino et al., 2015, 2016, 2017; Haber et al., 2019) and relied on beekeepers' estimations of this role instead of standardized measurements through an adequate monitoring at colony level (Beyer et al., 2018; Jacques et al., 2017; Haber et al., 2019). Moreover, none of these studies considered whether varroacidal treatments were implemented as recommended (Oberreiter and Brodschneider, 2020) or established a direct link between treatment and *V. destructor* infestation levels (Oberreiter and Brodschneider, 2020) or colony mortality (Giacobino et al., 2015, 2016, 2017; Haber et al., 2019). To better link the correct implementation in applying the alternative mite control concept (i.e., the application of the correct number of organic acid treatments at the correct time), with their intended goal of reducing *V. destructor* infestation rates and colony losses, we enrolled 30 Swiss hobby beekeepers. We asked them to record the number of treatment applications they performed and the time at which they were applied, as well as to provide access to their colonies to trained field assistants for sample and data collection. These assistants recorded *V. destructor* infestation rates in each of the 10 colonies per apiary, colony survival over two consecutive winters, and the amount of brood reared in the colonies. The last parameter, together with the amount of honey harvested per apiary (López-Uribe and Simone-Finstrom, 2019), facilitated the assessment of potential negative side-effects of the treatments on brood survival (Tihelka, 2018; Elzen et al., 2004) and the identification of economic incentives potentially affecting compliance with recommendations. After colony losses of the first year were linked to compliance, we communicated the results to the beekeepers to monitor putative improvements in compliance and colony mortality in the second year.

We tested the following hypotheses: (i) Lack of compliance with the recommended control concept decreases treatment effectiveness, leading to increased *V. destructor* infestation rates of winter workers and colony mortality; (ii) Showing the link between compliance and colony mortality to beekeepers can increase compliance in the future; (iii) Reducing the number of treatment applications reduces the negative side-effects on colonies and leads to larger brood size; and (iv) Lack of compliance reduces apiary productivity. From the results, we derived suggestions to improve beekeeper compliance with the recommended treatment regimen for controlling *V. destructor*, with the aim of fostering the health of managed honey bees.

2. Material and methods

2.1. Beekeeper enrolment, study area, and experimental period

Colony monitoring was performed over two years (from August 2018 to April 2020) in 30 apiaries located in the Jura, Bern, and Vaud cantons, Switzerland. Eligible beekeepers were identified by calls to participate in the study relayed by the beekeeper associations in the cantons and through two information meetings. Beekeepers between the ages of 18 and 70 were eligible to participate. Enrollment was stopped after 30 participants were recruited for the study. The 30 apiaries hosted 10 colonies each. The type of apiculture performed by these volunteers was typical for Swiss beekeepers and, thus, this sample can be considered representative of the beekeeping community in this country. The beekeepers initially agreed to follow the recommended *V. destructor* treatment regimen. After the first year of study, the relationship between colony survival and implemented mite control was communicated to the beekeepers during a meeting session and through email.

The 300 colonies (*Apis mellifera carnica*) used in this study were kept in Dadant and Swiss Bürki beehive systems and headed by queens between 1 and 1.5 years of age. All apiaries were monitored three times per year. The first visit occurred early August before the honey summer harvest and formic acid treatment. This was followed by a second visit in October before wintering and oxalic acid treatment and a third at the beginning of April of the following year, when colonies came out of the wintering period and started their development (Fig. 1).

2.2. Data collection

2.2.1. Capped brood size

During the August and October visits, the amount of capped brood produced in the colonies was quantified to monitor the side-effects of the treatments applied. This quantification was obtained using the ColEval method (Hernandez et al., 2020). Briefly, a calibrated estimation of the percentage comb surface area occupied by capped brood was performed and, subsequently, converted into number of cells.

2.2.2. Honey harvest

The mass of honey harvested per apiary was recorded by the beekeepers each year (Fig. 1) and used as a proxy for colony productivity and health.

2.2.3. V. destructor infestation rates

At the August and October visits (Fig. 1), adult honey bee workers (mean : 300, SD : 50) were sampled from the open brood frames of each

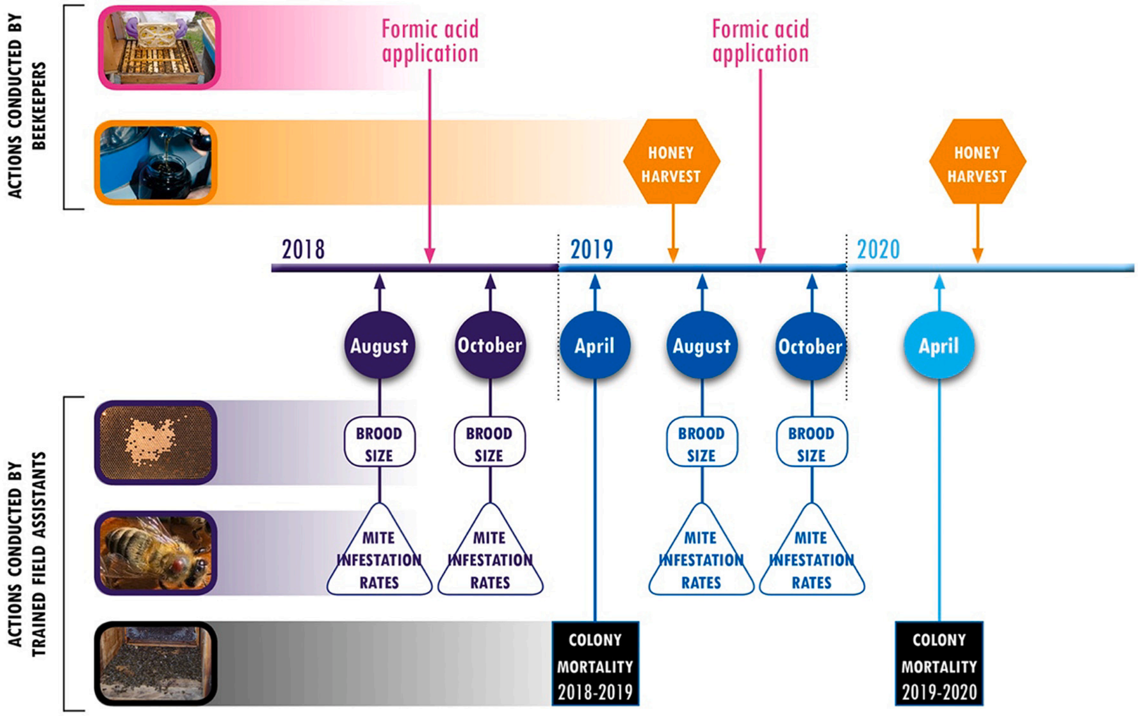

Fig. 1. Summary of data collection indicating who from the beekeepers or the researchers performed a given action or measurement.

colony for *V. destructor* infestation rate assessment (Dietemann et al., 2013; Lee et al., 2010a, 2010b). The samples were placed in plastic zip bags and kept on ice before being brought to the laboratory for storage at −20 °C until analysis. Before analysis, the samples were weighted to determine the number of honey bees they contained. Each sample was then washed with soapy water to dislodge the mites for counting, following a standard protocol (Dietemann et al., 2013). From these data, the number of mites per 100 workers was calculated (Dietemann et al., 2013).

2.2.4. *Colony mortality*

Colony mortality was recorded in April, after the overwintering period (Fig. 1). In case of colony losses, beekeepers replaced the dead colonies with nuclei prepared in the spring of the previous season, and new colony identity numbers were attributed to them.

2.3. *V. destructor control regimen*

In Switzerland, the recommended *V. destructor* treatment regimen includes three product applications. The first application of formic acid immediately after the honey harvest, between July 25th and August 10th, uses long-term dispensers. This is followed by a second application between August 25th and September 15th. Between November and December, when the colonies stop rearing brood, the application of oxalic acid is recommended. If more than five mites fall per day on the hives' bottom boards four weeks after this treatment, a second oxalic acid application is required (Apiservice, 2021). Several formic acid dispensers are available on the Swiss market [Apidea, FAM, Liebig, MAQS, or Nassenheider PRO (Apiservice, 2021)]. These models show a similar efficacy (Imdorf et al., 2003), and participating beekeepers were free to use any of them. Similarly, several equally efficacious oxalic acid application modes are available [spraying, trickling, or sublimation (Rosenkranz et al., 2010; van der Steen and Vejsnæs, 2021)], and beekeepers were also free to choose their preferred mode.

2.4. *Classification of compliance according to the recommended V. destructor treatment regimen*

To determine the influence of compliance on *V. destructor* infestation rates, brood size, honey harvest, and colony mortality, the beekeepers were asked to record the number of formic and oxalic acid applications, as well as the dates on which these were performed, through a mobile application (ApiNotes©). The number and timing of the treatment applications were used to determine compliance categories. The "compliant" category included beekeepers who correctly followed the control concept (i.e., who applied the correct number of treatments at the appropriate time). The "almost-compliant" category grouped beekeepers who did apply the required number of treatments but at inappropriate times. The "noncompliant" category characterized beekeepers who applied fewer treatments than recommended.

2.5. *Statistical analysis*

Our primary hypothesis was that colonies belonging to compliant beekeepers experience lower mortality compared to those belonging to almost-compliant and to noncompliant beekeepers. We ran a series of simulations to assess the sample size requirements. The simulations revealed that 30 apiaries with 10 colonies each (300 colonies in total) were sufficient to detect a true difference of 20% colony mortality per year in compliant beekeepers compared to the proportion of 40% in almost-compliant beekeepers with 80% power at 95% confidence level, assuming an intra-cluster correlation coefficient (i.e., colonies clustered in apiaries) of 0.2 and a similar number of beekeepers in each compliance category. The power to detect a difference between the complier and noncomplier groups was above 95%, assuming a true colony mortality of 50%.

To analyze the effect of compliance on the variables measured and to verify our hypotheses, we used generalized estimating equations and structural equation models (Overall and Tonidandel, 2004; Lefcheck, 2016; Pugesek and Tomer, 2003). We combined these models because the generalized estimating equations rely on well-known regression models, which account for clustered observations (i.e., colonies in apiaries) and provide reliable estimates even if some assumptions are slightly violated, whereas the structural equation models, although relying on more complex assumptions, account for the complex relationships between variables. In these structural equation models, the same variable can be both an independent and a dependent variable, allowing the identification of possible causal–effect relationships. It thus becomes possible to analyze the *V. destructor* infestation rate as both an endpoint of the implementation of the control concept and as a cause for colony mortality.

Generalized estimating equation models with an exchangeable correlation structure (Overall and Tonidandel, 2004) were thus used to analyze the effect of noncompliance (almost-compliant and noncompliant categories) on colony mortality, *V. destructor* infestation rates, and the number of capped brood cells in October, taking as reference the compliant category. These models used robust sandwich variance estimators to account for the correlations within clusters (i.e., colonies clustered in apiaries). For binary outcomes (mortality variable), we estimated the odds ratios using a logit-link function. Continuous outcomes (number of mites per adult bees and number of capped brood cells) were log-transformed prior to the analysis.

Changes in compliance over the years were assessed using a generalized estimating equation model for clustered ordinal data.

The honey mass harvested did not follow any theoretical distribution. Therefore, the impact of compliance on this variable was analyzed with a conditional version of the nonparametric Kruskal–Wallis test.

These analyses were conducted in R version 4.0.3 using the "geepack" and 'coin' packages (R Core Team (2019).

Because organic acid application can affect the *V. destructor* infestation rates and colony brood size through both direct and indirect pathways simultaneously, we used generalized structural equation models (Lefcheck, 2016; Pugesek and Tomer, 2003) to quantify the strength of the relationships in a single network and examine each of these pathways simultaneously while accounting for the correlations between multiple response variables (Grace et al., 2015). We present the results using a path diagram, together with the estimated coefficients for each path. Compliance was dummy-coded using "compliant" as the reference category, and colony mortality was modeled as a binary variable with a logit link function. All other variables were assumed to have approximately normally distributed error terms. The model was adjusted for year as a fixed effect and for colonies nested in apiaries as random effects. As generalized structural equation models do not provide standardized coefficients, we provided the unstandardized coefficients. Their interpretation is the same as in linear or logistic regression; that is, the coefficient on the path from a numeric variable toward a binary variable represents the log of the odds ratio associated with each unit increase in the variable at the start of the path. The model was implemented in Stata version 15.0 using the "gsem" command (StataCorp, 2017). No imputation of missing data was done.

We assessed the validity of various model assumptions (normally distributed errors, approx. linear relationships, homoscedasticity, no influential outlier) by visual inspections of regression diagnostic plots (residual vs leverage and QQ plots).

3. Results

3.1. Compliance categories

Of the 30 beekeepers enrolled in this study, two did not provide sufficient information in 2018 and three in 2019. These cases were thus excluded from the analysis. All beekeepers applied winter oxalic acid treatments at the right time. Compliance was thus restricted to the number and timing of formic acid treatments performed (Table 1).

Table 1
Compliance categories defined according to the number of formic acid treatments performed and their application time.

Compliance categories	Number and timing of formic acid applications
Compliant	Two applications within the recommended period[a]
Almost-Compliant	Two applications but at least one outside the recommended period[a]
Noncompliant	Less than two applications

[a] Between July 25th and August 10th.

In 2018, 25% of the colonies were treated in compliance with the recommendations, 43% were treated in an almost-compliant manner, and 32% were treated in a noncompliant manner (Fig. 2). A significantly higher percentage of colonies were treated according to compliant and almost-compliant regimens in 2019 with 26% in the compliant, 70% in the almost-compliant, and only 4% in the noncompliant categories ($p = 0.005$, Fig. 2). The improvement was mainly driven from the 2018 noncompliant beekeepers becoming compliant or almost-compliant in 2019 and occurred despite more than half the 2018 compliant beekeepers reducing their compliance level to almost-compliant in the second year (Fig. 2).

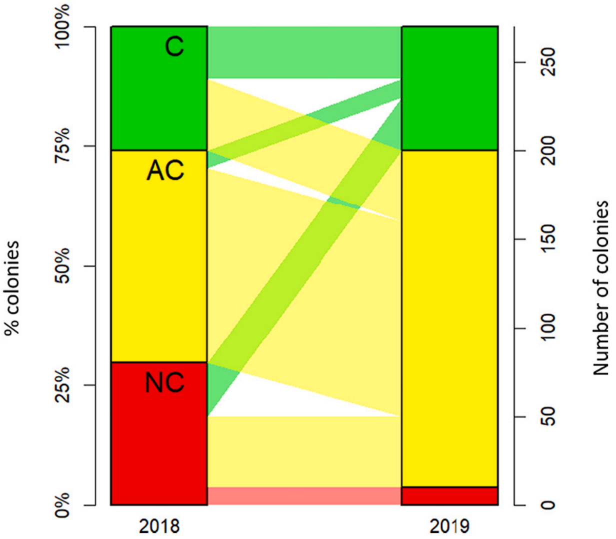

Fig. 2. Changes in compliance categories between 2018 and 2019 expressed in percent and number of colonies treated according to the various compliance regimens.

3.2. Effect of compliance on honey bee colony mortality, *V. destructor* infestation rates, brood size, and honey harvest

3.2.1. Effect of compliance on honey bee colony mortality

Overall, 28% of the colonies died in 2018 and 15% died in 2019 (Tables 2 and 3). With 2% colonies lost, compliant beekeepers experienced a significantly lower mortality rate compared to the 20% almost-compliant beekeepers (odds ratio: 11.9, 95% CI: 2.6–55.2, $p = 0.002$) and to the 55% noncompliant beekeepers (odds ratio: 50.4, 95% CI: 9.7–262.5, $p < 0.0001$; Tables 2 and 3). For noncompliant beekeepers, the probability of colony loss increased rapidly with the infestation rate in October, with a 50% probability of death for an infestation of 10 mites per 100 adult honeybee workers (Fig. 3).

3.2.2. Effect of compliance on *V. destructor* infestation rates

Overall, compliant beekeepers had a 26% lower mite infestation rate in October compared to August and this effect was more pronounced in 2018 than in 2019. In contrast, the mean infestation rate increased in the two other compliance categories (Table 2, Fig. 4). The statistical model comparing infestation rates among compliance categories is presented in Table 3. We observed a marginally significant lower log number of mites in colonies of compliant beekeepers compared to the noncompliant

Table 2
Colony mortality, *Varroa destructor* infestation rate per 100 adult honey bee workers, capped brood cell number, queen age, and honey harvest for each compliance category in total and for each year separately.

	Total			2018			2019		
	Compliant	Almost-compliant	Non-compliant	Compliant	Almost-compliant	Noncompliant	Compliant	Almost-compliant	Non-compliant
N colonies	140	310	100	70	120	90	70	190	10
Colony mortality[a] [%]	2	20	55	3	19	60	1	21	10
V. destructor August [mean (SD)]	3.3 (5.0)	4.6 (8.6)	3.8 (4.3)	3.8 (5.9)	3.0 (5.3)	3.6 (4.2)	2.7 (3.6)	5.7 (10.0)	6.0 (4.0)
V. destructor October [mean (SD)]	2.5 (3.9)	4.9 (9.8)	6.8 (9.8)	2.4 (2.6)	5.7 (13.5)	7.5 (10.4)	2.6 (5.0)	4.4 (6.1)	2.6 (3.1)
Relative difference [%]	−26	5	77	−37	92	110	−5	−23	−57
Brood cells August [mean (SD)]	8694 (4981)	9058 (5176)	8966 (5112)	10,397 (4665)	9863 (4845)	8685 (5252)	6991 (4726)	8556 (5323)	11,460 (2675)
Brood cells October [mean (SD)]	2055 (1556)	1702 (1600)	1446 (1550)	2154 (1589)	1526 (1308)	1158 (1247)	1954 (1526)	1813 (1754)	3920 (1754)
Relative difference [%]	−76	−81	−84	−79	−85	−87	−72	−79	−66
Honey harvest (kg per apiary) [mean(SD)]	194.2 (101.4)	180.5 (137.6)	68.0 (87.2)	130.7 (55.9)	83.9 (38.2)	63.0 (94.4)	257.7 (99)	240.9 (143.4)	98.3 (NA)
Queen age (years) [mean (SD)]	1.0 (0.6)	1.0 (0.6)	0.9 (0.7)	0.9 (0.5)	1.0 (0.6)	0.8 (0.7)	1.0 (0.6)	1.0 (0.6)	0.9 (0.5)

[a] One colony with missing data in 2018 for almost-compliant category.

Table 3
Analysis of the effect of beekeepers' compliance with the recommended *Varroa destructor* control concept on colony mortality, *V. destructor* infestation rates (in mites per 100 adult honeybee workers in October), and amount of brood (in number of capped brood cells in October) using generalized estimating equation models.

Colony mortality	Dead colonies	Odds ratio	95% CI	p
Compliant	3/140	ref		
Almost-compliant	62/309	11.9	2.6–55.2	0.002
Non-compliant	55/100	50.4	9.7–262.5	<0.0001
2018	72/279	ref		
2019	41/270	0.76	0.3–1.7	0.49
***V. destructor*infestation rates**	**log(mites + 1)**	**Difference**	**95% CI**	**p**
Compliant	0.9	ref		
Almost-compliant	1.2	0.3	−0.2–0.7	0.21
Non-compliant	1.5	0.6	0–1.2	0.07
2018	1.2	ref		
2019	1.1	0.0	−0.3–0.3	0.89
Number of capped brood cells	**log(cells + 1)**	**Difference**	**95% CI**	**p**
Compliant	6.7	ref		
Almost-compliant	5.6	−1.1	−2.3–0.1	0.07
Non-compliant	5.2	−1.3	−2.5–0	0.04
2018	5.6	ref		
2019	6.1	0.4	−0.7–1.5	0.47

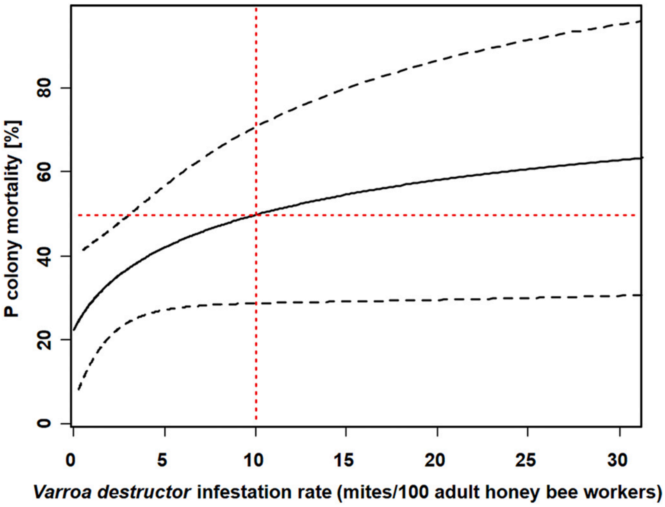

Fig. 3. Probability of colony mortality depending on *Varroa destructor* infestation rate in October. The figure is a visualization of the predicted values resulting from a logistic regression in noncompliant beekeepers back-transformed to the probability scale. Dashed lines represent the 95% confidence band around the prediction line.

category (difference$_{\text{log scale}}$ 0.6, 95% CI 0–1.2, $p = 0.07$). The difference between compliant and almost-compliant beekeepers was not significant (difference$_{\text{log scale}}$ 0.3, 95% CI -0.2 to 0.7, $p = 0.21$).

3.2.3. Effect of compliance on brood size

We observed the highest mean number of capped brood cells in October in the colonies of compliant beekeepers (Table 2). This was marginally significantly higher than the mean value measured in colonies of almost-compliers (difference$_{\text{log-scale}}$ = 1.1, 95% CI: −0.1 to 2.3, $p = 0.07$; Tables 2 and 3) and significantly higher than that in colonies of noncompliers (difference$_{\text{log-scale}}$ = 1.3, 95% CI: 0.0–2.5, $p = 0.04$; Tables 2 and 3).

3.2.4. Effect of compliance on apiary productivity

Honey harvest differed significantly among compliance groups with mean yields of 194, 180, and 68 kg in compliant, almost-compliant, and noncompliant groups, respectively (Kruskal–Wallis Test, chi-squared = 6, $p = 0.04$).

3.2.5. Path analysis of simultaneous direct and indirect effects of compliance on V. destructor infestation rates, colony mortality, and brood size

The structural equation path model was defined according to the relationships between the variables measured, as depicted in Fig. 5 (see also Appendix 1). The infestation rate in October was not linked to that in early August before the treatments were applied but was affected by the compliance category. Almost-compliance and noncompliance were associated with a significant positive effect on *V. destructor* infestation rates in October (0.31 logarithm units for almost-compliant and 0.6 logarithm units for noncompliant cases). The log-transformed *V. destructor* infestation rate in October had a significant positive impact on colony mortality. The odds of colony death increased by 1.88 with each log unit increase in the *V. destructor* infestation rate in October. In addition, the infestation rate measured in October had a

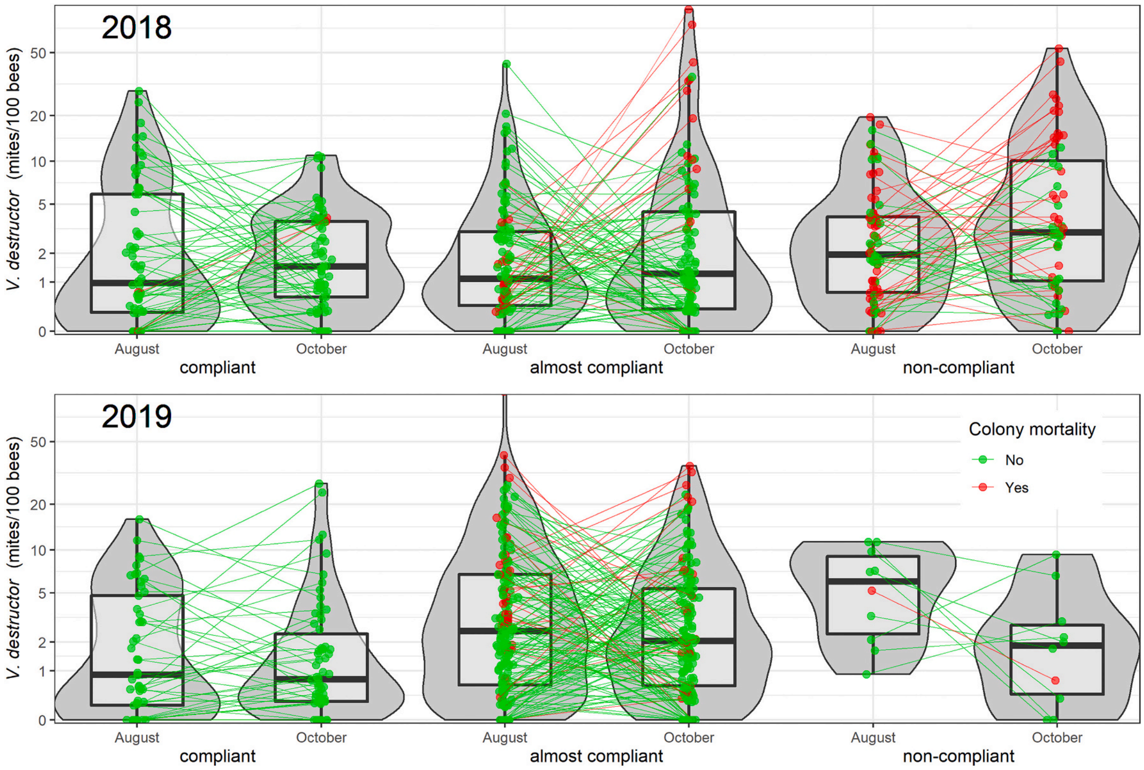

Fig. 4. *Varroa destructor* infestation rates in August and October, before and after treatments, respectively, and colony mortality over the following winter according to compliance categories in 2018 and 2019. The violins-plots show the probability density curve of the infestation rate values, and boxplots indicate the median of the data (thick horizontal line) and the interquartile range (box).

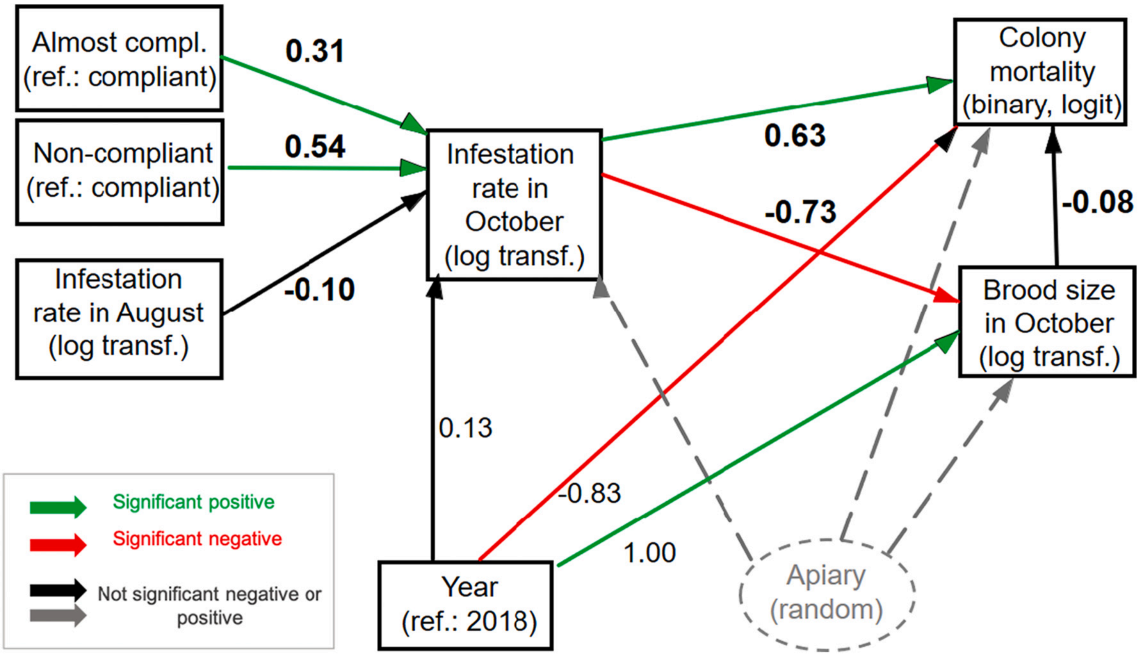

Fig. 5. Direct and indirect effects of compliance categories on *Varroa destructor* mite infestation rates, colony mortality, and amount of brood generated by the structural equation model. The standardized regression coefficient for each path is given next to the corresponding arrow. The model was adjusted for clustering of colonies within apiaries (random effect) and year (fixed effect).

significant negative effect on the number of brood cells (−0.73 log units). In this month, brood size had no significant effect on colony mortality (Appendix 1). The factor year had no significant effect on mite infestation rates in October but a significant effect on colony mortality and brood size.

4. Discussion

We showed that compliance with the recommended *V. destructor* treatment regimen reduced infestation rates in the colonies, which drastically increased colony survival and apiary productivity. The potential negative side-effects of treatment application on the brood did not significantly decrease winter colony survival, but brood size was significantly negatively impacted by the lack of compliant treatment. Communicating the negative impact of noncompliance on colony survival and productivity to participating beekeepers increased compliance of a fraction of the noncompliant beekeepers in the following year. However, a decrease in compliance was observed in half of the previously compliant beekeepers.

4.1. Compliance effects on V. destructor infestation rates, brood size, and colony mortality

We showed a strong link between compliance with the recommended *V. destructor* control concept and colony mortality. The results from the generalized equation model showed that if treatment regimens only deviated slightly from the recommended concept (i.e., were almost-compliant), a colony had 10 times higher risks of dying compared to a compliant treatment (20% vs. 2% colony losses) and 25 times higher risks if the beekeeper was noncompliant (i.e., applied less than two treatments) (55% vs. 2% colony losses, odds ratio 11 and 50, respectively, Table 3). The decrease in mite infestation after treatment application (i.e., between August and October) was higher in the compliant group, confirming the superior effectiveness of the recommended control concept. This lower infestation is likely to have contributed to the decreased colony losses experienced in this group. Despite the generalized equation modeling showing only a marginally significant infestation decrease in October comparing compliant to noncompliant groups (Table 3), the structural equation modeling showed a significant interaction effect (Fig. 5). This effect indicates that an increase in *V. destructor* infestation rates in October due to deviations from the recommendations is directly linked to increased colony mortality (Fig. 5). The negative coefficient between mite infestation rates in August and October (Fig. 5, Appendix 1) in structural equation modeling and the higher infestation rate in noncompliant treatment regimens compared to compliant ones (7 vs. 2.5 in mites per 100 adult workers, Table 2) indicate that formic acid applications decouple the number of mites in August from that in October. The number of mites measured in October was mainly determined by the level of compliance (Fig. 5).

In the case of noncompliance, an infestation rate of 10 mites per 100 adult honeybee workers in October led to a 50% chance of colony death over winter (Fig. 3). This result is in line with the previous literature, according to which the mortality rates of colonies infested in the 10 to 20 mites per 100 workers range in autumn could reach 20% to 50% on average (Genersch et al., 2010; Liebig, 2001; Guzmán-Novoa et al., 2010).

The analysis of the effect of *V. destructor* infestation on the amount of brood and colony mortality with the two models strongly supported the hypothesis that healthy winter honey bee workers, which were not parasitized during their pre-imaginal development, are crucial to ensure colony survival over winter. This was especially the case with the structural equation model, which allows for deriving the possible causal–effect relationships because of the model taking into account the complex relationships between the variables. Thus, our results represent the most tangible evidence, to date, that healthy winter honey bee workers are crucial to ensuring colony survival over winter. However, the effect can be smaller than our estimate because we cannot rule out the possibility that brood size itself also had a direct negative impact on infestation rates.

The positive effect of high compliance on colony survival occurred despite the potential negative side-effects of formic acid on brood survival (Gregorc et al., 2004; Strachecka et al., 2012). In addition, a reduced number of treatment applications by noncompliant beekeepers did not lead to a higher amount of brood in their colonies compared to compliant beekeepers (Tables 2 and 3). Instead, they experienced a significant decrease in the amount of brood in October. This decrease was likely due to the higher number of mites infesting the colonies (Table 3, Fig. 5). Thus, there is a stronger negative impact of *V. destructor* infestation than that of formic acid on the brood, infirming the hypothesis that the negative side-effects of the repeated formic acid applications (Tihelka, 2018) can exceed their positive effects. In addition, the amount of brood in October showed no noteworthy association with mortality (Fig. 5). There is thus no benefit in refraining from applying two formic acid treatments as recommended.

The importance of factors other than *V. destructor* infestation in causing colony mortality was indicated by significant effects of the factor year on brood size and colony mortality. The factor year includes the effect of variables not measured in our study. For example, interannual variations in weather can affect resource acquisition and brood rearing (Beyer et al., 2018; Bagheri and Mirzaie, 2019; Nürnberger et al., 2019), which in turn can affect the population dynamics of *V. destructor*, treatment effectiveness and thus colony mortality (Nürnberger et al., 2019; Calovi et al., 2021). However, no year effect on infestation rates was observed during our study (Fig. 5), indicating the involvement of other variables. Although our results have clearly shown the importance of reducing *V. destructor* infestation rates with correctly implemented control methods to reduce colony losses, we have not considered the role of other possible causes of mortality. Further variables will be considered in a follow-up study by extending our measures and observations to following years and by investigating land-use factors (e.g., pesticide use, agricultural management, and resource availability) in the vicinity of the apiaries, as well as the effect of other pathogens such as viruses, bacteria, and fungi, with the aim of acquiring a more holistic view on the various causes for colony losses.

4.2. Promoting compliance and limitations of the control concept

The lack of compliance observed in a proportion of the participating beekeepers may be due to them being less experienced and lacking sufficient knowledge about the recommended treatment regimen. Several studies have shown that a beekeeper's training background and practices are the main factors promoting honey bee colonies' health (Jacques et al., 2017; Thoms et al., 2019). To improve these situations, authorities or associations in many countries strive to provide information and training to beekeepers (e.g., Switzerland, Germany, Austria, Sweden, Denmark, and Netherlands; van der Steen and Vejsnæs, 2021). Our results, however, show that despite readily available information and training, compliance can be prone to self-interpretation. Compliance was increased in the framework of our experiment through a high personal involvement of beekeepers and ready access to hard data showing the consequences of ones' acts, even when occurring several months after the act itself. Personal involvement in the framework of a research project, with access to systematically acquired data can be considered informal training (Adams, 2018), and is an efficient means to improve colony health. However, such an approach may not be applicable to the wider beekeeping community. Improved compliance can be fostered by including results such as ours as an example of the consequence of deviating from the recommendations (i.e., an increased mortality risk) in formal training to make the latter less theoretical and more relatable to personal experience (Adams, 2018).

An additional incentive to promote compliance with recommendations can be of an economic nature, through the main motivation of most beekeepers (i.e., the honey harvest) (López-Uribe and Simone-Finstrom, 2019). Compliant beekeepers benefitted from three times higher harvests than noncompliers, whereas the harvests of almost compliers were only marginally smaller than those of compliers (Table 2). Showing the positive economic effect of implementing the control regimen as recommended is likely to motivate beekeepers to improve their *V. destructor* control strategies, despite the complexity of the recommended regimen.

Although the general level of compliance increased significantly from 2018 to 2019 (Fig. 2; Appendix 2), the proportion of fully compliant beekeepers remained stable over the two years. This was due to an increase in the compliance of the previously less-compliant beekeepers being compensated by a decrease in the level of compliance of the initially compliant beekeepers (Fig. 2, Appendix 2). This decrease is unlikely due to lack of knowledge or poor concept acceptance, since these participants were compliant in the first year. This decrease may be due to constraints in implementing the complex treatment regimen. These constraints should be identified to foster colony health, possibly with the help of the social sciences, but we can speculate that they originate from the need to apply treatments at a given time, determined by ambient temperatures (Rosenkranz et al., 2010; Steube et al., 2021). This timing might conflict with other commitments, especially for hobby beekeepers whose main activity might take precedence on the care of their honey bee colonies. The frequency of such conflict can be exacerbated by climate changes with increasing periods of extreme temperatures or increasing deviation from usual weather patterns (Steube et al., 2021), which do not allow formic acid application at the appropriate time, when it can reduce damages to the winter honey bee workers effectively. This phenomenon is suggested by the anecdotal reports gathered during this study. We occasionally observed a reluctance to apply the second formic acid treatment, which was described as too stressful for the colonies. A complicating factor was the recurrent summer heatwaves, which made the second application of formic acid more challenging to perform when the right conditions prevailed (application above 29 °C led to excessive negative side-effects (Rosenkranz et al., 2010; Steube et al., 2021). To overcome this issue, some participating beekeepers implemented biotechnical methods (queen caging, brood interruption, and hyperthermia (Büchler et al., 2020; Apiservice, 2021) to avoid the second application of formic acid, while others acted directly on the diffusion mode of the second formic acid application by modifying the evaporation quantity, possibly affecting the effectiveness of treatment. This clearly reveals the personal appropriation of the treatment concept against *V. destructor*. This phenomenon has also been observed in Austria with the application of unexpected *V. destructor* treatment regimens by beekeepers with detrimental effects on colony health (Oberreiter and Brodschneider, 2020). Further research is required to better understand the motivations and constraints faced by beekeepers that lead to a lack of compliance and increased colony losses.

Given that, irrespective of the intention to comply, not all constraints can be overcome, identifying which elements of the concept are more crucial to ensure colony health can lead to a “next best strategy” as a compromise between realistic implementation in the field and promotion of colony health. Here, we showed that deviations from the recommended treatment application time (almost-compliant) led to fewer colony losses than renouncing to one of the formic acid applications altogether (noncompliant) and allowed honey harvests almost as high as those of compliant beekeepers. Concept formulation could, therefore, be adapted by setting the priority on performing two formic acid applications, even if the appropriate timing cannot be held precisely. This represents a short-term solution to mitigating colony losses due to *V. destructor*. However, monitoring the precise implementation of *V. destructor* control methods in the field can contribute to developing new and sufficiently effective concepts better adapted to a constantly evolving context, be it climate changes or changes in social trends and personal constraints.

Our results also highlight the need to consider how *V. destructor* treatments are implemented (i.e., conformity to manufacturer instructions or compliance with recommendations) when surveying beekeepers to determine the role of management in colony health. All beekeepers in our study would have declared treating against the mite, but the data showed wide variations in treatment implementation and in their efficacy.

5. Conclusion

Although *V. destructor* is not the only cause for colony losses (Steinmann et al., 2015; Smith et al., 2013; Van Esch et al., 2020), our results support the view that the correct implementation of varroacidal treatments drastically improves colony survival over winter. We also showed that improved communication of the negative consequences of deviations from the recommended treatment regimens can lead to improved compliance and calls for new paradigms in beekeeper training. Integrating principles of social sciences into training can foster the acceptance of, and compliance with, recommendations. Social sciences can also contribute to identifying the constraints inherent to the complexity of the alternative control methods, which seems to limit compliance. In case such constraints are unavoidable, our results suggest that performing the treatment applications at suboptimal dates results in fewer honey bee colony losses than renouncing a treatment altogether. Alternatively, constraints to treatment implementation can be reduced with the development of simpler yet effective treatments against *V. destructor*.

Funding

This research was performed in collaboration with the Fondation Rurale Interjurassienne (FRI), Direction Générale de l’Agriculture, de la Viticulture et des affaires vétérinaires (DGAV), ProConseil, and financed by the Swiss federal office of agriculture (OFAG), and by the Cantons Vaud, Jura, and Bern.

Data statement

The datasets generated and analyzed during the current study are not publicly available due to their use in ongoing primary research, but subsections may be made available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We would like to thank all participating beekeepers for providing us with data and the field assistants (Gérald Buchwalder, Véronique Froidevaux, François Brunet and Cédric Reymond) who helped to collect the data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2021.12.025.

References

Adams, E.C., 2018. How to become a beekeeper: learning and skill in managing honeybees. Cult. Geogr. 25, 31–47. https://doi.org/10.1177/1474474016682345.

Apiservice , abeilles ch · le portail de l’’apiculture en S, 2021. Lutte contre le varroa [WWW Document]. Abeillesch · Portail Apic. En Suisse. https://www.abeilles.ch/themes/sante-des-abeilles/lutte-contre-le-varroa.html (accessed 5.21.21).

Bagheri, S., Mirzaie, M., 2019. A mathematical model of honey bee colony dynamics to predict the effect of pollen on colony failure. PLoS One 14, e0225632. https://doi.org/10.1371/journal.pone.0225632.

Berthoud, H., Imdorf, A., Haueter, M., Radloff, S., Neumann, P., 2010. Virus infections and winter losses of honey bee colonies (*Apis mellifera*). J. Apic. Res. 49, 60–65. https://doi.org/10.3896/IBRA.1.49.1.08.

Beyer, M., Junk, J., Eickermann, M., Clermont, A., Kraus, F., Georges, C., Reichart, A., Hoffmann, L., 2018. Winter honey bee colony losses, *Varroa destructor* control strategies, and the role of weather conditions: results from a survey among beekeepers. Res. Vet. Sci. 118, 52–60. https://doi.org/10.1016/j.rvsc.2018.01.012.

Bogdanov, S., Charrière, J.-D., Imdorf, A., Kilchenmann, V., Fluri, P., 2002. Determination of residues in honey after treatments with formic and oxalic acid under field conditions. Apidologie 33, 399–409. https://doi.org/10.1051/apido:2002029.

Brodschneider, R., Gray, A., Adjlane, N., Ballis, A., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M.F., Dahle, B., Graaf, D.C., de Dražić, M.M., Evans, G., Fedoriak, M., Forsythe, I., Gregorc, A., Grzęda, U., Hetzroni, A., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Medina-Flores, C.A., Mutinelli, F., Raudmets, A., Ryzhikov, V.A., Simon-Delso, N., Stevanovic, J., Uzunov, A., Vejsnæs, F., Wöhl, S., Zammit-Mangion, M., Danihlík, J., 2018. Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. J. Apic. Res. 57, 452–457. https://doi.org/10.1080/00218839.2018.1460911.

Brodschneider, R., Brus, J., Danihlík, J., 2019. Comparison of apiculture and winter mortality of honey bee colonies (*Apis mellifera*) in Austria and Czechia. Agric. Ecosyst. Environ. 274, 24–32. https://doi.org/10.1016/j.agee.2019.01.002.

Büchler, R., Uzunov, A., Kovačić, M., Prešern, J., Pietropaoli, M., Hatjina, F., Pavlov, B., Charistos, L., Formato, G., Galarza, E., Gerula, D., Gregorc, A., Malagnini, V., Meixner, M., Nedić, N., Puškadija, Z., Rivera-Gomis, J., Jenko, M.R., Škerl, M.I.S., Vallon, J., Vojt, D., Wilde, J., Nanetti, A., 2020. Summer brood interruption as integrated management strategy for effective Varroa control in Europe. J. Apic. Res. 59, 764–773. https://doi.org/10.1080/00218839.2020.1793278.

Calovi, M., Grozinger, C.M., Miller, D.A., Goslee, S.C., 2021. Summer weather conditions influence winter survival of honey bees (*Apis mellifera*) in the northeastern United States. Sci. Rep. 11, 1553. https://doi.org/10.1038/s41598-021-81051-8.

Charrière, J.-D., Imdorf, A., Fluri(EidgFuer(FAM), P.F.M., 1997. [With Formic Acid Dispensers against *Varroa jacobsoni*]. Agrar. Switz.

Charrière, J.-D., Neumann, P., 2010. Surveys to estimate winter losses in Switzerland. J. Apic. Res. 49, 132–133.

Conte, Y.L., de Vaublanc, G., Crauser, D., Jeanne, F., Rousselle, J.-C., Bécard, J.-M., 2007. Honey bee colonies that have survived *Varroa destructor*. Apidologie 38, 566–572. https://doi.org/10.1051/apido:2007040.

Conte, Y.L., Ellis, M., Ritter, W., 2010. Varroa mites and honey bee health: can Varroa explain part of the colony losses? Apidologie 41, 353–363. https://doi.org/10.1051/apido/2010017.

Dainat, B., Evans, J.D., Chen, Y.P., Gauthier, L., Neumann, P., 2012. Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. Appl. Environ. Microbiol. 78, 981–987. https://doi.org/10.1128/AEM.06537-11.

Dietemann, V., Pflugfelder, J., Anderson, D., Charrière, J.-D., Chejanovsky, N., Dainat, B., de Miranda, J., Delaplane, K., Dillier, F.-X., Fuch, S., Gallmann, P., Gauthier, L., Imdorf, A., Koeniger, N., Kralj, J., Meikle, W., Pettis, J., Rosenkranz, P., Sammataro, D., Smith, D., Yañez, O., Neumann, P., 2012. *Varroa destructor*: research avenues towards sustainable control. J. Apic. Res. 51, 125–132. https://doi.org/10.3896/IBRA.1.51.1.15.

Dietemann, V., Nazzi, F., Martin, S.J., Anderson, D.L., Locke, B., Delaplane, K.S., Wauquiez, Q., Tannahill, C., Frey, E., Ziegelmann, B., Rosenkranz, P., Ellis, J.D., 2013. Standard methods for varroa research. J. Apic. Res. 52, 1–54. https://doi.org/10.3896/IBRA.1.52.1.09.

Elzen, P.J., Baxter, J.R., Spivak, M., Wilson, W.T., 2000. Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. Apidologie 31, 437–441. https://doi.org/10.1051/apido:2000134.

Elzen, P.J., Westervelt, D., Lucas, R., 2004. Formic acid treatment for control of *Varroa destructor* (Mesostigmata: Varroidae) and safety to *Apis mellifera* (Hymenoptera: Apidae) under southern United States conditions. J. Econ. Entomol. 97, 1509–1512. https://doi.org/10.1603/0022-0493-97.5.1509.

Fries, I., Rosenkranz, P., 1996. Number of reproductive cycles of *Varroa jacobsoni* in honey-bee (*Apis mellifera*) colonies. Exp. Appl. Acarol. 20, 103–112. https://doi.org/10.1007/BF00051156.

Fries, I., Aarhus, A., Hansen, H., Korpela, S., 1991. Comparison of diagnostic methods for detection of low infestation levels of *Varroa jacobsoni* in honey-bee (Apis mellifera) colonies. Exp. Appl. Acarol. 10, 279–287.

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg, S., Ritter, W., Mühlen, W., Gisder, S., Meixner, M., Liebig, G., Rosenkranz, P., 2010. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41, 332–352. https://doi.org/10.1051/apido/2010014.

Giacobino, A., Molineri, A., Cagnolo, N.B., Merke, J., Orellano, E., Bertozzi, E., Masciángelo, G., Pietronave, H., Pacini, A., Salto, C., Signorini, M., 2015. Risk factors associated with failures of Varroa treatments in honey bee colonies without broodless period. Apidologie 46, 573–582. https://doi.org/10.1007/s13592-015-0347-0.

Giacobino, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Pacini, A., Salto, C., Signorini, M., 2016. Key management practices to prevent high infestation levels of *Varroa destructor* in honey bee colonies at the beginning of the honey yield season. Prev. Vet. Med. 131, 95–102. https://doi.org/10.1016/j.prevetmed.2016.07.013.

Giacobino, A., Pacini, A., Molineri, A., Bulacio Cagnolo, N., Merke, J., Orellano, E., Bertozzi, E., Masciangelo, G., Pietronave, H., Signorini, M., 2017. Environment or beekeeping management: what explains better the prevalence of honey bee colonies with high levels of *Varroa destructor*? Res. Vet. Sci. 112, 1–6. https://doi.org/10.1016/j.rvsc.2017.01.001.

Grace, J.B., Scheiner Jr., S.M., D, R.S., 2015. Structural equation modeling: building and evaluating causal models: Chapter, 8, pp. 168–199.

Gray, A., Adjlane, N., Arab, A., Ballis, A., Brusbardis, V., Charrière, J.-D., Chlebo, R., Coffey, M.F., Cornelissen, B., da Costa, C.A., Dahle, B., Danihlík, J., Dražić, M.M., Evans, G., Fedoriak, M., Forsythe, I., Gajda, A., de Graaf, D.C., Gregorc, A., Ilieva, I., Johannesen, J., Kauko, L., Kristiansen, P., Martikkala, M., Martín-Hernández, R., Medina-Flores, C.A., Mutinelli, F., Patalano, S., Raudmets, A., Martin, G.S., Soroker, V., Stevanovic, J., Uzunov, A., Vejsnaes, F., Williams, A., Zammit-Mangion, M., Brodschneider, R., 2020. Honey bee colony winter loss rates for 35 countries participating in the COLOSS survey for winter 2018–2019, and the effects of a new queen on the risk of colony winter loss. J. Apic. Res. 59, 744–751. https://doi.org/10.1080/00218839.2020.1797272.

Gregorc, A., Pogacnik, A., Bowen, I., 2004. Cell death in honeybee (*Apis mellifera*) larvae treated with oxalic or formic acid. Apidologie 35, 453–460. https://doi.org/10.1051/apido:2004037.

Guichard, M., Dietemann, V., Neuditschko, M., Dainat, B., 2020. Three decades of selecting honey bees that survive infestations by the parasitic mite *Varroa destructor*: outcomes. Limitations and Strategy. https://doi.org/10.20944/preprints202003.0044.v1.

Guzmán-Novoa, E., Eccles, L., Calvete, Y., Mcgowan, J., Kelly, P.G., Correa-Benítez, A., 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. https://doi.org/10.1051/apido/2009076.

Haber, A.I., Steinhauer, N.A., vanEngelsdorp, D., 2019. Use of chemical and nonchemical methods for the control of *Varroa destructor* (Acari: Varroidae) and associated winter Colony losses in U.S Beekeeping Operations. J. Econ. Entomol. 112, 1509–1525. https://doi.org/10.1093/jee/toz088.

Hernandez, J., Maisonnasse, A., Cousin, M., Beri, C., Le Quintrec, C., Bouetard, A., Castex, D., Decante, D., Servel, E., Buchwalder, G., Brunet, F., Feschet-Destrella, E., de Bellescize, K., Kairo, G., Frontero, L., Pédehontaa-Hiaa, M., Buisson, R., Pouderoux, T., Aebi, A., Kretzschmar, A., 2020. ColEval: honeybee COLony structure EVALuation for field surveys. Insects 11, 41. https://doi.org/10.3390/insects11010041.

Imdorf, A., Charrière, J.-D., Maquelin, C., Kilchenmann, V., Bachofen, B., 1996. Alternative varroa control. Am. Bee J. 136, 189–193.

Imdorf, A., Charrière, J.-D., Kilchenmann, V., Bogdanov, S., Fluri, P., 2003. Alternative strategy in Central Europe for the control of *Varroa destructor* in honey bee colonies. Apiacta 38.

Jacques, A., Laurent, M., Consortium, E., Ribi, M., 2017. A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. PLoS One 17.

Korpela, S., Aarhus, A., Fries, I., Hansen, H., 1992. *Varroa jacobsoni* Oud. in cold climates: population growth, winter mortality and influence on the survival of honey bee colonies. J. Apic. Res. 31, 157–164. https://doi.org/10.1080/00218839.1992.11101278.

Lee, K.V., Moon, R.D., Burkness, E.C., Hutchison, W.D., Spivak, M., 2010a. Practical sampling plans for Varroa destructor (Acari: Varroidae) in Apis mellifera (Hymenoptera: Apidae) colonies and apiaries. J. Econ. Entomol. 103, 1039–1050. https://doi.org/10.1603/EC10037.

Lee, K., Reuter, G., Spivak, M., 2010b. Standardized sampling plan to detect varroa density in colonies and apiaries. Am. Bee J. 150, 1151–1155.

Lefcheck, J.S., 2016. piecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. 7, 573–579. https://doi.org/10.1111/2041-210X.12512.

Liebig, G., 2001. How many varroa mites can be tolerated by a honey bee colony. Apidologie 32, 482–484.

López-Uribe, M.M., Simone-Finstrom, M., 2019. Special issue: honey bee research in the US: current state and solutions to beekeeping problems. Insects 10, 22. https://doi.org/10.3390/insects10010022.

Maggi, M.D., Ruffinengo, S.R., Mendoza, Y., Ojeda, P., Ramallo, G., Floris, I., Eguaras, M. J., 2011. Susceptibility of *Varroa destructor* (Acari: Varroidae) to synthetic acaricides in Uruguay: Varroa mites' potential to develop acaricide resistance. Parasitol. Res. 108, 815–821. https://doi.org/10.1007/s00436-010-2122-5.

Milani, N., 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie 30, 229–234. https://doi.org/10.1051/apido:19990211.

Nürnberger, F., Härtel, S., Steffan-Dewenter, I., 2019. Seasonal timing in honey bee colonies: phenology shifts affect honey stores and varroa infestation levels. Oecologia 189, 1121–1131. https://doi.org/10.1007/s00442-019-04377-1.

Oberreiter, H., Brodschneider, R., 2020. Austrian COLOSS survey of honey bee Colony winter losses 2018/19 and analysis of hive management practices. Diversity 12, 99. https://doi.org/10.3390/d12030099.

Overall, J.E., Tonidandel, S., 2004. Robustness of generalized estimating equation (GEE) tests of significance against misspecification of the error structure model. Biom. J. 46, 203–213. https://doi.org/10.1002/bimj.200210017.

Pugesek, B.H., Tomer, A., von Eye, A., 2003. Structural Equation Modeling: Applications in Ecological and Evolutionary Biology. Cambridge University Press.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria., n.d..

Ritter, W., 1981. Varroa disease of the honeybee *Apis Mellifera*. Bee World 62, 141–153. https://doi.org/10.1080/0005772X.1981.11097838.

Rosenkranz, P., Aumeier, P., Ziegelmann, B., 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103, S96–S119. https://doi.org/10.1016/j.jip.2009.07.016.

Smith, K.M., Loh, E.H., Rostal, M.K., Zambrana-Torrelio, C.M., Mendiola, L., Daszak, P., 2013. Pathogens, pests, and economics: drivers of honey bee colony declines and losses. EcoHealth 10, 434–445. https://doi.org/10.1007/s10393-013-0870-2.

StataCorp, L.L.C., 2017. Stata statistical software: release 15 College Station, TX, 2017. Www Stata Comfeaturesdocumentationlast Accessed 1 March 2018.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M.-P., vanEngelsdorp, D., 2018. Drivers of colony losses. Curr. Opin. Insect Sci., Ecology Parasites/Parasitoids/Biological control 26, 142–148. https://doi.org/10.1016/j.cois.2018.02.004.

Steinmann, N., Corona, M., Neumann, P., Dainat, B., 2015. Overwintering is associated with reduced expression of immune genes and higher susceptibility to virus infection in honey bees. PLoS One 10, e0129956. https://doi.org/10.1371/journal.pone.0129956.

Steube, X., Beinert, P., Kirchner, W.H., 2021. Efficacy and temperature dependence of 60% and 85% formic acid treatment against *Varroa destructor*. Apidologie. https://doi.org/10.1007/s13592-021-00859-5.

Strachecka, A.J., Paleolog, J., Borsuk, G., Olszewski, K., 2012. The influence of formic acid on the body surface proteolytic system at different developmental stages in *Apis mellifera* L. workers. J. Apic. Res. 51, 252–262. https://doi.org/10.3896/IBRA.1.51.3.06.

Thoms, C.A., Nelson, K.C., Kubas, A., Steinhauer, N., Wilson, M.E., vanEngelsdorp, D., 2019. Beekeeper stewardship, colony loss, and *Varroa destructor* management. Ambio 48, 1209–1218. https://doi.org/10.1007/s13280-018-1130-z.

Tihelka, E., 2018. Effects of synthetic and organic acaricides on honey bee health: a review. Slov. Vet. Res. 55 https://doi.org/10.26873/SVR-422-2017.

Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., McAfee, A., 2020. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends Parasitol. 36, 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

van der Steen, J., Vejsnæs, F., 2021. Varroa control: a brief overview of available methods. Bee World 98, 50–56. https://doi.org/10.1080/0005772X.2021.1896196.

van Dooremalen, C., Gerritsen, L., Cornelissen, B., Steen, J.J.M., van der Langevelde, F., van Blacquière, T., 2012. Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. PLoS One 7, e36285. https://doi.org/10.1371/journal.pone.0036285.

Van Esch, L., De Kok, J.-L., Janssen, L., Buelens, B., De Smet, L., de Graaf, D.C., Engelen, G., 2020. Multivariate landscape analysis of honey bee winter mortality in Wallonia. Belgium. Environ. Model. Assess. 25, 441–452. https://doi.org/10.1007/s10666-019-09682-w.

vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. J. Invertebr. Pathol. 103, S80–S95. https://doi.org/10.1016/j.jip.2009.06.011.