6.3 Survey study on fruit pollination practices and their impact on honeybee health in the Flemish region (2012-2013)

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Abstract

Background: The purpose of this study was to examine if there is a difference in honeybee mortality between bees that are used for pollination or come into contact with commercial fruit plantations on the one hand, and bees that never forage on commercial fruit plantations at the other hand. Therefore we conducted a survey amongst Flemish beekeepers.

Results: The majority of surveyed beekeepers (>60%) indicated that their bees come into contact with commercially grown fruit. However, no significant differences in colony losses between different beekeeper groups with a different 'fruit contact status' were obtained. Different contact distances to commercially grown fruit, or differences between beekeepers who had or who had not delivered pollination services were not found to be significant factors in predicting colony loss rates. Also specific foraging history on apple (in which a preflowering treatment with the neonicotinoid imidacloprid was allowed and common practice in Flemish pome fruit growing at the timing of this survey) did not significantly correlate with higher colony losses. On the other hand, for several other factors including presence of *Varroa* and *Nosema,* significant correlations with colony losses were found.

Conclusions: Based on the data of this survey study no detrimental effects of commercially fruit production and its current crop protection schedules on fruit crop foraging/pollinating honeybees could be identified.

Keywords: honeybee, survey, mortality, fruit, pollination, foraging

Introduction

The last decade substantial honeybee losses have been reported in different regions worldwide (Chauzat et al., 2013; Stokstad 2007; Pettis & Delaplane 2010; Potts et al. 2010). A number of possible causes for reduced overwinter survival of managed honey bees have been put forward in both scientific literature and popular media, including pests and parasites, bacteria, fungi, viruses, pesticides, nutrition, management practices, and environmental factors (vanEngelsdorp et al. 2010; vanEngelsdorp and Meixner 2010). Bee pollination is essential for the production of a variety of agricultural crops, especially in commercial fruit growing. Despite the successful implementation of Integrated Pest Management (IPM) -an approach that uses all available techniques in an organized program to suppress pest populations in effective, economical and environmentally safe ways- and the fact that newly developed compounds go through a rigorous registration process that includes assessment of toxicity to honey bees, exposure to pesticides is still considered as one of the factors potentially responsible for the honeybee population declines (Chauzat et al., 2009). Though, good agricultural practice with crop protection treatments according to product label directions reduce the chance of acute lethal bee poisoning incidents to a minimum. Potential sublethal intoxications caused by exposure to either non-lethal compounds or metabolites from lethal compounds are, however, difficult to exclude. The purpose of this study was to evaluate if crop protection agents (including neonicotinoids) used in IPM schedules in commercial fruit growing do have an impact on the colony development and health of honeybees that are used to pollinate fruit crops. Therefore we examined if there is a difference in honeybee decline or winter mortality between bees that are used for pollination or come into contact with commercial fruit plantations on the one hand, and bees that never forage on commercial fruit plantations at the other hand, by conducting a large-scale survey amongst Flemish beekeepers between November 2012 and May 2013.

Experimental methods

Design of the survey

A semi-structured survey was conducted with multiple choice questions as well as open questions. In the first part of the survey the questions aimed at determining to which group the beekeepers belong (contact/no contact with commercially fruit growing). In the second part of the survey the questions were directed to the various aspects of beekeeping. The aim was to find out if there were (significant) differences between the different beekeeper groups regarding general bee health and mortality and beekeeper practices.

Survey data collection

The survey was conducted between November 2012 and May 2013. A response of minimal 200-300 filled-in surveys was targeted at. In order to ensure qualitative data input a number of winter meetings of various local beekeeper organizations (to encourage participation and to assist the participants by giving additional information wherever needed) was attended by the executers of this study. With the exception of a few returned completed surveys via mail, all surveys were filled in under guidance of an involved researcher ensuring that the questionnaires were filled in with care. The survey recorded 273 responses, of which 16 did not provide sufficient information to calculate winter loss. Hence, the analytic sample size was 257.

Calculations and Statistical analyses

The percentage colony losses was calculated by dividing the number of colonies lost during the winter by the total number of colonies at the start of the winter x 100. Two statistical programs were used for statistical analyses: the Unistat Statistical Package, version 6.0 (Unistat Ltd. 2011, London, England) and 'R' statistics software (version 3.0.1 for Windows, 64 bit; R Core Team, 2013). Descriptive statistics (Lower 95%, upper 95% confidence intervals of means, medians, variances, standard deviations; histograms, fitting of distribution functions) were executed using Unistat 6.0. The sample size requirement was analysed as described by Bartlett et al. (2001). Potential differences between groups of the responding beekeepers were explored by a nonparametric test, the Kruskal-Wallis test (similar as described in VanEngelsdorp et al., 2012). This test is used to evaluate the degree of association between samples. It is assumed that the samples have similar distributions at a 95% significance level. All cases in all samples are ranked together and then the rank sum of each sample is found. Multiple comparisons (Dunn) tests were executed for checking of potential differences between groups. Right-Tail Probabilities less than 5% indicate significance (0.05).

In order to explore the correlation between the proportion of winter losses and potential relevant factors, a statistical modelling procedure using R statistics software was followed. The statistical model describes a mathematical relation between the probability of colony losses and the (presence of a) specific factor. In this study we used logistic regression models. Generalized linear models (GLM) with quasi-binomial distribution of the dependent variable (in this case the proportion of colony losses) and the 'logit' as link function were constructed. In the procedure one starts from a given model and takes a series of steps by deleting a term already in the model, and afterwards tests (with ANOVA F-test) if the new model is significantly better that the previous model. At first instance we constructed models with only one factor and tested whether they were significantly better than the 'no factor model' (model without any factor). Each time the Residual Deviance = the deviance of the model with single factor expressed as level of goodness-of-fit; and P(>F): ANOVA p-value F-test for testing significant difference between the model with single factor and the corresponding 'no factor model', was calculated. At second instance we constructed multifactor models. A series of steps was executed in which each time a factor was deleted from the complete multiple factor model. The resulting models were every time tested by comparing them to the corresponding complete multiple factor model (ANOVA, F-test) using R statistics software. The followed procedure is in conformity with the procedures described by Van der Zee et al. (2013), Rodriguez (2006), and Kindt and Coe (2005).

Results and discussion

Distribution of beekeepers and beehives over different groups according to their 'fruit contact' status

Table 1 displays an overview of the distribution of beekeepers and beehives over different groups according to their 'fruit contact' status. The majority of surveyed beekeepers (60.31%) indicated that their bees come into contact with commercially grown fruit. A substantial part of them (33.85%) travels to fruit crops for pollination services. Around 20 % of the surveyed beekeepers have their apiary within 100 m of commercial fruit parcels, and about 23% within foraging distance (3000 m). When we look at the number of beehives of the different beekeeper groups with distinct 'fruit contact status' it is noticeable that apiaries coming into contact with commercial grown fruit have clearly more beehives than apiaries without any (known) contact with commercial fruit production sites (mean of ~23 vs ~7 beehives per beekeeper, respectively). When only the beekeepers providing pollination services (travelling to fruit) are taken into account, the mean number of beehives increases to ~36 per beekeeper.

Table 1 Distribution of beekeepers and beehives over different groups according to their 'fruit contact' status

	Number of surveyed beekeepers (%)	Number of beehives (%)	Mean number of beehives per beekeeper*	Lower 95%*	Upper 95%*	Standard Deviation
All surveyed beekeepers	257 (100)	4297 (100)	16.7	11.8	21.7	40.2
No (known) contact with commercial fruit	101 (39.30)	674 (15.69)	6.7	5.6	7.7	5.2
Contact (in general) with commercial fruit	155 (60.31)	3623 (84.31)	23.3	15.3	31.4	50.6
Travelling to fruit (pollination services)	87 (33.85)	3103 (72.21)	35.6	21.9	49.4	64.5
Distance between beehives and commercial fruit <100m	53 (20.62)	1221 (28.42)	23.0	6.9	39.2	58.6
Distance between beehives and commercial fruit >100m and <3000m	60 (23.35)	1155 (26.88)	19.3	5.1	33.4	54.8
Foraging on apple	121 (47.08)	2961 (68.91)	24.5	14.5	34.5	55.6
Foraging on pear	89 (43.63)	2347 (54.62)	26.4	12.9	39.7	63.6
Foraging on cherry	113 (43.97)	2836 (66.00)	25.1	15.1	35.1	53.5
Foraging on strawberry	61 (23.74)	2513 (58.48)	41.2	21.5	60.8	76.7
Foraging on raspberry	29 (11.28)	1378 (32.07)	47.5	10.8	84.3	96.6
Foraging on on berries	42 (16.34)	1763 (4.03)	41.9	14.3	69.7	88.9

* t-interval

With a little less than half of the surveyed beekeepers (~47%) who indicated that their bees come into contact with commercially grown apple orchards, apple turned out to be the most visited fruit crop. As the number of beehives of this group is considerably higher than the mean number of beehives of all surveyed beekeepers (~25 vs ~17 per beekeeper), the percentage of beehives coming into contact with apple even increases to almost ~69% of all beehives involved in this study. Also a large part of the beehives (~66%) forages on (or is in foraging distance with) commercially grown cherries, followed by strawberries (~58%), pears (~55%) and raspberries (~32%). Also noteworthy is that the mean number of beehives foraging on soft fruit (strawberry, raspberry, berries) is substantially higher than the mean number of beehives foraging on pit and stone fruit (apple, pear, cherries) (~41-48 vs ~24-26). However, there was a large variation in the number of beehives per beekeeper within all different indicated groups.

Colony losses

Table 2 displays an overview of the mean percentages colony losses of different groups of beekeepers according to their 'fruit contact' status. The overall mean colony loss percentage is 18.2 %. Most of the groups have mean colony loss percentages around 18%. Notably exceptions are the group of beekeepers that provides pollination services (only 13.3 %) and the group of beehives foraging on raspberries (somewhat higher, 25.9 %). There is, however, also a large variation in the percentage colony losses within all different groups (standard deviations 18-26%).

Table 2 Distribution of beekeepers, beehives and mean percentage colony losses over different groups according to their 'fruit contact' status

	Number of surveyed beekeepers (%)	Number of beehives (%)	Mean percentage colony losses*	Lower 95%*	Upper 95%*	Standard Deviation
All surveyed beekeepers	257 (100)	4297 (100)	18.2 %	15.2	21.2	24.2
No (known) contact with commercial fruit	101 (39.30)	674 (15.69)	17.7 %	12.8	22.5	24.2
Contact (in general) with commercial fruit	155 (60.31)	3623 (84.31)	18.5 %	14.7	22.4	24.3
Travelling to fruit (pollination services)	87 (33.85)	3103 (72.21)	13.3 %	9.4	17.3	18.5
Distance between beehives and commercial fruit <100m	53 (20.62)	1221 (28.42)	19.3 %	12.2	26.5	25.9
Distance between beehives and commercial fruit >100m and <3000m	60 (23.35)	1155 (26.88)	17.9 %	14.6	21.2	23.9
Foraging on apple	121 (47.08)	2961 (68.91)	18.1 %	14.0	22.2	22.6
Foraging on pear	89 (43.63)	2347 (54.62)	21.3 %	16.1	26.5	24.7
Foraging on cherry	113 (43.97)	2836 (66.00)	17.1 %	12.7	21.5	23.5
Foraging on strawberry	61 (23.74)	2513 (58.48)	20.2 %	14.0	26.5	24.5
Foraging on raspberry	29 (11.28)	1378 (32.07)	25.9 %	15.7	36.2	26.9
Foraging on on berries	42 (16.34)	1763 (4.03)	18.8 %	11.8	25.7	22.4

* t-interval

In Figure 1 the histogram of variable '% colony losses' of the whole group of surveyed beekeepers is shown, with six fitted distribution functions (Normal, Student's t, Chi-Square, Binomial, Negative Binomial, Discrete Uniform). It is clear that the percentage colony losses is not normally distributed. In fact, by far most of the colony loss percentages belong to the first (lowest) class [0-10%]. The best fit was retrieved by negative binomial distributions. All different 'fruit contact status' groups displayed the same type of distribution (data not shown). Consequently, a binomial type of distribution (quasi-binomial) was also used for model fitting and factor analyses (see further).

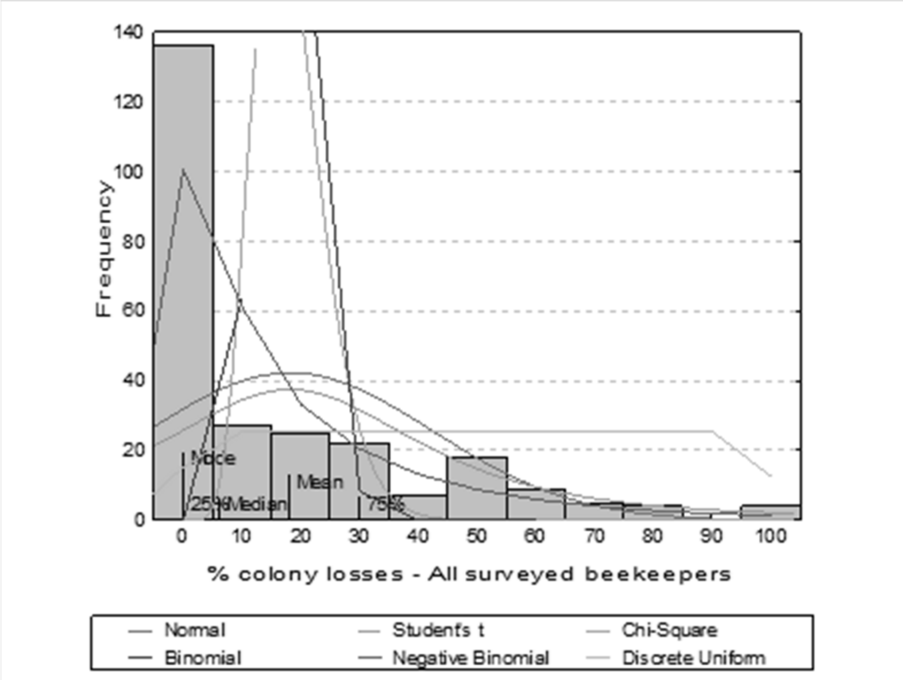

Figure 1 Histogram of variable '% colony losses' of the whole group of surveyed beekeepers, with six fitted distribution functions (Normal, Student's t, Chi-Square, Binomial, Negative Binomial, Discrete Uniform)

Statistical comparisons between colony losses of beekeepers with distinct 'fruit contact status'

Potential differences between sub-groups of the responding beekeepers were explored by a nonparametric test, the Kruskal-Wallis test. This test is used to evaluate the degree of association between samples. It is assumed that the samples have similar distributions (in this case a binomial like distribution, see above). All cases in all samples are ranked together and then the rank sum of each sample is found. In this test the null hypothesis is 'the percentage colony losses is the same in all different 'fruit contact' beekeepers groups' at a 95% significance level. For the different subgroups of beekeepers with beehives foraging on specific indicated fruit crops (apple, pear, etc.) also a Multiple comparisons (Dunn) test was executed for checking of potential differences between them. For none of all executed tests the Right-Tail Probability was less than 5% (0.05). Hence the null hypothesis is accepted in all cases. Thus we can conclude that there is no significant difference of colony losses between the different 'fruit contact status' beekeeper groups.

Colony losses and factor analyses

Single factor analyses

The single factor GLM model is expressed as:

GLM(Proportion of colony losses ~ Factor)

Using the GLM procedure in R with quasi-binomial distribution of the proportion of colony losses as dependent variable and the 'logit' as link function, a number of potential factors was modelled into a single factor model. The resulting single factor models were each time tested by comparing them to the corresponding 'no factor' model (ANOVA, F-test). In Table 3 (first part) the results for the 'fruit contact' factors are displayed. For instance, the model with 'Foraging on commercially grown Fruit (YesNo)' as potential declaring factor is not significantly better than the 'no factor model' (P=0.2735). Hence, foraging on commercially grown fruit is not a relevant factor to predict colony losses. This is in agreement with the Kruskal-Wallis tests. Also more specific for the different contact distances (<100m, <3000m) with commercially grown fruit, or the fact whether or not the beekeeper had delivered pollination services for commercially grown fruit, no significant effect could be found. Since a preflowering treatment of the neonicotinoid imidacloprid in apple is very common in Flemish pome fruit growing (estimated >85% of Flemish apple growers) we also specifically tested the factor 'Foraging on Apple'. However, also for this group no significant effect could be found.

On the other hand, for several other factors this single factor modelling approach did identify significant effects (see Table 3, second part). For instance the model with '*Varroa* problems (YesNo)' as potential declaring factor is turned out to be significant better than the corresponding 'no factor model'. Hence, *Varroa* problems is a relevant factor to predict colony losses. For the presence of '*Nosema*' even a very strong effect was found (P = 0.00094). Other factors meaningfully deviating from the 'no factor model' are 'Control action against disease of pest (YesNo)', 'TOTAL Number of colonies start winter', 'Number of small colonies (<4combs) start winter', 'Queens from Larva relocation project', 'Bought virgin queens' and 'No honey harvesting'.

Table 3 Results output of the GLM single factor analyses. 1 Factor models compared with the corresponding 'no factor' model.

Factor	Residual Deviance	F value	F value P(>F)	Significant effect?
Foraging on commercially grown Fruit (YesNo)	353.95	1.2041	0.2735	No
Contact fruit < 100m	353.31	0.7314	0.3932	No
Contact fruit < 100m and > 3000m	352.48	1.3357	0.2489	No
Contact fruit travelling (pollination services)	353.95	0.2751	0.6004	No
Contact fruit during past.year (YesNo)	354.18	0.1069	0.744	No
Foraging on Apple	350.63	2.681	0.1028	No
Significant factors				
Nosema	339.16	11.21	0.0009369	Yes, strong
Varroa problems (YesNo)	337.10	3.7084	0.05529	Yes
Control action against disease of pest (YesNo)	351.23	3.1872	0.07541	Yes
TOTAL Number of colonies start winter	347.51	4.8349	0.02879	Yes
Number of small colonies (<4combs) start winter	345.92	5.0593	0.02536	Yes
Queens from Larva relocation project	351.77	2.788	0.0962	Yes
Bought virgin queens	350.36	3.8311	0.0514	Yes
No honey harvesting	342.19	3.6839	0.0561	Yes

Residual Deviance: the deviance of the model with single factor expressed as level of goodness-of-fit.
P(>F): ANOVA p-value F-test for testing significant difference between the model with single factor and the corresponding 'no factor model'.

Multiple factor analyses

The multiple factor GLM model is expressed as:

GLM(Proportion of colony losses ~ Factor1 + Factor2 + Factor3 + etc.)

When we take into account all significant factors as derived from the single factor analyses (see 6.3.1) the GLM model is as follows:

GLM (Proportion of colony losses ~ *Nosema* + *Varroa* problems (YesNo) +
Control action against disease of pest (YesNo) + TOTAL Number of colonies start winter +
Number of small colonies (<4combs) start winter + Queens from Larva relocation project +
Bought virgin queens + No honey harvesting, family = quasibinomial(link = 'logit'),
data = bijenenquetedefdata)

This model was programmed in R statistics software. Table 4 displays the output.

Table 4 Details of the GLM multiple factor model with 8 factors.

Factor	Coefficient Estimate	Std. Error	t value	P(>|t|)
(Intercept)	-0.425909	0.255945	-1.664	0.09743
Nosema	1.378.936	0.516019	2.672	0.00806
Varroa problems (YesNo)	0.308885	0.297360	1.039	0.29998
Control action against disease of pest (YesNo)	0.358587	0.392924	0.913	0.36238
TOTAL Number of colonies start winter	0.003748	0.005359	0.699	0.48496
Number of small colonies (<4combs) start winter	0.036548	0.026040	1.404	0.16177
Queens from Larva relocation project	0.401599	0.290747	1.381	0.16851
Bought virgin queens	1.726.511	0.806351	2.141	0.03329
No honey harvesting	-0.848484	0.394550	-2.151	0.03254

Min	1Q	Median	3Q	Max
-21.422	-10.482	0.4713	10.893	17.391

Null deviance: 336.27 on 243 degrees of freedom
Residual deviance: 297.55 on 235 degrees of freedom

The proportion colony losses correlates positively with '*Nosema*', 'Control action against disease of pest (YesNo)', 'TOTAL Number of colonies start winter', 'Number of small colonies (<4combs) start winter', 'Queens from Larva relocation project' and 'Bought virgin queens'.

For the first two factors it seems logical that if *Nosema* is present or there is a clear requirement for control actions against diseases of pests, the colonies are weaker and as a consequence colony losses are higher. Concerning the positive correlation of the total number of colonies and the number of small colonies going into the winter. This could be explained by the fact that the more colonies a beekeeper has to handle, the higher the probability that the colonies are not optimally prepared for winter. Certainly the small colonies (<4 combs) have a higher chance to get lost during winter. The fact that the factors 'Queens from Larva relocation project' and 'Bought virgin queens' also positively correlate with colony losses is more surprising. Possibly this reflects the fact that queens from breeding programs are often selected for 'non-aggressiveness'. This 'calmness' might result in bees that are more susceptible to pests (*Varroa*, etc.) and diseases than bees naturally selected by the environment.

The 'No honey harvesting' status correlates negatively with the proportion colony losses. This means that beekeepers that do not harvest honey have lower colony losses rates, which can be explained by the fact that their own honey is the best food for bees to survive the winter. With its high nutrients content honey is an important element in the diet of honeybees.

Subsequently, a series of steps was executed in which each time a factor was deleted from the multiple 8-factor model. The resulting models were tested every time by comparing them to the corresponding full 8-factor model (ANOVA, F-test). The results are shown in Table 5. It is clear that mainly '*Nosema*', 'Bought virgin queens' and 'No honey harvesting' are the determining factors in this multiple factor model. The other factors individually have no significant additional value in the model. With other words: in order to predict the proportion of colony losses the factors '*Nosema*', 'Bought virgin queens' and 'No honey harvesting' are absolutely required. The other factors make the model better, but might be linked somehow to the other factors, as they have on their own no significant contribution in a model in which all other factors are already included.

Table 5 Results output of the GLM multiple factor analyses. Multiple Factor models compared with models with one factor less.

Factor	Residual Deviance	F value	F value P(>F)
(Multiple factor model)	297.55		
Nosema	306.17	6.8091	0.009652
Varroa problems (YesNo)	298.66	0.8782	0.349654
Control action against disease of pest (YesNo)	298.41	0.6812	0.410023
TOTAL Number of colonies start winter	298.13	0.4557	0.500288
Number of small colonies (<4combs) start winter	300.10	2.0097	0.157624
Queens from Larva relocation project	299.52	1.5584	0.213135
Bought virgin queens	303.90	5.0180	0.026021
No honey harvesting	302.51	3.9176	0.048950

Residual Deviance: the deviance of the corresponding multiple model without the particular factor expressed as level of goodness-of-fit. P(>F): ANOVA p-value F-test for testing significant difference between the multiple factor model and the corresponding model without the particular factor.

The here created multiple 8-factor model (residual deviance = 297.55) can only partially explain the observed variability in the colony losses rates between the different beekeepers. With other words: there have to be also other factors or reasons determining the degree of colony losses, which were not included in this modeling approach.

The model could further be improved by also considering interactions between the different factors (not executed in this study). Also addition of other factors not evaluated in this study or factors of which too few data were collected in this study most probably will improve the model.

Conclusions

In summary, in this survey study no significant differences in colony loss rates between different beekeeper groups with different 'fruit contact status' were obtained. Different contact distances (<100m, <3000m or no contact: >3000m) with commercially grown fruit, or the fact whether or not the beekeeper had delivered pollination services for commercially grown fruit were not found to be significant factors in predicting colony losses rates. Also specific foraging on apple (in which a preflowering treatment of the neonicotinoid imidacloprid is very common in Flemish pome fruit growing) did not significantly correlate with higher colony losses, based on the data and statistical analyses from this survey study. On the other hand, mainly '*Nosema*', 'Bought virgin queens' and 'No honey harvesting' were found to be determining factors for predicting colony losses.

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