Parasitol Res (2017) 116:S109–S122 DOI 10.1007/s00436-017-5497-8

Original Article

Evaluation of the Efficacy and Safety of Flumethrin 275 mg Bee-hive Strips (PolyVar Yellow®) against *Varroa destructor* in Naturally Infested Honey Bee Colonies in a Controlled Study

Tjeerd Blacquière[1*], Gertraut Altreuther[2*]✉, Klemens J. Krieger[2]

[1] Wageningen University & Research Centre, 6708 PB Wageningen, Netherlands
[2] Bayer Animal Health GmbH, 51368 Leverkusen, Germany

Corresponding author:
Gertraut Altreuther
✉ E-mail: gertraut.altreuther@bayer.com

[*] These authors have contributed equally to this work.

Abstract

A controlled, randomized, partially blinded study was conducted to evaluate target animal safety and efficacy of a late summer/autumn treatment with flumethrin 275 mg bee-hive strips (PolyVar yellow®, Bayer) against natural infestation with *Varroa destructor* in honey bee colonies. Thirty colonies received the test product applied as a "gate" at the hive entrance over 116 days, a positive control product (flumethrin bee-hive strips for in hive use, Bayvarol®) over 42 days or remained untreated as a negative control. On day 117 a follow-up treatment with coumafos solution (Perizin®) was applied to all three groups. For the efficacy evaluation dead mites were counted frequently up to 2 weeks after application of the follow-up treatment. For the safety evaluation dead bees were collected during treatment and follow-up treatment period by dead bee traps and several colony examinations were conducted until the following summer. Efficacy of the test product against *Varroa* was clearly demonstrated by a 99.9 % mite count reduction with superiority over the negative control ($p = 0.0008$). Survival of colonies treated with the test product or the positive control product was 80 % and 90 %, respectively, while survival in the negative control group was only 30 %. Significantly lower numbers of dead bees were observed in the treated groups compared to the negative control. Also, no differences in colony

development were observed between the groups that were considered clinically relevant.
Thus, flumethrin 275 mg bee-hive strips were confirmed to be an efficacious and safe treatment for varroosis in honey bees caused by flumethrin-sensitive *Varroa destructor*.

Introduction

Much research has been undertaken in past years to investigate potential causes of extensive colony losses in honeybees (Potts et al. 2010). The problem appears to be multifactorial (Oldroyd 2007, Kluser et al. 2010) but infestation with *Varroa destructor*, has been concluded to play a significant role (Neumann and Carreck 2010, Smith et al. 2014).
Sustainable *Varroa* control is a labour intensive process requiring a combination of different measures, e.g., monitoring of mite fall, cutting of drone brood and application of miticides. Such "integrated pest management" needs to consider the population dynamics of *Varroa* as well as the bee colony so that measures can be applied at appropriate times of the year (Rosenkranz et al. 2010). To avoid development of resistance of the *Varroa* mites against miticides alternating use of actives with different modes of action should be practiced, though not simple in a mainly non-professional beekeepers community (Rosenkranz et al. 2010). An example for population dynamics of *Varroa* mites in honeybee colonies is shown, together with numbers of adult bees and brood, in Fig. 1. Late summer and autumn is a critical period for honeybee colonies and *Varroa* infestation in various ways. With decline of honey and pollen flow in late summer colony size decreases through death of the short-lived summer bees and cessation of brood rearing activity so that relative mite load strongly increases. Robbing behaviour of bee colonies occurs especially during this time of the year and may result in significant horizontal mite transfer between colonies and apiaries often affecting rather strong colonies (Rosenkranz et al. 2010, Frey and Rosenkranz 2014, Seeley and Smith 2015). Also, at this time the long-lived winter bees are reared (Mattila and Otis 2007), which are pivotal for survival of the colony until the next brood rearing season in the following spring. Therefore, to keep the mite burden as low as possible a miticide treatment in late summer/early autumn has become a regular part of *Varroa* management in most parts of Europe. However, timing of treatment can be challenging and available approved miticides so far do not cover the complete period until end of flight activity so that significant horizontal mite transfer with resulting re-infestation can occur despite of a successful *Varroa* treatment especially in regions with intense beekeeping (Frey and Rosenkranz 2014). Flumethrin 275 mg bee-hive strips (PolyVar Yellow®) were developed as a treatment alternative which – with a treatment duration of up to four months - could cover the especially critical time between end of honey flow and end of flight activity towards winter. The strips are applied at the hive entrance and have holes through which the bees enter and leave the hive thereby getting exposed to the active ingredient. For the clinical development of this product, several studies were conducted to address the various aspects of testing described in the "Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees" (EMA 2011). The study described herein was conducted as a combined dose confirmation and target animal safety study to evaluate efficacy and safety.

Materials and methods

The study was conducted as a monocentric, negative and positive controlled, randomized, partially blinded efficacy and target animal safety study according to the standards of "Good Clinical Practice" based on VICH GL9 (July 2001) and

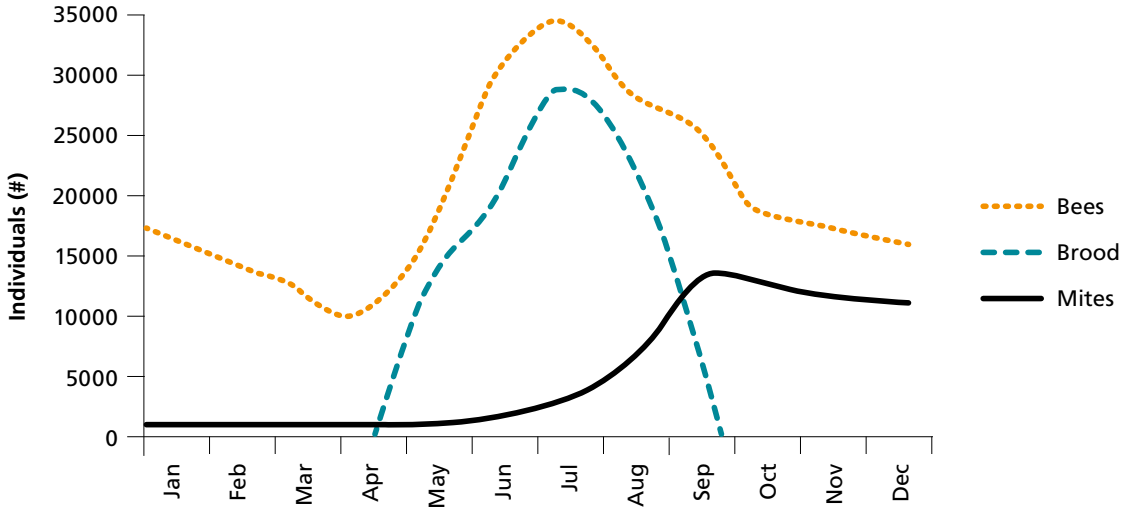

Fig. 1 Exemplary colony development for adult bees, worker brood, and *Varroa destructor* mites. The number of individual adult bees and worker brood was modelled over one year. The number of mites was modelled as being the second year of mite infestation with a starting population of 100 mites in the first year. [redrawn from Martin (1998)]

Fig. 2 Apiary and hive positioning

the guidelines on *Varroa* control: "Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees" (EMA 2011) and the "Technical guideline" (2011).

Study animals

Thirty *Apis mellifera mellifera* honeybee colonies that were naturally infected with *Varroa destructor* were included in the study. Queens were up to 2 years of age. Colonies showed

Table 1 Study groups, treatments and timing of treatments

Group		Treatment	Treatment period	Follow-up treatment
1	IVP	Flumethrin 275 mg bee-hive strips	day 0 - 116	Perizin® (coumafos), day 117
2	CP	Bayvarol® (flumethrin)	day 0 - 42	Perizin® (coumafos), day 117
3	Negative control	No treatment	n.a.	Perizin® (coumafos), day 117

normal behavior and no signs of infectious diseases, occupied 7–10 combs with presence of all stages of brood development and had at least 3 combs with capped brood cells. No acaricidal treatment of the colonies was allowed for at least 4 months prior to study start. As the colonies were owned by the Wageningen Plant Research institute no informed consent was required.

Housing and management

The colonies were housed in one-storey Simplex hives with ten or eleven frames. The hives were equipped with a bottom board with a screened *Varroa* tray. The apiary was located in the experimental fields of Wageningen Plant Research in Wageningen, The Netherlands (Fig. 2). Another apiary was located at ~ 400 m to the south. No other significant apiaries were present within one kilometre. Large apiaries were located at > 4.5 km distance. During periods without a significant honey flow colonies were provided with sugar dough (Fondabee, Belgosuc nv, Beernem, Belgium) or with liquid sugar (Apiinvert, Südzucker AG, Mannheim, Germany). For the study the hives were marked with numbers one through thirty. Queens that died (see results section) were replaced with mated queens.

Group allocation and treatment

The colonies were randomly allocated to three groups of ten on study day -1 (Table 1). Group 1 received the investigational veterinary product (IVP), at a dose of two bee-hive strips, each containing 275 mg flumethrin in a polymer matrix formulation applied at the hive entrance between study day 0 (22 August) and 116 (16 December) (Fig. 3). Group 2 was treated with authorized flumethrin bee-hive strips for use inside the hive (Bayvarol® 3.6 mg bee-hive strips, Bayer Animal Health GmbH, Germany) at a dose of four strips per colony between day 0 and 42 as per instruction of the manufacturer as a positive control product (CP). This group was included for the evaluation of safety. Evaluation of the efficacy (mite count reduction) of the CP was not deemed appropriate due to the time gap of 75 days between end of treatment and application of follow-up treatment, however some supplemental analyses were conducted for information purposes. Group 3 served as negative control for the evaluation of efficacy and safety, and therefore remained untreated. All colonies received a follow-up treatment with coumafos solution for application by trickling (Perizin® 32 mg/ml) according to the manufacturer's instruction on day 117 to determine the number of residual mites.

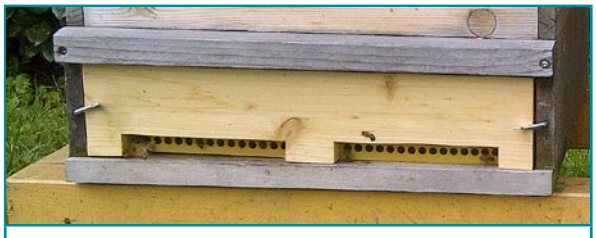

Fig. 3 Fixation of the IVP at the hive entrance (picture not taken within this study)

Mite counts

Dead *Varroa* mites were collected using disposable sticky boards with grid lines ("Biosignaalplaten"/"sticky traps", Royal Brinkman, 2690 AA 's-Gravenzande, Netherlands and "Bug-scan dry", Biobest,

2260 Westerlo, Belgium) that had been placed in the *Varroa* trays of the hives.

Sticky boards were collected 3 times per week between day -3 and 124 with a maximum of 2 calendar days between collections. A final sticky board was entered into the hives on day 124 and removed on day 131. On day 0 and 117 the sticky boards were changed before treatment or follow-up treatment. Counting of mites was performed away from the apiary by lab personnel who had no access to the group allocation and thus were blinded.

Colony examination

The colonies were examined once on day -3, day 14, day 62 and day 116 using the Liebefeld estimation method described by Imdorf and Gerig (2001). A further examination took place in the following spring on day 228 (April), and a final examination was conducted in June on day 298.

As it was not possible to complete evaluations of all colonies in the early morning before flight activity started colonies were estimated alternately in each study group in order to compensate the fluctuations of the flying activity. The evaluation comprised the estimation of colony strength (i.e. surface covered by live bees present), capped brood surface and capped drone brood surface. Also presence and appearance of eggs and open brood were documented. In deviation to Imdorf and Gerig (2001) only the brood surface of capped brood was estimated while the surface taken by open brood was left undetermined to minimize disturbance of the bee colony. This was regarded as justified as effects on open brood are expected to become evident indirectly in capped brood and colony size. It was also attempted to search for the queen, however if the queen could not be identified the presence of eggs was taken as an indication that the queen was present. The following factors were used for conversion of the surface estimations to numbers of individuals according to the review by Delaplane et al. (2013):

- Adult live bees: 125 bees per dm^2
- Capped worker brood: 400 cells per dm^2
- Capped drone brood: 260 cells per dm^2

In addition, the hives were generally inspected from the outside for abnormalities or any signs for unusual events three times per week between day -2 and 124 and once on day 131.

Dead bee counts

Intra hive mortality was determined by using dead bee traps (Muenster traps). These traps have been described and evaluated in Illies et al. (2002). Undertaker bees carrying dead bees have to pass through metal netting, which causes them to lose the dead bee, which falls down into a drawer (Fig. 4). Dead bees were collected from the traps three times per week between day -2 and 124 with a maximum of 2 calendar days between collections and counted directly differentiating the type of bee (pupa, queen, worker, drone). From day 12 onwards also the number of dead worker bees with signs of deformed wing virus (DWV) infection was recorded.

Data analysis

Efficacy per colony was analysed based on the critical evaluation stated in the Guideline on veterinary medicinal products controlling *Varroa destructor* (EMA, 2011) according to the following formula:

$$\%\ \text{mite count reduction} = \frac{\text{N mites killed between day 0 and 117} \times 100}{\text{N mites killed between day 0 and 131}}$$

Only colonies that completed the IVP treatment period (day 0 to 117) and follow-up treatment period (day 117 to 131) and that had demonstrated a minimum infestation of 300 mites were included in the analysis.

Results of mite count reduction were further assessed for the IVP group versus the negative control using the non-parametric Wilcoxon-Mann-Whitney-U test. Superiority was defined

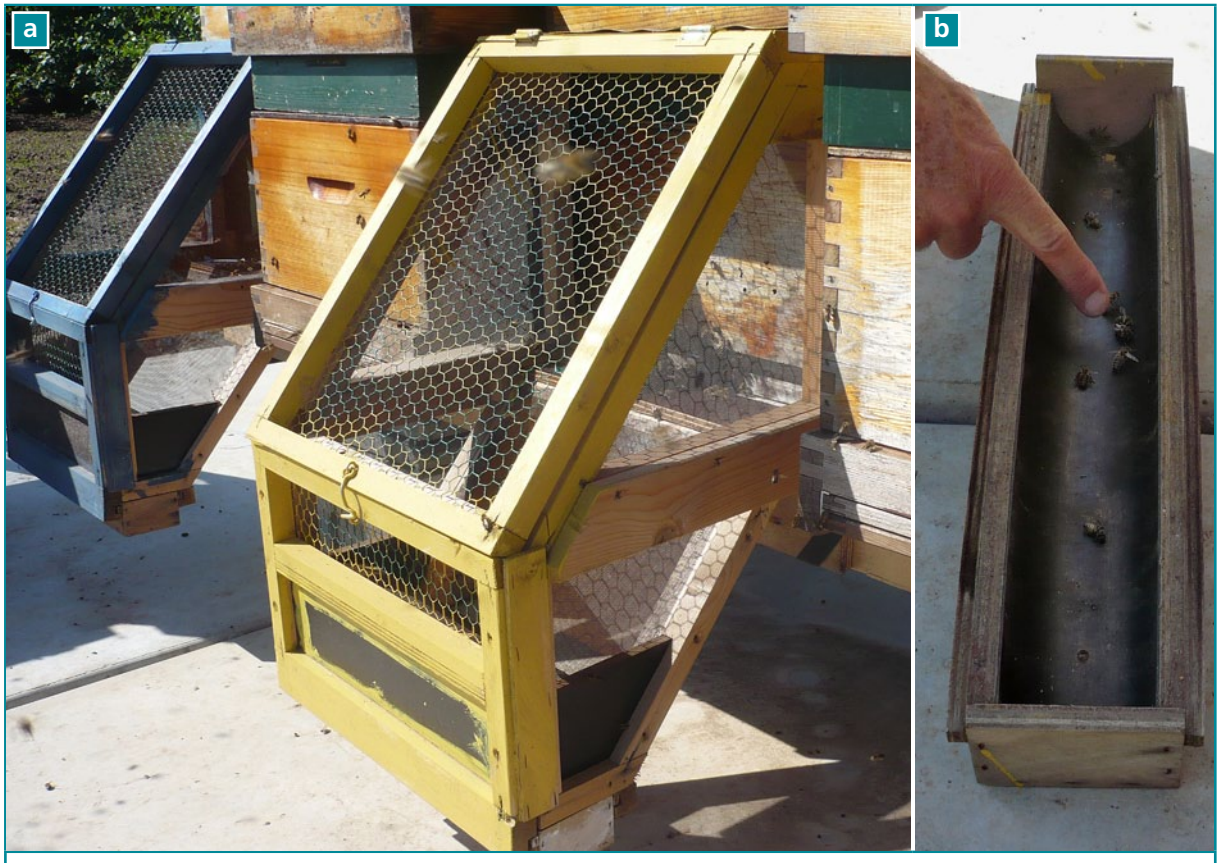

Fig. 4 Dead bee trap (Muenster trap):
a) trap mounted at hive; b) removable drawer for collection of dead bees at the bottom of the trap

for a Mann Whitney (MW)-measure of 0.50. Thus H0 was MW ≤ 0.50 and H1 was MW > 0.50. As an additional efficacy parameter the number of residual mites after treatment per 100 bees (infestation rate) was calculated per colony using the following formula:

$$\text{Residual mite burden per 100 bees} = \frac{\text{N mites killed between day 117 and 131} \times 100}{\text{N bees on day 116}}$$

Safety parameters were analysed descriptively. Dead bee counts were also analysed using the above mentioned Wilcoxon-Mann-Whitney-U test for superiority.

The analyses were performed using Report Version 6.7 and Testimate software Version 6.5 (IDV Gauting, Germany).

Results

Efficacy

Mite count and efficacy data are summarized in Tables 2 and 3 and mitefall over time is shown in Fig. 5. All colonies that reached day 131 were adequately infected with at least 1,272 mites thus well above the requirement of 300 mites per colony. Efficacy of the flumethrin 275 mg bee-hive strips was 99.9 % and superiority over the negative control was statistically proven ($p = 0.0008$). Clear differences between the two treatment regimens and the negative control were also evident in the number of residual mites per 100 bees (Table 3). Finally, 95 % of mites in the IVP group fell within the first 5 weeks while only 19 % of mites in the negative control group had fallen within this time.

Table 2 Mean mite numbers (range) for different time periods during the study

Group	Pre-treatment period (day -3 to 0)		IVP treatment period (day 0 to 117)		Follow-up treatment period (day 117–131)		IVP treatment and follow-up treatment period (day 0 to 131)	
1	174	(20–617)	4,434	(1,750–11,183)	5	(0–15)	4,439	(1,752–11,183)
2	135	(13–557)	3,529	(1,180–6,835)	94	(27–211)	3,623	(1,272–6,943)
3	85	(9–400)	5,955	(2,294–8,674)	821	(66–1,274)	6,776	(4,031–8,740)

Table 3 Results efficacy analysis: mean % efficacy (95 % confidence interval), mean number (range) of residual mites per 100 bees after day 117 and proportion of colonies with residual mite loads >4 %

Group	% efficacy	Number of residual mites per 100 bees after day 117		Proportion of colonies with residual mite loads >4 %	
1	99.9 (99.8–100)	0.1	(0–0.4)	0 %	(0/8 colonies)
2	n.a.[a]	2.7	(0.6–6.5)	20 %	(2/10 colonies)
3	84.9 (70.5–99.3)	20.4	(7.0–31.0)	100 %	(5/5 colonies)

[a]not calculated because follow-up treatment was applied only 75 days after end of treatment with Bayvarol on day 42

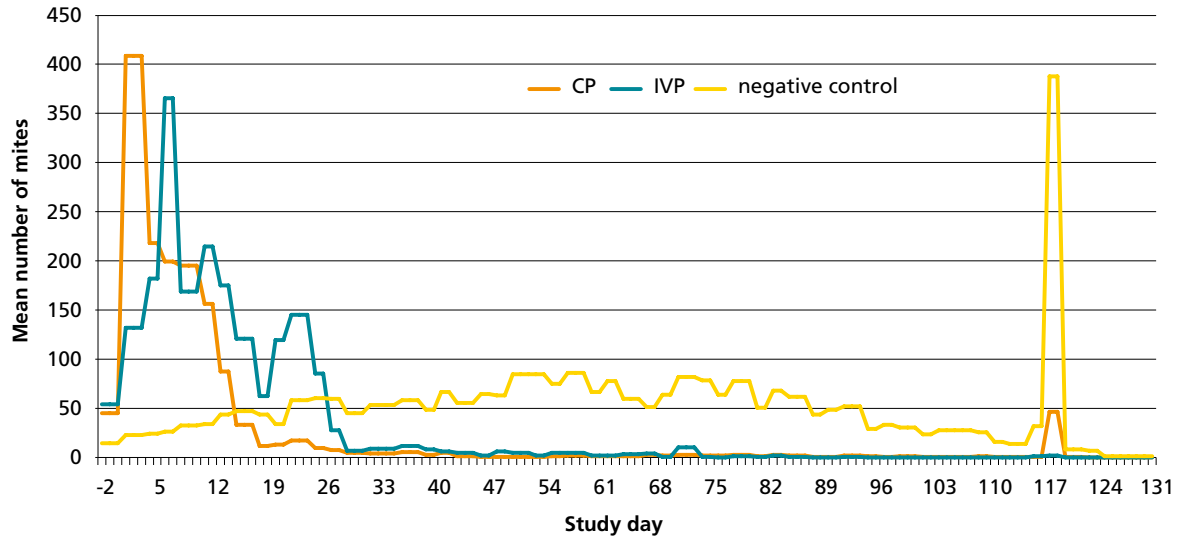

Fig. 5 Mean number of fallen mites per day. Mites were collected every 2 to 3 days, for better overview mite number per day was calculated by dividing number at mite collection by the number of days covered by the respective mite collection. Note: line plot was preferred over column graph for clarity

Safety

Colony survival

Overall colony survival is shown in Table 4. Until application of the follow-up treatment on day 117 five colonies of the negative control died or were euthanized because of strong decrease in colony size and heavy *Varroa* infestation. Also two colonies of the IVP group were euthanized during this time due to small colony size. One of these colonies had been the weakest of the colonies and had been already in the process of decline due to heavy *Varroa* infestation at baseline and the other

Table 4 Results safety evaluation: overall survival, number of dead or euthanized colonies and number of dead queens

Group	Overall colony survival	Death/euthanasia [N]		Dead queen found in dead bee trap [N]	
		Day 0–116	After day 116	Day 0–116	Day 117–124
1	80 %	2	0	0	1
2	90 %	0	1	2	1
3	30 %	5	2	0	0

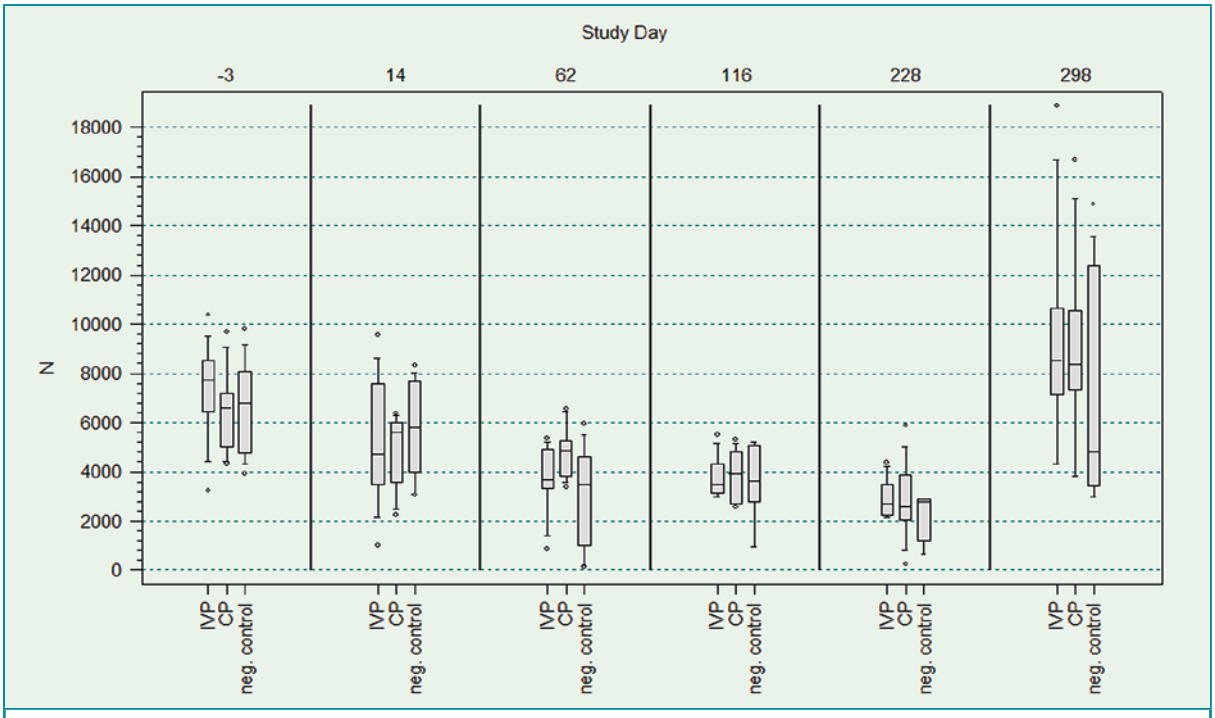

Fig. 6 Number of adult bees at the colony estimations on day -3, 14, 62, 116, 228 and 298 (boxplot shows 10th and 90th percentile)

was considered to have suffered from queen problems that had likely been present already at baseline. A relation of IVP treatment to these colonies' weakness therefore was considered to be not likely. Three further colonies of the negative control and one colony of the CP group did not survive until the end of the study.

Colony examinations

Colony development during the study is summarized by Fig. 6 (adult bees) and Fig. 7 (capped brood cells). Colony development was similar across groups with no differences that were regarded as clinically relevant. However, only 3 colonies of the negative control group survived for the colony examinations in spring and summer.

Dead bee counts

Dead bee counts are summarized in Figs. 8 and 9 showing the time course from day 0 to day 124. The time course of dead bee counts did not reveal any signs for acute toxicity after start of treatment. Also there was no indication for a trend in the IVP group which could indicate toxicity building up over time. Total numbers of trapped dead worker bees in the IVP and CP group were proven significantly lower than in the negative control group ($p \leq 0.03$). The groups that received IVP or CP were also proven

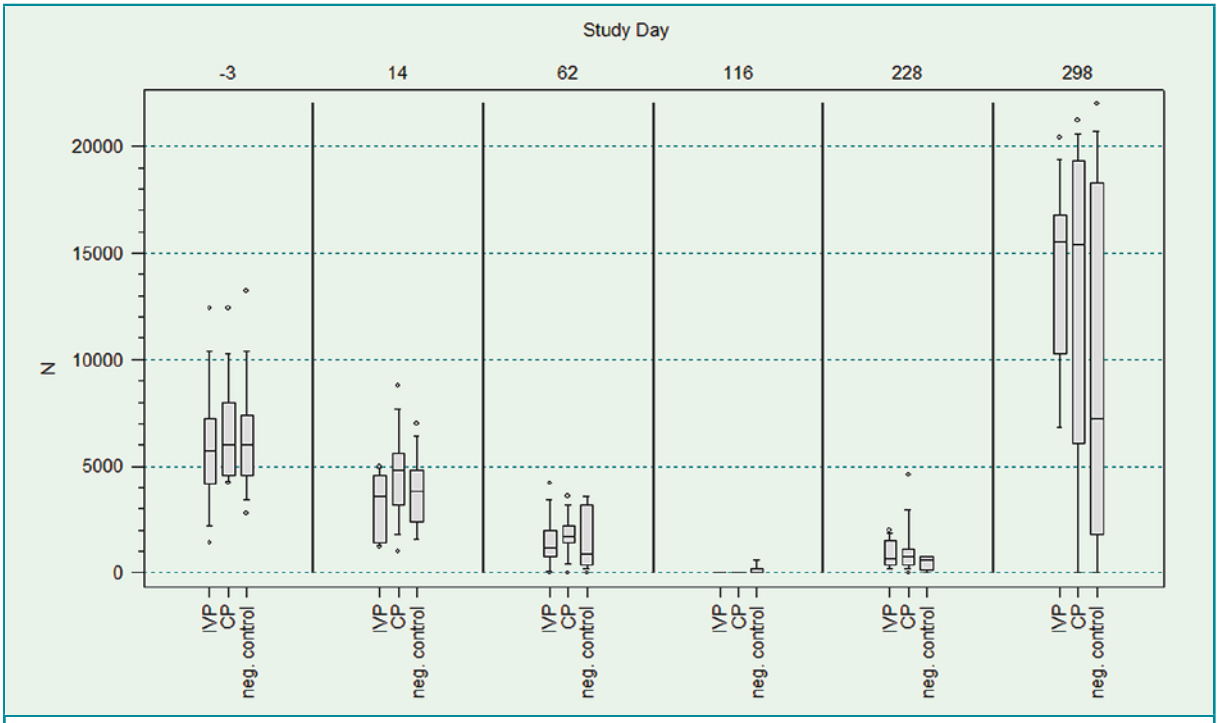

Fig. 7 Number of capped brood cells at the colony estimations on day -3, 14, 62, 116, 228 and 298 (boxplot shows 10th and 90th percentile)

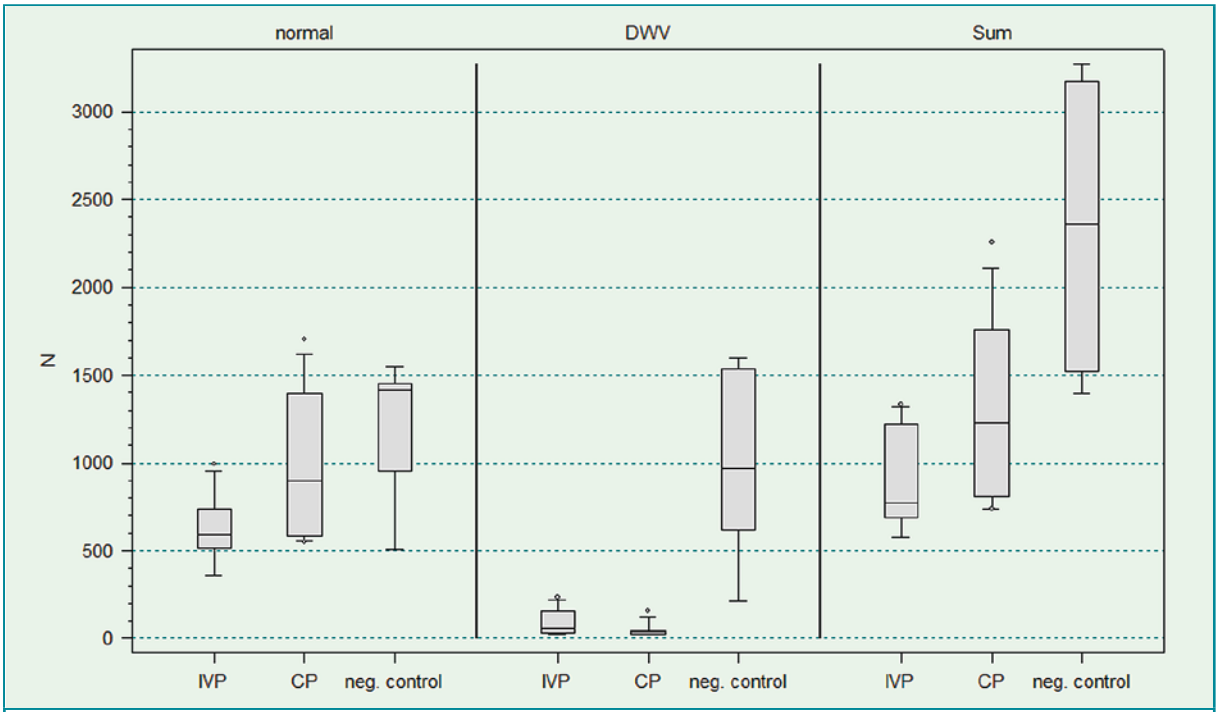

Fig. 8 Number of dead workers from study day 0 to 124 for categories "normal": dead workers with normal wing shape, "DWV": dead workers with deformed wings, "Sum": N normal + N DWV, (boxplot shows 10th and 90th percentile)

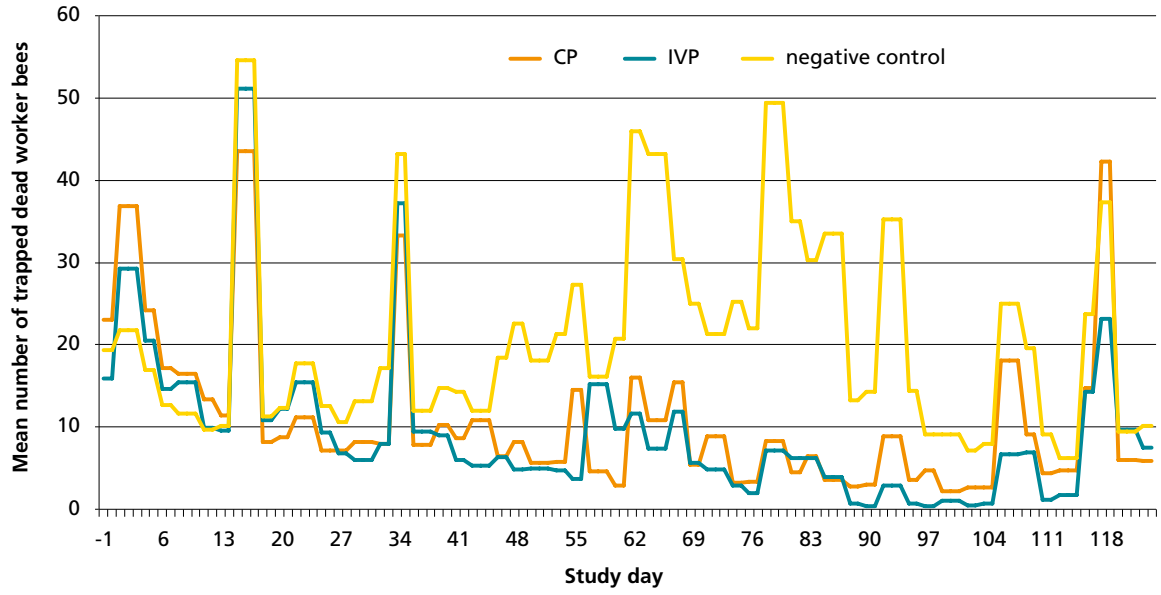

Fig. 9 Mean number of dead worker bees collected in Muenster traps per day. Bees were collected every 2 to 3 days, for better overview number per day was calculated by dividing number at collection by the number of days covered by the respective collection. Note: line plot was preferred over column graph for clarity

to have significantly lower numbers of dead worker bees with signs of DWV infection ($p \leq 0.003$).

Several dead queens (one in the IVP group and three in the CP group) were trapped as shown in Table 4 and death was considered to be related to manipulations associated with colony examinations or follow-up treatment that occurred in close timely connection except for one of the queens in the CP group. However, in this case a relation to treatment with the CP appeared unlikely as the dead queen was observed only on day 96, i.e. 54 days after termination of treatment with CP.

Trapped dead pupae were observed in low numbers with no differences between groups that were considered relevant. Trapped dead drones were not observed in significant numbers in any of the groups.

Discussion

The study demonstrated very high efficacy of flumethrin 275 mg bee-hive strips. Seemingly high natural mite mortality in the negative control group is considered to be caused by the extremely long observation period of four months which was required to ensure conservative conditions for the evaluation of safety. According to Rosenkranz et al. (2010) one replication cycle of *Varroa* mites is approximately 13 days so that multiple replication cycles of mites can be expected to have occurred, which is supported by the presence of capped brood in the negative control during most of the treatment period (Fig. 7). Under field conditions an average number between two and three reproductive cycles can be expected in the lifetime of a female mite (Fries and Rosenkranz 1996, Martin 1998, Martin and Kemp 1997). Thus several generations of mites may have emerged and died during the treatment period accumulating to a large number of mites while no significant replication was possible in the treatment groups due to the treatment effect in the first few weeks of the study.

Very high efficacy in comparison to the negative control is also evident by the residual mite load per 100 bees at the end of the treatment

period (Table 3). Evaluation of the infestation rate after treatment is considered a useful complementary method to evaluate efficacy and state of colonies from a clinical parasitological perspective. Also, this parameter can be used to determine whether a winter treatment is necessary, indeed infestation rates above 4 % can be considered critical (e.g., Honey bee health coalition 2017). Infestation rates in the IVP group were well below 1 % in all colonies while mite loads above 4 % were seen in 20 % of the colonies in the CP group which may illustrate that despite good efficacy of a late summer treatment re-infestation and replication of mites surviving treatment can occur in significant numbers until the end of flight activity towards winter.

Some minor differences in the initial mite fall between the IVP and CP group may be explained by the different way of application (Fig. 5). Application of a strip within the hive may lead to faster exposure of the bees to the active ingredient in comparison to an application at the hive entrance where exposure may be more influenced by weather conditions causing variable numbers of bees to enter and leave the hive and to be near the hive entrance.

Ninety-five percent of the mites that were observed during treatment and follow-up treatment fell within 5 weeks. However, due to the long evaluation period such an analysis may be influenced by several factors like mites re-infesting colonies during treatment so that this should be regarded as estimation. In a multicentre field study conducted for the same product similar calculations indicated that 95 % of the mites fell at least within 9 weeks (Bayer Animal Health, unpublished data).

Effectiveness and significance of treatment was further illustrated by the survival rates: colony survival of the groups treated with IVP or CP was 80 and 90 %, respectively, while survival in the negative control group was only 30 %. Fifty percent of the negative control colonies had already died before application of the follow-up treatment on day 116. Acute safety was confirmed by the dead bee counts with significantly lower numbers of dead bees in the IVP or CP group in general, but also significantly lower numbers of dead bees with signs of DWV infection were observed in the IVP or CP group compared to the negative control illustrating the indirect importance of effective *Varroa* treatment for the health of a colony.

Several circumstances can be considered relevant for the interpretation of the study results with regard to efficacy as well as safety. Treatments against *Varroa* in this study started only towards end of August which was relatively late compared to the advised *Varroa* control schedules in the Netherlands which are recommended to start in July already (Cornelissen et al. 2013). To meet the demand of no acaricidal treatment at least four months before start of the study no *Varroa* treatment could be applied to the colonies in July so that they had been treated the last time in the winter preceding the study. This may have implicated that the onset of production of winter bees in all colonies coincided with high *Varroa* loads which may have impacted colonies in all treatment groups (Van Dooremalen et al. 2012). A very early and favourable spring preceding the study may further have added to a high *Varroa* load in the colonies, for which in the Netherlands a warning of unpreceded high *Varroa* infestations was sent out to all beekeepers in August by the Dutch beekeepers association. The possible damage caused by the high *Varroa* load already at start of the study may thus have resulted in the safety evaluation being rather conservative. Among a few colonies already weakened at study start which had to be euthanized before follow-up treatment due to weakness was one colony from the IVP group which had been the weakest at baseline. However, for the reasons above death of this colony was not attributed to treatment. Dead bee traps and Liebefeld estimations may have represented further stressors to the colonies. However, changes in colony strength were within the expected

ranges under the study conditions with strong reductions in colony size and cessation of brood rearing towards winter. The spring season following treatment was tough and started late in the area where the study was conducted. From Figs. 6 and 7 it can be seen that during the colony estimation in spring (April, day 228) the bee population in all three groups had still not really grown compared to December (day 116). However this is comparable to data of Van Dooremalen et al. (2012) which relate to a similar late spring. At the end of the study on day 298 the colonies had started to build up well.

In conclusion, excellent efficacy and safety of flumethrin 275 mg bee-hive strips was confirmed in a positive and negative controlled study. The introduction of the guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees (EMA 2011) states that *Varroa* control implies a number of integrated pest management measures, which include routine beekeeping maintenance methods and the use of approved miticides and that veterinary medicinal products should therefore be regarded as an integrated part of *Varroa* control. Flumethrin 275 mg bee-hive strips may provide a significant contribution to such integrated *Varroa* control.

Acknowledgements

The authors thank B. Cornelissen, C. Hok-Ahin, D. Panziera, L. Poley, J. van der Steen (all Wageningen Plant Research, The Netherlands) and M. Ocak (MD research, Germany) for their help in the conduct or analysis of the study.

Ethical Standards

The study was performed in compliance with current national laws and regulations.

Funding

The study was funded by Bayer Animal Health GmbH, Germany.

Conflict of Interest

Gertraut Altreuther and Klemens Krieger are employees of Bayer Animal Health GmbH. Tjeerd Blacquière is an employee of Wageningen Plant Research, part of WUR.

References

Cornelissen B, Blacquière T, van der Steen JJM (2013) Effectieve bestrijding van Varroa (Tweede, licht gewijzigde druk). Plant Research International. http://library.wur.nl/WebQuery/wurpubs/450973. Accessed 20 March 2017

Delaplane KS, van der Steen J, Guzman-Novoa E (2013) Standard methods for estimating strength parameters of *Apis mellifera* colonies. J Apic Res. doi 10.3896/IBRA.1.52.1.03

EMA (2011) Guideline on veterinary medicinal products controlling *Varroa destructor* parasitosis in bees. EMA/CVMP/EWP/459883/2008. http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500099137. Accessed 19 April 2017

Frey E, Rosenkranz P (2014) Autumn invasion rates of *Varroa destructor* (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. J Econ Entomol 107: 508–515. doi: 10.1603/EC13381

Fries I, Rosenkranz P (1996) Number of reproductive cycles of *Varroa jacobsoni* in honey-bee (Apis mellifera) colonies. Experimental & Applied Acarology 20, 103–112

Honey bee health coalition (2017) Tools for *Varroa* management–a guide to effective *Varroa* sampling & control. Sixth edition April 2017. http://honeybeehealthcoalition.org/wp-content/uploads/2017/04/HBHC-Guide_Varroa_Mgmt_6thEd_7April2017_c.pdf. Accessed: 18 April 2017

Illies I, Mühlen W, Dücker G, Sachser N (2002) The influence of different bee traps on undertaking behaviour of the honey bee (*Apis mellifera*) and development of a new trap. Apidologie 33: 315–326

Imdorf A, Gerig L (2001) Course in determination of colony strength (Swiss Bee Research Centre, Swiss Federal Dairy Research Institute, Liebefeld, CH-3003 Bern), cited in EMA (2011) above.

Kluser S, Neumann P, Chauzat MP, Pettis JS (2010) UNEP emerging issues: global honey bee colony disorder and other threats to insect pollinators. United Nations Environment Programme. https://wedocs.unep.org/rest/bitstreams/14378/retrieve. Accessed 11 April 2017

Martin S (1998) A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. Ecol Model 109: 267–281

Martin SJ, Kemp D (1997) Average number of reproductive cycles performed by *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. J Apicult Res 36: 113–123

Mattila HR, Otis GW (2007) Dwindling pollen resources trigger the transition to broodless populations of long-lived honeybees each autumn. Ecol Entomol 32: 496–505

Neumann P, Carreck NL (2010) Honey bee colony loss. J Apic Res 49 (special issue): 1–6

Oldroyd BP (2007) What's killing American honey bees? PLoS Biol 5: e168. doi:10.1371/journal.pbio.0050168

Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones R, Neumann P, Settele J (2010) Declines of managed honey bees and beekeepers in Europe. J Apicult Res 49: 15–22

Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology 103, 96–119

Seeley TD, Smith ML (2015) Crowding honeybee colonies in apiaries can increase their vulnerability to the deadly ectoparasite *Varroa destructor*. Apidologie 46: 716–727

Smith KM, Loh EH, Rostal MK, Zambrana-Torrelio CM, Mendiola L, Daszak P (2014) Pathogens, Pests, and Economics: Drivers of Honey Bee Colony Declines and Losses. EcoHealth. doi: 10.1007/s10393–013–0870–2

Technical guideline for the evaluation of treatments for control of *Varroa* mites in honey bee colonies, Recommendations from the CA 3686. Document prepared during discussions within the CA 3686 working group: "Evaluation of treatment for control of *Varroa* mites in honey bee colonies". (2011)

Van Dooremalen C, Gerritsen L, Cornelissen B, van der Steen JJM, van Langevelde F, Blacquière T (2012) Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. PLoS ONE 7(4): e36285. doi:10.1371/journal.pone.0036285