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Autumn Invasion Rates of *Varroa destructor* (Mesostigmata: Varroidae) Into Honey Bee (Hymenoptera: Apidae) Colonies and the Resulting Increase in Mite Populations

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ABSTRACT The honey bee parasite *Varroa destructor* Anderson & Trueman can disperse and invade honey bee colonies by attaching to "drifting" and "robbing" honey bees that move into nonnatal colonies. We quantified the weekly invasion rates and the subsequent mite population growth from the end of July to November 2011 in 28 honey bee colonies kept in two apiaries that had high (HBD) and low (LBD) densities of neighboring colonies. At each apiary, half (seven) of the colonies were continuously treated with acaricides to kill all *Varroa* mites and thereby determine the invasion rates. The other group of colonies was only treated before the beginning of the experiment and then left untreated to record *Varroa* population growth until a final treatment in November. The numbers of bees and brood cells of all colonies were estimated according to the Liebefeld evaluation method. The invasion rates varied among individual colonies but revealed highly significant differences between the study sites. The average invasion rate per colony over the entire 3.5-mo period ranged from 266 to 1,171 mites at the HBD site compared with only 72 to 248 mites at the LBD apiary. In the untreated colonies, the *Varroa* population reached an average final infestation in November of 2,082 mites per colony (HBD) and 340 mites per colony (LBD). All colonies survived the winter; however, the higher infested colonies lost about three times more bees compared with the lower infested colonies. Therefore, mite invasion and late-year population growth must be considered more carefully for future treatment concepts in temperate regions.

KEY WORDS honey bee, *Varroa destructor*, invasion rate, population growth, horizontal transmission

The parasitic mite *Varroa destructor* Anderson & Trueman is considered the most destructive threat of the honey bee *Apis mellifera* L. Recently it has been identified as one of the major reasons for periodical colony losses worldwide (Boecking and Genersch 2008, Brodschneider et al. 2010, Chauzat et al. 2010, Guzmán-Novoa et al. 2010). Even moderate *Varroa* infestation rates in autumn significantly increase the risk of colony losses during winter (Genersch et al. 2010). These results clearly indicate that the production of healthy and long-living winter bees (Amdam et al. 2004) is negatively affected by an infestation with *V. destructor*. Under temperate climatic conditions, long-living winter bees are produced in autumn when brood rearing is ceased for several months. These winter bees should not only survive the broodless period but also collect the first pollen and establish a new brood nest in spring.

This is already true at the level of the individual host bee where a *Varroa* infestation changes important physiological parameters of the winter bees (Amdam et al. 2004). At the colony level, the problem is intensified through an inverse population dynamic of host and parasite in late summer and autumn: although the population of bees and brood decrease substantially during this time of the year, the total number of mites increases exponentially throughout the whole period when the colony has brood (Fries et al. 1994, Calis et al. 1999, Wilkinson and Smith, 2002, DeGrandi-Hoffman and Curry 2004). This leads to continuously increasing infestation rates in the remaining brood and consequently increases damage in the emerging winter bees.

Under temperate climatic conditions, yearly treatments against the parasite are therefore indispensable to prevent damage of infested honey bee colonies. The effective control of *V. destructor* after the honey yield, but before the production of winter bees, is a crucial element of sustainable treatment concepts (Imdorf et al. 1996, Rice et al. 2004, reviewed in Rosenkranz et al. 2010).

However, there are frequent reports from beekeepers who complain of colony damage and high numbers of *Varroa* mites during winter treatment, although the recommended treatment has been performed in late summer (Le Conte et al. 2010). Such problems might

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in part be related to an insufficient efficacy of some treatments against mites that are protected within the capped bee brood cells (Sammataro et al. 2004). But, even after an effective summer treatment, there remain certain risks of colony damage, mainly caused by two factors. First, *Varroa* mites from other infested colonies that are not yet treated might invade into the treated colonies. The invasion rates seem to depend on the robbing activity (i.e., internest thievery of honey) and bee density within and around the apiary (Sakofski et al. 1990, Greatti et al. 1992, Goodwin et al. 2006, Frey et al. 2011). Secondly, the remaining and invading *Varroa* females will reproduce and might build a new parasite population that might reach the damage threshold before the wintering of the colony. There are few quantitative data concerning the interaction of *Varroa* invasion and the increase of mite population between summer treatment and wintering of the colony. Vetharaniam (2012) discussed the density effects on the mite population increase, i.e., a small *Varroa* starting population will show rapid growth rates compared with situations where *Varroa* growth rate is suppressed by high *Varroa* infestation and therefore limited availability of unparasitized brood cells.

Therefore, we quantified the mite invasion rate and the increase of *Varroa* population in nearly mite-free colonies between summer treatment and the start of the overwintering period. We made our observations in honey bee colonies at two apiaries situated in regions that had significant differences in the density of honey bee colonies. Because of the different colony densities, we assume the invasion pressure of *Varroa* mites differed between the apiaries. Furthermore, we quantified the effect of mite infestation rates in late summer on the overwintering ability of the honey bee colonies.

Materials and Methods

Study Sites. The experiment was conducted at two apiaries characterized by low and high bee densities in the southern part of the Baden state in southwest Germany, the region with the highest density of honey bee colonies in Germany (4.35 colonies per square kilometers; statistical information provided by the Baden State Beekeeping Association).

The study site with low bee density (LBD) was situated in the foothills of the Black Forest at 360 m above sea level (48° 6′1″ N, 8° 3′21″ O). Because of the mountainous structure of the region and the usually long and snowy winter, there are few permanent apiaries. After the late nectar flow in July 2011 was completed, all migrating beekeepers left the valley and only 50 nucleus colonies of one commercial beekeeper were left within the flight range (2.5 km) of our experimental colonies. These commercial colonies were treated twice with formic acid before the start of our experiment (two times 30 ml of formic acid 60% per colony, evaporated on a sponge), and therefore were considered as having a low mite infestation rate.

The study site with high bee density (HBD) was at the outskirts of the Upper Rhine Plain at 177 m above sea level (48° 12′ N, 7° 46′ O). As a result of the mild winter climate, this part of the Rhine Valley is a preferred region for overwintering of honey bee colonies and hosts a large number of local and migratory beekeepers. According to the list of registered beekeepers from the Veterinary office where all beekeepers are obliged to register and an additional survey together with the local beekeeper organization of Emmendingen, we identified >300 colonies with unknown status of *Varroa* treatment within the flight range of our experimental apiary.

Because of the abundance of *Impatiens glandulifera* Royle (an annual invasive plant in southern Germany) and several *Solidago* species, the autumn pollen supply was sufficient at both study sites. Beginning in August, all colonies were provided with additional sugar syrup for winter stores. During the experimental period, the average temperature and precipitation were recorded 2 m above the ground for both study sites once a day at a nearby weather station (Center for Agriculture and Technology, Augustenberg).

Colony Setup and Experimental Groups. We used 28 honey bee colonies of approximately the same population, headed by queens of the local Hohenheim breeding line (*Apis mellifera carnica* Pollman) and kept in Hohenheim two-story standard hives with 10 Zander frames per storey. The hives were fitted with movable sticky bottom boards protected by a wire grid of 2 mm in diameter. Without opening the hives, dead mites could be counted on the bottom boards after falling down through the wire grid. The boards were covered with an oil-soaked layer of paper towel that reliably prevents ants and earwigs from removing dead mites. To ensure equal start conditions, all colonies were treated before the experiment against *Varroa* with two highly effective acaricides: CheckMite (active ingredient: 1.36 mg coumaphos; Bayer HealthCare AG, Leverkusen, Germany) and Bayvarol (active ingredient: 4.0 mg flumethrin 90%; Bayer HealthCare AG). One strip of each acaricide was used per storey of hive with brood.

The 28 colonies were divided randomly into four groups of seven colonies each. At each of the two study sites, two groups were established. In one group at each study site, the CheckMite and Bayvarol treatments were continued from the start of the experiment on 26th July until the winter treatment in December (hereafter referred to as "treated colonies"). The two different acaricides were applied simultaneously to ensure efficacy and to help prevent the development of acaricide resistance (Rice et al. 2004). This continuous application should have killed all invading mites before they were able to enter a brood cell for reproduction. In the other group at both study sites, the acaricides were removed after 2 wk of treatment (= one sealed brood cycle) at the start of the experiment on 26th July (hereafter referred to as "nontreated colonies"). Therefore, invading mites should have been able to reproduce within these colonies. On 18th October, the nontreated colonies were again treated with CheckMite and Bayvarol for 3 wk to determine the mite invasion up to that time. At the

beginning of December, a final oxalic acid treatment (trickling Oxuvar according to the manufacturer´s recommendations; Andermatt BioVet GmbH, Lörrach, Germany) was performed on all 28 colonies to remove any remaining mites.

Evaluation of Colony Development. At 3-wk intervals (nontreated colonies) and 6-wk intervals (treated colonies), the numbers of bees and brood cells of all colonies were estimated according to the Liebefeld method (Imdorf et al. 1987). In February 2012, the number of bees of all 28 colonies was estimated for the final time to identify the loss of the adult bee population over winter.

Two combs each from the colonies of the treated and nontreated groups were removed in September and analyzed for residues of coumaphos (LOQ 0.5 mg/kg) and flumethrin (LOQ 1 mg/kg) in the beeswax according to the analytical method developed at our residue laboratory (accredited according to DIN EN ISO/IEC 17025).

Evaluation of Invasion and Infestation Rates of *V. destructor*. In the treated colonies, the invading mites that were killed by the acaricides were counted on the sticky bottom boards once a week from 26th July until 5th November. The expected mite-free status of these colonies was confirmed during the experiment by analyzing bee and brood samples taken every 6 wk. We quantified *Varroa* infestation by counting the adult female mites.

To estimate the growth of the *Varroa* infestation within the nontreated colonies, samples of adult worker bees (≈150 bees per colony) and sealed brood (≈200 worker brood cells containing pink-eyed pupae) were analyzed at 3-wk intervals during the experiment. The final mite infestation was determined according to the number of mites killed by the treatments in October and December (see above).

Data Analysis. The nonparametric Kruskal–Wallis test was used to compare bee and brood populations between groups at the start of the experiment. A repeated-measures analysis of variance (ANOVA) was used to compare the amount of brood in the nontreated colonies between the LBD and HBD sites over the entire experimental period; sites was the independent factor in the ANOVA. The Mann–Whitney *U* test was used for the comparisons of the weekly mite invasion rates in the treated groups. In October, the *Varroa* infestation levels of the nontreated colonies revealed large differences between the apiary sites. For the analysis of the decline of the bee population over winter (bee populations in October vs. bee populations in February), we used one-way ANOVA with repeated measures for the LBD and HBD sites separately. All tests were performed with the SPSS 20.0 statistics software.

Results

Climatic Conditions at Both Study Sites. The course of the average daily temperature did not differ between the areas with HBD and LBD, suggesting similar foraging conditions at both apiaries. The 2011 season was characterized by warm and stable weather conditions until mid-October followed by a short cold spell and a warm autumn. During the 100-d experimental period, on Day 92 (LBD) and Day 89 (HBD) the maximum daytime temperatures exceeded 12°C, which is considered the minimum threshold value for honey bee flight activity. On only Days 5 (LBD) and 2 (HBD) did the precipitation exceed 0.5 mm per hour, which might have prevented the bees from leaving their hives.

Colony Development. At the start of the experiment, the four experimental groups were homogenous in number of bees ($P = 0.45$; Kruskal–Wallis) and brood ($P = 0.09$; Kruskal–Wallis) with average bee populations ranging from ≈17,000–22,000 for the different groups. By the end of the season and the start of the wintering period in October, the average adult bee populations decreased in all groups from ≈20,000 bees per colony to 10,000–14,000 bees per colony. Of the 28 experimental colonies, 25 had adult bee populations of >8,000 bees in October and only three colonies had populations between 7,000 and 8,000 bees per colony. Honey bee colonies with >8,000 bees in October are considered well-prepared for successful overwintering in temperate climates (Imdorf et al. 2008).

After the start of the experiment, the brood production was significantly greater at the HBD site than at the LBD site ($F = 23.2$; $P < 0.01$; repeated measures ANOVA), probably due to a more varied range of pollen sources (Fig. 1). Overall averages of the number of brood cells in the nontreated colonies were 109,000 ± 4,471 brood cells per colony at the HBD site and 75,000 ± 3,635 brood cells at the LBD site.

***V. destructor* Invasion Rates.** The number of mites invading the treated colonies differed between the LBD and HBD sites (Fig. 2). During the entire experimental period, with the exception of the calendar Weeks 30, 31, and 33, the invasion rates into the HBD colonies were significantly higher compared with the LBD colonies ($P < 0.05$ for calendar Weeks 32 and 34 and $P < 0.01$ for calendar Weeks 35–44; *U* test). At the HBD apiary, a striking increase of the invasion rate was recorded after calendar Week 33, while at the LBD apiary, it remained continuously low over the whole period (Fig. 2).

The average invasion rates over the entire 3.5-mo period were 462 ± 74 mites per colony with a range between 266 and 1,171 mites at the HBD apiary and 126 ± 16 mites per colony with a range between 72 and 248 mites per colony at the LBD apiary. The maximum number of invaded mites per colony in a week was 109 for HBD (calendar Week 35) and 47 for LBD (calendar Week 30). For all seven colonies at each of the two apiaries, these numbers add up to totals of 3,238 introduced mites at the HBD apiary compared with only 880 mites at the LBD apiary.

The analysis of bee and brood samples of the treated colonies (taken three times during the experimental period in July, September, and October) confirmed the efficacy of the applied acaricides. We found only

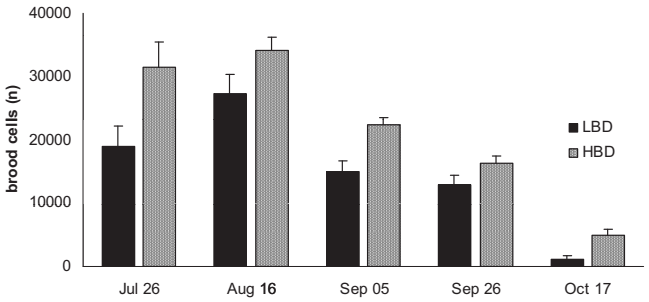

Fig. 1. Average number of worker brood cells of the nontreated colonies at study sites with LBD and HBD (means ± SE). In October, two of the LBD colonies were without brood. The differences in brood production were significant between the two sites ($P < 0.01$; repeated measures ANOVA).

eight mites in 5 of the 42 analyzed bee samples (corresponding to 8,334 analyzed bees), and only 30 mites in 15 of the 41 examined brood samples (corresponding to 7,606 analyzed worker brood cells).

***V. destructor* Infestation Rates of the Nontreated Colonies.** The average *V. destructor* infestation rate of adult bees at the LBD apiary increased from 0.0% at the start of the experiment to 4.3 ± 1.1% in mid-October (Table 1). The brood infestation rate increased from 1.1% in July to 16.0 ± 3.8% in October (Table 1). The colonies at the HBD apiary started with an average mite infestation on adult bees of 0.2% and reached a final bee infestation of 18.0 ± 3.7% 3 mo later. The brood infestation rate of this group revealed an extreme increase from 0.7% in July to 50.8 ± 10.5% in October (Table 1).

The colonies of the nontreated groups received *Varroa* treatments with CheckMite and Bayvarol in October followed by a final oxalic acid treatment in December. As a result of mite invasion and subsequent reproduction of those mites, the *Varroa* populations in these nontreated colonies reached average final infestation levels of 864–6,028 mites per colony at the HBD apiary (on average 2,028 mites per colony) but only 190–488 mites per colony at the LBD apiary (on average 340 mites per colony; Table 2). Thus, the numbers of *Varroa* mites per apiary ($n = 7$ colonies each) added up to 14,577 (HBD) and 2,380 (LBD), respectively.

The final oxalic acid treatment of the treated colonies confirmed the efficacy of the combined Bayvarol and CheckMite treatment: very low numbers of mites (3, 3, and 5) recorded in only 3 of the 14 colonies.

In the beeswax of the treated colonies, residues of coumaphos were detected in low concentrations (6.6 and 7.5 mg/kg), whereas flumethrin was not detected. In the samples of the nontreated colonies none of these active ingredients were found, indicating that the possibility of a residual effect from the previous treatment was rather low.

***Varroa* Infestation and Overwintering.** At the HBD site there was a significant difference between the treated and nontreated colony groups in the percentage loss of the adult bee population during the wintering period from October 2011 until the end of February 2012 ($F = 91.7$, df = 1, $P < 0.001$). The nontreated, highly mite infested colonies lost 58.1% of their bee population, while the treated colonies with fewer mites lost only 24% (Fig. 3). However, at the LBD site the decline of the bee population was similar and not significantly different ($F = 2.3$, df = 1, $P = 0.16$) between the moderately infested nontreated group (36.0%, on average) and the treated group (39.8%, on average).

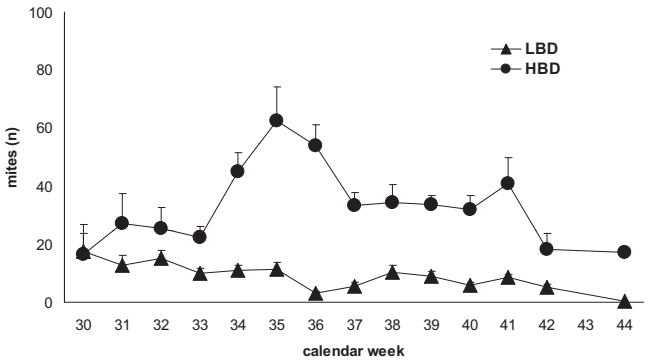

Fig. 2. Average number of *V. destructor* that invaded treated colonies at the LBD ($n = 7$) apiary and HBD ($n = 7$) apiary from the end of July until the beginning of November (means ± SE).

Table 1. Average *V. destructor* infestation rates (mites per 100 bees; mean ± SE) in sealed brood cells and on adult bees of the nontreated colonies at the LBD (n = 7) apiary and the HBD (n = 7) apiary

Sampling date	LBD		HBD	
	Bees	Brood	Bees	Brood
26 July	0.0 ± 0.0	1.1 ± 0.1[a]	0.2 ± 0.8	0.7 ± 0.4
16 Aug.	0.5 ± 0.2	0.9 ± 0.4[a]	0.9 ± 0.5	0.8 ± 0.3
05 Sept.	0.5 ± 0.1	2.6 ± 0.3	1.3 ± 0.9	6.5 ± 1.6
26 Sept.	1.2 ± 0.3	8.5 ± 1.1	6.5 ± 2.0	22.1 ± 8.8
17 Oct.	4.3 ± 1.1	16.0 ± 3.8[b]	18.0 ± 3.7	50.8 ± 10.5

[a] Brood samples taken from n = 6 colonies.
[b] Brood samples taken from n = 3 colonies.

Discussion

Under temperate climatic conditions, *Varroa* treatments have to be performed before the production of long-lived winter bees. Worker bees parasitized during development have a reduced life span (Amdam et al. 2004) and will presumably not survive until spring. In addition, a high *Varroa* infestation in the colony leads to a higher transmission of viruses among the bees (Francis et al. 2013). The close correlation between mite infestation in late autumn and winter mortality of honey bee colonies has also been confirmed by a large German bee monitoring project (Genersch et al. 2010). An effective *Varroa* control in late summer is crucial for the successful overwintering of honey bee colonies when mite infestations are threatening, and therefore is an indispensable part of an integrated pest management (IPM; Delaplane 2011, Dietemann et al. 2012).

However, the time window available for these late summer treatments is rather narrow because chemical treatments can only be started when the last honey harvest is completed (Currie and Gatien 2006). Our study indicates that the horizontal transmission of *Varroa* mites could additionally jeopardize the IPM performed by the beekeepers. We used two neighboring study sites to quantify the invasion rates of *Varroa* mites in relation to the density of honey bee colonies. The two experimental apiaries were only 21 km apart and provided nearly identical conditions in terms of ambient temperature and rainfall. At both apiaries, the provision with nectar and pollen throughout the experimental period was sufficient to stimulate brood production and prevent robbing. The crucial difference between both apiaries was the number of honey bee colonies within the foraging range. The LBD apiary was situated within an isolated valley with a low number of treated nucleus colonies in the proximity. The HBD apiary was located within a region preferred by beekeepers for overwintering their hives and represents a region with one of the highest density of honey bee colonies in Germany. Therefore, we assume a substantially higher invasion pressure of *Varroa* mites at the HBD site than at the LBD site. For the quantification of the weekly invasion rates into the experimental colonies, we used a continuous treatment with two different acaricides. The repeated sampling of adult bees and brood for *Varroa* and the nearly mite-free status of these colonies at the end of the experiment confirmed that *Varroa* mites were killed immediately after invading these colonies.

Over the entire 3.5-mo period, we recorded a total mite invasion rate of >3,200 mites into the seven treated colonies at the HBD site. This was a nearly fourfold higher number compared with the LBD apiary, although significant site-specific differences in the weekly invasion rates were identified only after mid-August. The latter confirms earlier studies assuming that *Varroa* invasion is triggered by cessation of the nectar flow in late summer and the subsequent increase in robbing among honey bee colonies (Sakofski et al. 1990, Greatti et al. 1992, Frey et al. 2011). However, our weekly invasion rates were clearly lower compared with the >30 mites per day per colony described from Italy ≈20 yr ago (Greatti et al. 1992). This difference might be explained by the fact that, in our research area, there were no indications of feral bee colonies and that, during our experimental period, there were no reports of collapsing colonies. Furthermore, because of intensive extension services and the well-organized beekeeping association in this state, local beekeepers are very much aware of the need for late summer treatments and the vast majority try to follow these recommendations.

Despite these good advisory services and long-term experience of beekeepers in treating *Varroa*, we still recorded a dangerously high invasion pressure at the HBD site from mid-August through mid-October. This leads to reinvasion of mites in previously treated colonies as is demonstrated by our largely mite-free experimental colonies. One can assume that the invading *Varroa* mites come from more highly infested colonies of neighboring apiaries. Invasion, coupled with subsequent reproduction by invading mites, can be a substantial problem for beekeepers who treated their colonies earlier, giving them a false sense of security. It also might explain, at least in part, unexpected winter mortality of colonies belonging to experienced beekeepers who have performed *Varroa* treatments according to recommendations (Le Conte et al. 2010).

At both our study sites, half of the colonies were only treated before the start of the experiment, and thereafter left untreated until a final treatment in

Table 2. Final mite infestation of the non-treated colonies at the LBD apiary and the HBD apiary in November resulting from *Varroa* invasion and subsequent reproduction during the experimental period

	LBD							HBD						
Colony no.	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Mites (n)	342	319	488	449	292	300	190	933	1,412	1,846	1,232	864	2,262	6,028

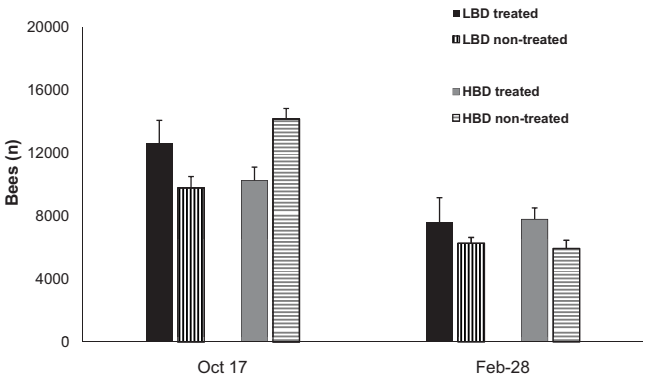

Fig. 3. Number of bees of the treated and nontreated colonies at the apiaries with LBD (moderate *Varroa* infestation) and HBD (high *Varroa* infestation) before and after overwintering (means ± SE).

November. Invaded mites could therefore reproduce over the entire 3.5-mo period. Because all experimental colonies had the same genetic background and were of similar size, we assume invasion rates in the nontreated colonies were similar to those in the continuously treated colonies in the same apiary.

There were large differences between the HBD and LBD sites for infestations of both brood and bees. At the HBD site, the average brood infestation exceeded 6% at the beginning of September. At the end of September, the average infestation rate exceeded 22%, meaning that a quarter of the hatching winter bees were already weakened. At the HBD site, the infestation level of the adult bees was comparatively low until mid-September but then clearly exceeded the autumn economic threshold of 3–5 mites per 100 adult bees suggested for the United States and Canada (Delaplane and Hood 1999, Strange and Sheppard 2001, Currie and Gatien 2006). At the LBD site, both the bee and brood infestations remained consistently below the damage threshold. However, at the last examination in October, the bee infestation was only slightly lower than the threshold level of 6 mites per 100 bees determined by the long-term monitoring of winter losses in Germany (Genersch et al. 2010).

The site-specific differences in the final infestation, as determined by treatments starting at the beginning of November and performed with three different acaricidal compounds (flumethrin, coumaphos, and oxalic acid), were large. In the colonies at the HBD site, we recorded between 933 and 6,028 mites per colony, whereas at the LBD site, we found a clearly lower range of 190 and 488 mites per colony. These values demonstrate the large variation in the infestation levels of colonies kept under identical beekeeping management conditions. In addition, it demonstrates the significant reproductive capacity of *Varroa* mites in autumn when environmental conditions allow brood rearing within the colonies. An exact calculation of the rate of *Varroa* reproduction in our colonies is not possible because invasion rates and subsequent reproduction cannot be separated within the same colony. However, if we compare the absolute number of invading mites with the average final infestation, we see a large difference at both study sites: 126 versus 340 mites at the LBD and 462 versus 2,088 at the HBD site, indicating a substantial multiplication of the invading mites. The ratios between final infestation and invading mites (2.7 at the LBD and 4.5 at the HBD site) indicate that at the HBD site the mites invaded earlier, resulting in more reproductive cycles, or that these colonies provided better conditions for *Varroa* reproduction. As the availability of brood is a crucial factor for *Varroa* population growth (Wilkinson and Smith 2002, Vetharaniam 2012), a higher reproductive rate could at least partly be explained by the higher brood production of the HBD colonies during the experimental period. Daily mite population growth rates between 0.01 and 0.025 are suggested by models (Martin, 1998, Wilkinson and Smith, 2002). Such growth rates could explain our final infestation levels if we use the data on mite invasion from our experiment within a simple exponential model of *Varroa* reproduction.

In terms of relevance for beekeeping practice, we could demonstrate that effective *Varroa* treatment at the end of July, when undertaken alone, is not sufficient for successful overwintering if the mite invasion pressure is high. It is likely that a high density of *Varroa* infested honey bee colonies within flight range will increase the invasion pressure. However, other factors like ineffective *Varroa* treatments might also influence the invasion rates independently from the colony density. Even colonies that are largely mite-free at the beginning of August can build up threatening *Varroa* populations by the beginning of winter. Our data on overwintering also emphasize the risk of high *Varroa* infestations late in the year. At the LBD apiary, where both experimental groups were either noninfested (continuously treated) or moderate infested, the average decline of the bee population from October till February was <40%. Such values are within the range reported from overwintering colonies in temperate regions (Free and Racey 1968, Imdorf et al. 2008). However, at the HBD site the heavily infested colonies lost, on average, nearly 60% of their bees, which represents a highly significant difference compared with the noninfested colonies at the same apiary. The reason that none of these highly infested colonies com-

pletely collapsed overwinter might be due to the high number of bees in October (>14,000, on average) and the fact that the *Varroa* population increased at a time of the year when a proportion of winter bees has already been produced. According to Mattila et al. (2001), the first winter bees appear at the end of August. At that time, none of the experimental colonies had a high *Varroa* infestation. Van Dooremalen et al. (2012) showed clearly that *Varroa* infested bees had a shorted lifespan and that successful overwintering depends strongly on the proportion of noninfested winter bees. Infested bees have a higher probability of being infected with bee viruses (Nguyen et al. 2011, Francis et al. 2013), which may have additionally contributed to the weakening of our colonies. Comparative virus analysis on bees from the different experimental groups before and after overwintering supports this assumption (McMahon et al. 2014).

We assume that the situation within our research sites largely corresponds to other regions with a temperate climate and intensive beekeeping activities. Because of anticipated changes in climate leading to higher autumn and winter temperatures in temperate regions (Linderholm 2006), we will increasingly be faced with conditions that support reinvasion into colonies of *Varroa* mites in autumn and their reproduction therein. Therefore, our study points out some general aspects that should be considered for the implementation of *Varroa* treatment concepts. First, IPM programs should be coordinated region-wide to reduce the *Varroa* reinvasion pressure. Second, additional *Varroa* diagnostic measures are recommended during the period after summer *Varroa* treatment. This is the only way for the beekeeper to detect and then react to unexpectedly high mite infestations.

Beside these practical recommendations, our results also point to a conflict between beekeeping practice and the selection of *Varroa* resistant honey bees. For many selection programs, colonies should be allowed to host a number of mites sufficient to demonstrate the capacity of the colony to control the growth of the mite population (Büchler et al. 2010). Therefore, *Varroa* treatments should not be performed too early and should depend on colony infestation levels. At least in regions with high bee densities, this will significantly increase the number of mites within the region and most likely, as a consequence, the *Varroa* reinvasion pressure and horizontal transmission of *Varroa* mites between colonies. Horizontal transmission of a pathogen is assumed to favor the development of a virulent host–parasite relationship (Fries and Camazine 2001). That is, *Varroa* mites that harm or even kill their colony have a realistic chance to find a new host colony for further reproduction. Hence, high bee densities combined with ineffective treatment will not only increase the risk of colony damage but might also select for more virulent *Varroa* mites.

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