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Original article

Efficacy and temperature dependence of 60% and 85% formic acid treatment against *Varroa destructor*

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Abstract – The ectoparasitic mite *Varroa destructor* is considered one of the main threats to the western honey bee (*Apis mellifera*). Efficient pest management is crucial, and the evaporation of formic acid (FA) is an active principle that could be adopted. However, the usage of FA has an extreme variable efficacy depending on several conditions, ambient temperature among them. Cooler conditions, as they usually occur in Central Europe in late summer and autumn, can negatively affect treatment success. Our study aims to evaluate factors that influence the efficacy of different FA treatments. Over a period of 8 years, we investigated the effect of ambient temperature, hive size and dispenser type on the treatment success with 60% and 85% FA and consolidated those factors in a linear regression model. Treatment with 60% FA shows higher variability, and often lowered efficacy, especially in double brood chamber hives. In contrast, 85% FA treatment achieves higher efficacy and lower variability and shows significantly diminished dependence on ambient temperature.

***Apis mellifera* / *Varroa destructor* / formic acid / mite / treatment efficacy**

1. INTRODUCTION

The mite *Varroa destructor* (Anderson and Trueman) is an almost globally distributed ectoparasite of the western honey bee (*Apis mellifera* L.). It originates in Southeast Asia, where it can be found in colonies of the Asian honey bee *A. cerana*. At the beginning of the twentieth century, by enhancing the efficacy of transport and trade, western honey bee colonies were imported to Asia and henceforth coexisted with Asian honey bee species (Oldroyd 1999). Around 1960, it was first discovered that *Varroa* successfully had switched to western honey bee populations through the sympatric cohabitation of both *Apis* species (Delfinado 1963). Global spread to other continents took place in the 1960s and 1970s via international shipping of honey bee colonies (de Guzman et al. 1997; De Jong et al. 1982). In regions without geographical barriers, the transfer of *V. destructor* to other colonies was further enhanced through robbing, drifting of drones and worker bee homing errors (Seeley and Smith 2015), but also via combining hives, transferring food stores between hives and high bee densities (Fries and Camazine 2001).

For a long time, it was assumed that *Varroa* was only one species described by A.C. Oudemans (1904) as *V. jacobsoni*. Only at the beginning of this millennium, the phylogenetic analysis of mtDNA data uncovered *V. destructor* as an independent species (Anderson and Trueman 2000). Currently, the genus *Varroa* is represented by at least four species, but the only mite of actual economic importance is *V. destructor* (Rosenkranz et al. 2010).

V. destructor parasitizes pupae and adult bees. During the larval development, it reproduces in

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capped brood cells where it feeds on the bees' fat body tissue (Ramsey et al. 2019). The loss of tissue during the ontogenetic development within the brood cell significantly decreases lipid synthesis, protein titres and the weight of the hatching bee (Bowen-Walker and Gunn 2001; Schneider and Drescher 1987). The weight loss and negative influence on the development of the hypopharyngeal gland increase with the number of mites (Schneider and Drescher 1987). In addition to the damage that is caused through feeding on the bees' tissue, *V. destructor* accelerates the transmission of viruses and microorganisms and acts as a vector for several diseases (Boecking and Genersch 2008; Bowen Walker et al. 1999; Di Prisco et al. 2011; Francis et al. 2013; Gliński and Jarosz 1992). Varroosis is considered to be the most serious threat to honey bees (Sammataro et al. 2000; Rosenkranz et al. 2010). It is assumed that the final breakdown of the colony is an effect of interactions between virus infections and other stress factors, rather than the effect of direct parasitization (VanEngelsdorp et al. 2009) and without any treatment colonies would collapse within 3 years (Fries et al. 2003, 2005).

Besides their importance for pollination of wildflowers, honey bees are one of the economically most valuable pollinators for pollination dependent crops in agriculture (Calderone 2012; Crane 1990; Southwick and Southwick 1992). Managed honey bees are a reliable solution for farmers to ensure crop pollination and have been shown to be more effective than alternative pollinators (Klein et al. 2007; Rader et al. 2009). Since honey bees can forage over large distances, they are very suitable for the pollination of large monocultures. Thus, the control of *V. destructor* plays not only a central role in beekeeping but is also crucial to for effective crop pollination.

The integrated pest management is a set of proactive methods that is used before the population of *V. destructor* reaches levels that threaten colony productivity and survival (Honey Bee Health Coalition 2018). Depending on the seasonal cycle of the colony, several of these methods can be combined (Roth et al. 2020). A chemical treatment option that is highly effective in capped brood cells and therefore affects not only mites that are parasitize adult bees but also mites in the reproductive stage is formic acid (FA) fumigation (Fries 1991; VanEngelsdorp et al. 2008). In contrast to lipophilic synthetic acaricides, FA is water soluble, and thus no residues in wax can be found. As the range between therapeutic benefits and toxic effects of organic acids is very narrow, the treatment of varroosis with FA must be carefully coordinated. In the temperate climate of Central Europe, problems with FA treatment in autumn are frequently reported by beekeepers. Poor evaporation due to cooler temperatures and thus insufficient treatment efficacy frequently leads to the loss of colonies in winter. The efficacy of different treatments for varroosis with FA has been addressed in several studies, often focusing exclusively on one factor like the form of application through different dispenser-types, the place of application within the hive, location of hives or the timing of treatment (Giovenazzo and Dubreuil 2011; Girisgin and Aydin 2010; Moosbeckhofer et al. 1997; Pietropaolo and Formato 2018; Satta et al. 2005). Formic acid has been evaluated in warmer seasons (Pietropaoli and Formato 2018) in different concentrations under laboratory conditions on mites, larvae and worker bees (Underwood and Currie 2003; Bolli et al. 1993, Lindberg et al. 2000).

This study aims to evaluate factors and their respective influence on the therapeutic efficacy of FA treatment against varroosis and combine these factors in one model. We investigated the effect of ambient temperature, hive size and type of dispenser on the efficacy of treatments with 60% and 85% FA. The results of this study will contribute to optimize FA treatment and serve as a guide for beekeepers to improve their *Varroa* management.

2. MATERIAL UND METHODS

2.1. Study site

The study was conducted from 2011 to 2019. In total, 340 treatments were implemented in *A. mellifera* colonies maintained in Zander bee hives at eight experimental apiaries in North Rhine Westphalia, Germany. All colonies were naturally infected with *V. destructor* . Natural mite mortality was monitored, and colony strength

(adult bees, brood, stored honey) was evaluated following the Liebefeld method within 7 days prior to treatment (Imdorf et al. 1987). Equalised colonies containing either approximately 8000 bees in one 10-frame brood chamber or approximately 13,000 bees in two 10-frame brood chambers were allocated randomly to treatment groups.

2.2. FA application methods

The Liebig Dispenser (Andermatt Biovet GmbH, Lörrach, Germany) and the Nassenheider *professional* (Joachim Weiland Werkzeugbau GmbH & Co KG, Hoppegarten, Germany) were tested based on the manufacturers' specifications within the late summer/autumn treatment. Treatments with the Nassenheider dispenser lasted 10–14 days. The Liebig Dispenser remained for 7 days in single brood chamber colonies, for 3 days in August and 7 days in September/October in double chamber colonies. Both are vacuum devices that consist of a plastic bottle to be filled with formic acid solution and a wick that allows a slow release of the liquid FA. The devices are placed on top of the frames of the brood chamber within an additional empty brood chamber. Formic acid is meant to evaporate successively over treatment days (long-term treatment). The dispensers were tested with 60% and 85% FA in single and double brood chamber hives.

2.3. Data recording

During the experiments, ambient temperature was recorded continuously at the apiary (DL-120 TH; DL-111 K; DL-210TH, Voltcraft, Conrad Electronic AG). Daily mean temperature was used for further calculations. In order to calculate treatment efficacy, the number of dead mites was counted daily. For this purpose, a bottom board with a metal screen that covered the entire hive bottom was used. These bottom boards were prepared with rapeseed oil soaked paper in a way that dead mites, fallen off from bees or out of cells, would adhere on the boards (Dietemann et al. 2013). As soon as the treatment started, for the duration of the treatment and for the subsequent 12 days, monitoring was carried out. This ensured that mites killed inside the brood cells could be included in the analysis.

The remaining number of mites was assessed in November/December with a follow-up treatment carried out with 3.5% oxalic acid solution (Oxuvar 5.7%, Andermatt Biovet GmbH, Lörrach, Germany). The oxalic acid dihydrate solution (50 ml) contains oxalic acid dihydrate (1.75 g), sucrose (30.015 g) and purified water (30.015 g), and the dose per bee-occupied frame side is 0.25 ml/dm^2. As the oxalic acid dihydrate solution is only effective on mites on the adult bee, it is trickled onto the cluster of bees in brood-free colonies. After the application of oxalic acid, mite numbers were recorded for additional 3 weeks.

Treatment efficacy was calculated as the percentage of mites killed during the formic acid treatment relative to the total number of mites in the colony, killed either during the formic acid treatment or by the follow-up treatment with oxalic acid. To calculate treatment efficacy (E), the following formula was used: E = (VT/ (VT+ FOLLOW-UP))*100, where VT is the number of killed mites counted during the FA treatment and VT + FOLLOW UP represents the total number of mites in the colony, killed either during the treatment or the follow-up treatment.

2.4. Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk test were used to analyse data distributions. To compare treatment efficacy, a one-way ANOVA model was used. Homogeneity of variances was determined by means of Levene test. The Welch robust test was applied according to homogeneity of variances. Games-Howell was used as a post hoc test. For linear regression, the examination of potential predictor variables and the collinearity between these variables was performed by a two-tailed Pearson's correlation. If two potential risk factors were associated, only one was included in the multivariable analysis. A linear regression with efficacy was adjusted for the previously tested significant factors. Variables with a $p < 0.05$, calculated using the one-tailed Pearson correlation, were maintained in the model. All statistical analyses were performed with IBM® SPSS

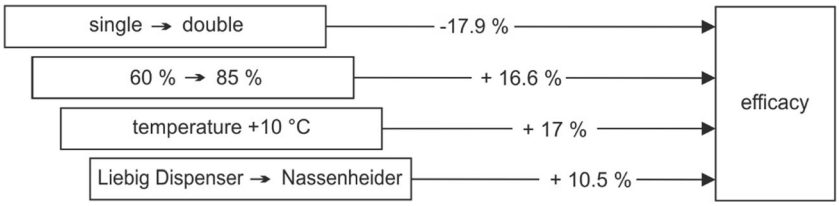

Figure 1. Model for the linear regression equation. Left boxes: independent predictor variables that show significant impact on efficacy (hive size, FA concentration, ambient temperature and dispenser type). Arrows: associated coefficients show the respective impact on efficacy if the independent variables are adapted as indicated in the left boxes. The variables are arranged in order of their importance according to standardized beta coefficients.

Statistics Version 24 (IBM Deutschland GmbH, Ehningen).

3. RESULTS

The study was conducted to evaluate the treatment efficacy depending on different variables. Average ambient temperature during treatments was 14.9 ± 3.4 °C (min 7.8 °C, max. 23.6 °C).

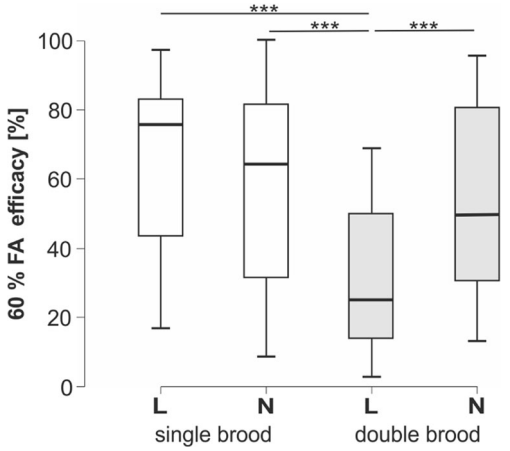

Figure 2. Sixty percent FA treatment efficacy (%) obtained for the four groups under study. Sixty percent formic acid was tested in single and double brood (white and grey bars, respectively) chamber hives with the Liebig Dispenser (L) and the Nassenheider (N). The treatment with Liebig Dispenser in double brood chamber hives resulted in significantly lower values than all other treatment groups (Games-Howell post hoc test, *** < 0.001). Data are shown as boxplots with the median; the edges indicate 25th and 75th percentiles. Whiskers represent × 1.5 the interquartile range, and circles indicate outliers.

3.1. Linear regression model

A significant correlation of hive size with stored honey and the number of adult bees (Pearson's $r = 0.488$, $p < 0.001$, $n = 298$ and 0.418, $p < 0.001$, $n = 312$, respectively) was found, and as a consequence, hive size was chosen as variable for the regression. The further examination of potential predictor variables revealed no significant relation of efficacy and adult bees (Pearson's $r = 0.021$, $p = 0.714$, $n = 299$), capped brood (Pearson's $r = 0.113$, $p = 0.051$, $n = 300$) or stored honey (Pearson's $r = -0.065$, $r = 0.266$, $n = 298$). Consequently, hive size, FA concentration, ambient temperature and type of dispenser remained as predictor variables in the linear regression model, as they all have a significant influence on the treatment efficacy (Fig. 1, $F(4335) = 27.61$, $p < 0.001$). R^2 for the overall model was 0.248 (adjusted $R^2 = 0.239$). The model revealed a decrease in efficacy of 18% if treatment was conducted in double brood chamber instead of single brood chamber colonies. Furthermore, regression coefficients indicated that 85% FA rather than 60% FA could increase efficacy by 17%; Nassenheider rather than Liebig dispenser results in 10.5% higher efficacy.

3.2. Sixty percent formic acid

Efficacy of 60% FA was determined in 182 treatments and achieved 51.9 ± 27.8%. The treatment with 60% FA was most efficient in single brood chamber hives regardless of the dispenser used (Fig. 2, Table I). The treatment was significantly less efficient for colonies in double brood chamber hives when the Liebig Dispenser was

Table I. Efficacy of the treatment groups with different dispenser types (Liebig Dispenser and Nassenheider), formic acid concentrations (60% and 85%) and brood chambers (1 and 2)

FA [%]	Dispenser	Hive size	*n*	Efficacy [%]		
				Min	Max	Mean ± SD
60	Liebig	1	31	16.8	97.2	66 ± 25
		2	40	3.0	68.7	31 ± 20
	Nassenheider	1	71	8.7	100.0	57 ± 28
		2	40	13.2	95.4	53 ± 26
85	Liebig	1	44	20.5	97.8	76 ± 19
		2	52	6.0	97.9	44 ± 27
	Nassenheider	1	33	27.0	100.0	78 ± 22
		2	29	17.3	98.8	75 ± 24

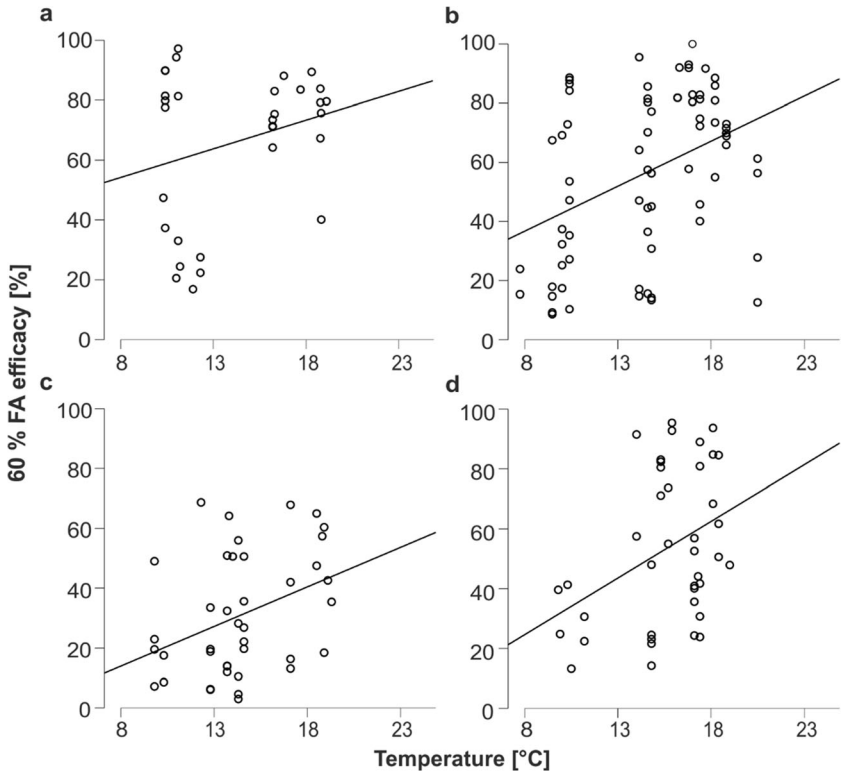

Figure 3. Treatment efficacy (%) across four different treatment combinations with 60% FA in relation to average ambient temperature. Regression lines are given in the graph. **a** Liebig Dispenser, single brood chamber (Pearson's $r = 0.266, p = 0.074, n = 31, R^2 = 0.071$). **b** Nassenheider, single brood chamber ($r = 0.392, p < 0.001, n = 71, R^2 = 0.154$). **c** Liebig Dispenser, double brood chamber ($r = 0.367, p = 0.010, n = 40, R^2 = 0.135$). **d** Nassenheider, double brood chamber ($r = 0.378, p = 0.008, n = 40, R^2 = 0.143$).

used (Games-Howell post hoc test, $p < 0.001$). Except for the treatment with the Liebig Dispenser in single brood chamber hives, treatment efficacy in colonies treated with 60% FA increased significantly with ambient temperature (Figs. 3 and 6).

3.3. Eighty-five percent formic acid

Efficacy of 85% FA was determined during 158 treatments and achieved 65.7 ± 27.6%, which is significantly higher than in 60% FA treatments (t (338) = - 4.594, $p < 0.001$). The significantly lowest treatment efficacy is obtained with the Liebig Dispenser in double brood chamber hives (Fig. 4, Games-Howell post hoc test, $p < 0.001$). No significant correlation between temperature and treatment efficacy was found (Figs. 5 and 6).

4. DISCUSSION

Our study examined a variety of influencing factors concurrently. We investigated and compared the efficacy of two FA concentrations which are used during the treatments carried out with two commercially available and industry-established dispensers in two different hive sizes. The duration of this study over several years and the high number of treatments enabled us to find a significant relationship between efficacy, concentration, ambient temperature, hive size and dispenser type. Additionally, we examined these factors according their relative importance. The amount of capped brood does not correlate with efficacy, but still this result was very narrow. If the regression was nevertheless carried out with capped brood as a variable for verification purposes, the variable remained without significant impact on the model and therefore on the efficacy.

In general, the treatment of colonies in single brood chamber hives resulted in higher efficacy than in double brood chamber hives. The regression model revealed a decrease in efficacy of 18% if treatment was conducted in the larger hive size. The smaller volume of the single brood chamber hives could have led to a faster achievement of the required dose of FA. Additionally, in single brood colonies, the dispenser is located directly on top of the brood nest, whereas in double brood chamber hives, there is a greater distance between the dispenser and the brood nest. We know from studies on thermoregulation capacities that temperature in the brood nest region is highly stable within a very narrow range (Jones et al. 2005; Kleinhenz et al. 2003; Kronenberg and Heller 1982). Temperature decreases with distance from the broodnest, and this consequently could also influence the evaporation of FA. Also, the amount of bees and brood differed between hive sizes, which might influence ventilation within the hive. The more bees that are in the hive, the more ventilation could take place. This could influence distribution of the FA and thus lead to a higher efficacy. But this effect is might be counteracted by the fact that in a smaller hive, the concentration is presumably reached faster, which encourages the bees to start ventilating faster compared to bigger hives. In double brood chamber hives, the Nassenheider evaporator showed a significantly higher efficacy over the Liebig Dispenser for both FA concentrations.

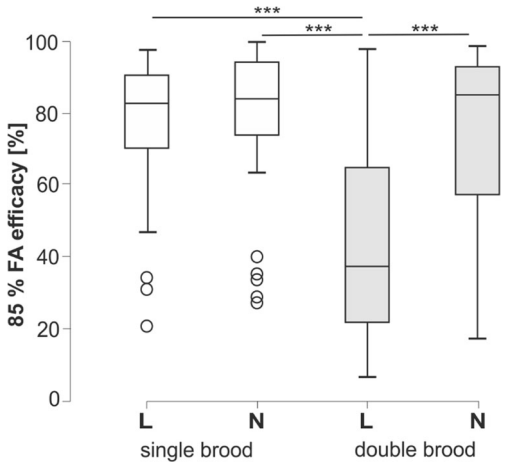

Figure 4. Box plot of 85% FA treatment efficacy (%) obtained for the four groups under study. 85% formic acid was tested in single and double brood (white and grey bars, respectively) chamber hives with Liebig Dispenser (L) and Nassenheider (N). The treatment with Liebig Dispenser in double brood chamber hives acquired significantly lower values than all other treatment groups (Games-Howell post hoc test, ***$p < 0.001$). Data are shown as boxplots with the median; the edges indicate 25th and 75th percentiles. Whiskers represent × 1.5 the interquartile range, and circles indicate outliers.

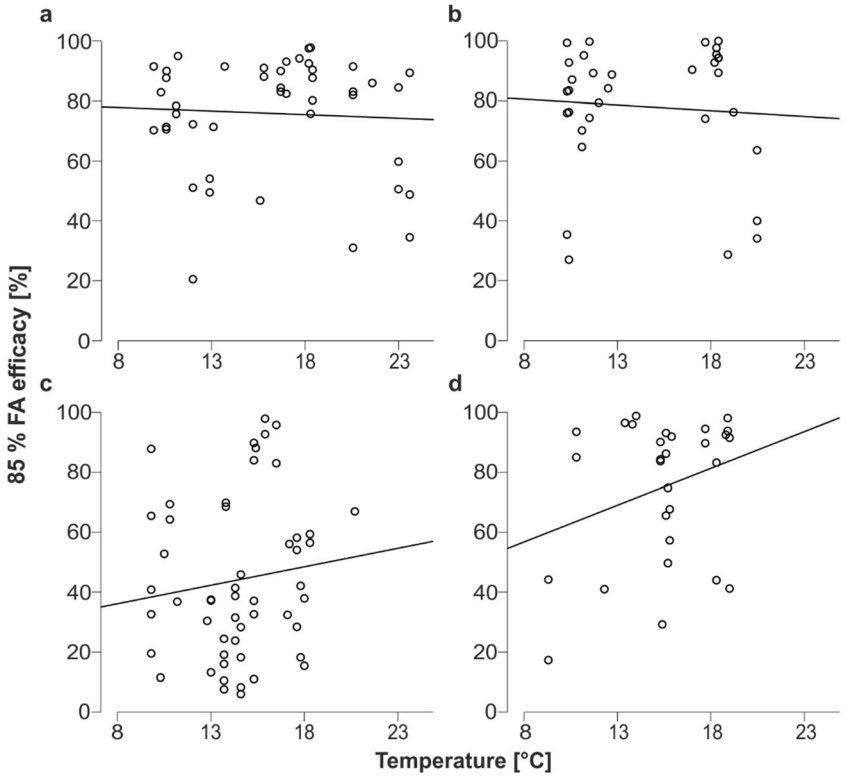

Figure 5. Treatment efficacy (%) across four different treatment combinations with 85% FA in relation to average ambient temperature. Regression lines are given in the graph. **a** Liebig Dispenser, single brood chamber (r = -0.055, p = 0.361, n = 44, R^2 = 0.003). **b** Nassenheider, single brood chamber (r = -0.069, p = 0.351, n = 33, R^2 = 0.005). **c** Liebig Dispenser, double brood chamber (r = 0.125, p = 0.189, n = 52, R^2 = 0.016). **d** Nassenheider, double brood chamber (r = 0.292, p = 0.062, n = 29, R^2 = 0.085). Correlation was done with single tail Pearson correlation.

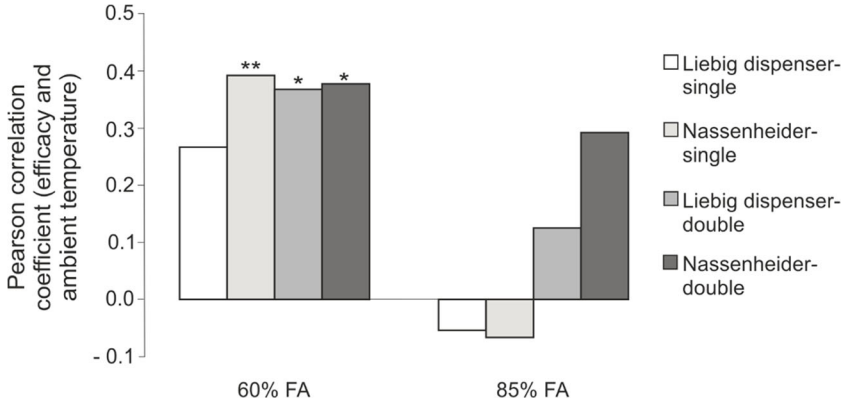

Figure 6. Bar chart showing Pearson correlation coefficients of efficacy and ambient temperature for 60% FA (left bars) and 85% FA (right bars). Asterisks indicate significances (*p < 0.05; **p < 0.01).

Probably the larger wick and therefore larger evaporation area of the Nassenheider has a positive effect on the evaporation.

A differentiation between the concentrations results in higher efficacy for 85% FA compared to 60% FA, which supports previous findings (Marinelli et al. 2007 cited in Pietropaoli and Formato 2018). Furthermore, we can show that treatments with 60% FA are highly ambient temperature dependent, whereas this is not the case for 85% FA treatments. The lesser influence of temperature on the efficacy of 85% FA treatment is clearly evident, as we did not observe significant correlation with regard to the outside temperature. Cooler temperatures could lead to difficulties in reaching the required dose with the 60% FA because compared to 85% FA, a higher amount must evaporate until air saturation occurs and the necessary drug dose is reached. Therefore, especially at lower temperatures (8–13 °C), 85% FA treatments produce more reliable results than 60% FA treatments. Overall, treatments with 60% FA showed a high degree of variability and often, especially in double brood chamber hives, too low efficacies. In contrast, 85% FA achieved higher efficacy and showed lower variability, and ambient temperature had a significantly lower influence.

It should be pointed out that our data on efficacy also show that there is an enormous residual variability that cannot be explained by the factors addressed in this study. This indicates strongly that there are additional variables affecting treatment success and that the process of evaporation of FA within the hive is not yet fully understood. Direct and continuous recordings of formic acid concentrations in the hive air are therefore necessary and currently underway.

Until now, the use of 85% FA has not been permitted in Germany to treat varroosis. However, this study has shown that in order to achieve sufficient treatment success even at low temperatures (8–13 °C), reconsidering a registration of 85% FA could be clearly increased efficacy of the crucial *Varroa* treatment. Until then, we highly recommend an integrated pest management that compromises the combination of different chemical and non-chemical treatment methods and the regular examination of the actual mite load to avoid too high population numbers. The late summer treatment in Central Europe should be conducted after the last honey harvest and in accordance with the weather forecast to avoid too low temperatures during FA application. Based on our data, we recommend that the expected average temperature of the forthcoming treatment days should not fall below ~ 16 °C in single brood chamber colonies. For double brood chamber colonies, average temperature should be 19 °C, and the Nassenheider dispenser is preferable to the Liebig dispenser.

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Code availability Not applicable.

AUTHORS' CONTRIBUTIONS

Steube X, Beinert P and Kirchner WH conceived this research and designed experiments. Data collection and analysis were performed by Steube X and Beinert P. Steube X, Beinert P and Kirchner WH wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

OPEN ACCESS

Efficacité et dépendance à la température d'un traitement à l'acide formique à 60 % et 85 % contre *Varroa destructor*.

***Apis mellifera* / *Varroa destructor* / Acide formique / Acarien / Efficacité du traitement.**

Wirksamkeit und Temperaturabhängigkeit von Behandlungen mit 60 %- und 85 %-iger Ameisensäure gegen *Varroa destructor*.

***Apis mellifera* / *Varroa destructor* / Ameisensäure / Milbe / Behandlungswirksamkeit.**

REFERENCES

Anderson DL, Trueman JWH (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol 24:165-189

Boecking O, Genersch E (2008) Varroosis - the ongoing crisis in bee keeping. J Verbrauch Lebensm 3:221-228

Bolli HK, Bogdanov S, Imdorf A, Fluri P (1993) Zur Wirkungsweise von Ameisensäure bei *Varroa jacobsoni* Oud und der Honigbiene (*Apis mellifera* L). Apidologie 24:51-57

Bowen-Walker PL, Martin SJ, Gunn A (1999) The transmission of Deformed Wings Virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. J Invertebr Pathol 73:101-106

Bowen-Walker PL, Gunn A (2001) The effect of the ectoparasitic mite, *Varroa destructor* on adult worker honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels. Entomol Exp Appl 101:207-217

Calderone NW (2012) Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992–2009. PLoS One 7:10

Crane E (1990) Bees and beekeeping: Science, practice and world resources, 1. publ. Heinemann Newnes, Oxford

De Guzman L, Rinderer TE, Stelzer JA (1997) DNA evidence of the origin of *Varroa jacobsoni* Oudemans in Americas. Biochem Genet 35:327-335

De Jong D, De Jong PH, Gonçalves LS (1982) Weight loss and other damage to developing worker Honeybees from infestation with *Varroa Jacobsoni*. J Apic Res 21:165-216

Delfinado MD (1963) Mites of the honeybee in South-East Asia. J Apic Res 2:113-114

Di Prisco G, Pennacchio F, Caprio E, Boncristiani HF, Evans JD, Chen Y (2011) *Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. J Gen Virol 92:151-155

Dietemann V, Nazzi F, Martin SJ, Anderson D, Locke B et al (2013) Standard methods for *Varroa* research. In V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS BEEBOOK, Volume II: standard methods for Apis mellifera pest and pathogen research. J Apic Res 52(1): doi:https://doi.org/10.3896/IBRA.1.52.1.09

Francis RM, Nielsen SL, Kryger P (2013) *Varroa*-virus interaction in collapsing honey bee colonies. PLoS One 8:10

Fries I (1991) Treatment of sealed honey bee brood with formic acid for control of *Varroa jacobsoni*. Am Bee J: 313-314

Fries I, Camazine S (2001) Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie 32:199-214

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

Fries I, Imdorf A, Rosenkranz P (2005) Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a nordic climate. Apidologie 37:564-570

Giovenazzo P, Dubreuil P (2011) Evaluation of spring organic treatments against *Varroa destructor* (Acari: Varroidae) in honey bee *Apis mellifera* (Hymenoptera:

Apidae) colonies in eastern Canada. Exp Appl Acarol 55(1):65

Girişgin AO, Aydın L (2010) Efficacies of formic, oxalic and lactic acids against *Varroa destructor* in naturally infested Honeybee (*Apis mellifera* L.) colonies in Turkey. Journal of the Faculty of Veterinary Medicine, University of Kafkas, Kars (Turkey): 941-945

Gliński Z, Jarosz J (1992) *Varroa jacobsoni* as a carrier of bacterial infections to a recipient bee host. Apidologie 23:25-31

Honey Bee Health Coalition (2018) Tools for *Varroa* management a guide to effective *Varroa* sampling & control. https://honeybeehealthcoalition.org/wpcontent/uploads/2018/06/HBHC Guide_Varroa_Interactive_7thEdition_June2018.pdf. Accessed 31 May 2020

Imdorf A, Buelmann G, Gerig L, Kilchenmann V, Wille H (1987) Examination of the method for estimating the brood area and number of worker bees in free-flying bee colonies. Apidologie 18:137-146. https://doi.org/10.5281/zenodo.3341580

Jones JC, Helliwell P, Beekman M, Maleszka R, Oldroyd BP (2005) The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. J Comp Physiol 191:1121-1129

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc Biol Sci 274:303-313

Kleinhenz M, Bujok B, Fuchs S, Tautz J (2003) Hot bees in empty broodnest cells: heating from within. J Exp Biol 206:4217-4231

Kronenberg F, Heller HC (1982) Colonial thermoregulation in honey bees (*Apis mellifera*). J Comp Physiol B 148:65-76

Lindberg CM, Melathopoulos AP, Winston ML (2000) Laboratory evaluation of miticides to control *Varroa jacobsoni* (Acari: Varroidae), a honey bee (Hymenoptera: Apidae) parasite. J Econ Entomol 93:189-198

Marinelli E, De Santis L, De Pace FM, Dell'Aira E, Saccares S et al (2007) Impiego del timolo e dell'acido formico per il controllo della varroatosi nel Lazio [Use of thymol and formic acid to control varroatosis in Latium region]. Apitalia 1:1-4

Moosbeckhofer R, Pechhacker H, Kohlich A (1997) 38. Test verschiedener Methoden einer Ameisensäure-Langzeitbehandlung zur Varroabekämpfung. Apidologie 28:195-196

Oldroyd BP (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. Trends Ecol Evol 14:312-315

Oudemans AC (1904) On a new genus and species of parasitic acari. Notes from the Leyden Museum 24:216-222

Pietropaoli M, Formato G (2018) Liquid formic acid 60% to control *Varroa* mites (*Varroa destructor*) in honey bee colonies (*Apis mellifera*): protocol evaluation. J Apic Res 57:300-307

Rader R, Howlett BG, Cunningham SA, Westcott DA, Newstrom-Lloyd LE, Walker MK, Teulon DAJ, Edwards W (2009) Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. J App Ecol 46:1080-1087

Ramsey SD, Ochoa R, Bauchan G, Gulbronson C, Mowery JD, Cohen A, Lim D, Joklik J, Cicero JM, Ellis JD, Hawthorne D, VanEngelsdorp D (2019) *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. PNAS 116:1792-1801

Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biologiy ad control of *Varroa destructor*. J Invertebr Pathol 103:96-119

Roth MA, Wilson JM, Tignor KR, Gross AD (2020) Biology and management of *Varroa destructor* (Mesostigmata: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies. J Integr Pest Manag 11:1-8

Sammataro D, Gerson U, Needham G (2000) Parasitic mites of honey bees: life history, implications, and impact. Annu Rev Entomol 45:519-548

Satta A, Floris I, Eguaras M, Cabras P, Garau VL, Melis M (2005) Formic Acidbased treatments for control of *Varroa destructor* in a Mediterranean area. J Econ Entomol 98:267-273

Schneider P, Drescher W (1987) Einfluss der Parasitierung durch die Milbe *Varroa jacobsoni* Oud. auf das Schlupfgewicht, die Gewichtsentwicklung, die Entwicklung der Hypopharynxdrüsen und die Lebensdauer von *Apis mellifera* L. Apidologie 18:101-110

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

Southwick EE, Southwick L (1992) Estimating the economic value of honey bees (Hymenoptera: Apidae) as agricultural pollinators in the United States. J Econ Entomol 85:621-633

Underwood RM, Currie RW (2003) The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apismellifera* (Hymenoptera: Apidae). Exp Appl Acarol 29:303-313

VanEngelsdorp D, Underwood RM, Cox-Foster DL (2008) Short-term fumigation of honey bee (Hymenoptera: Apidae) colonies with formic and acetic acids for the control of *Varroa destructor* (Acari: Varroidae). J Econ Entomol 101(2):256-264

VanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, Pettis JS (2009) Colony collapse disorder: a descriptive study. PLoS One 4:e6481