Dynamics of Falling Varroa Mites in Honeybee (*Apis mellifera*) Colonies Following Oxalic Acid Treatments

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Abstract

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The aims of the study were to establish the dynamics of the fallen mites after treating honeybee colonies and to establish the effectiveness of oxalic acid for the control of varroa in colonies during the bee-keeping season in order to reduce varroa populations to tolerable levels. This study presents data from the periodic checking of the number of mites that had fallen onto the bottom of hives in the pre-treatment periods and after treatments. Oxalic acid (2.9%) and sucrose-in-water solution (31.9%) (w/w) (OA) application in honeybee colonies triggered a significant increase ($p < 0.01$) in mite mortality. Two days after the August 8, 16 and 23 OA treatments, the mite mortality was estimated at 68.62 ± 12.29%, 65.31 ± 10.61%, and 33.35 % ± 13.99%, respectively. The mite mortality between the second and fourth day was estimated at 18.69 ± 7.43%, 22.98 ± 7.69%, and 14.06 ± 6.75%, respectively. Between the 2nd and 9th days after OA applications was a highly significant ($p < 0.001$) reduction and between the 9th and 11th days after the August 23 application the reduction in mite mortality was significant ($p < 0.05$). High correlation (R = 0.9896) was found between the natural mite-mortality and the number of mites that fell during three August OA treatments. Efficacy of treatments conducted in colonies with capped broods averaged 23.82 ± 1.52%. We can conclude that in colonies with less than 1 natural mite-death per day following treatments should reduce their mite population by approximately 40 %. Our results could be used to establish a mite treatment programme in honeybee colonies and to evaluate in further research.

Varroa destructor, mite mortality, alternative control, biological control, bee parasites

Varroa destructor, a parasite of *Apis mellifera*, can cause a collapse of untreated colonies within a few years. It is imperative to control the mite in order to maintain the populations of honeybee colonies in most bee-keeping regions around the world. The use of acaricides should be minimised in bee-keeping to avoid both the build-up of residues and their by-products in honey and wax (Wallner 1999) and to reduce the potential of acaricide-resistance (Ruijter 1994). Currently, there is little pressure to resist selecting “natural acaricides” such as organic acids (Milani 1999), which do not accumulate in wax and their residue build-up in honey is limited and toxicologically insignificant (Imdorf et al. 1996).

Counting the mites that drop from a colony onto a bottom board is a reliable diagnostic method to evaluate the efficacy of an acaricide treatment (Ritter 1981; Fries et al. 1991; Gregorc and Jelenc 1996; Poklukar 1999). A relationship has been found between the number of mites in a hive’s debris and the mite population of a colony (Liebig et al. 1984). Experiments have been conducted to evaluate oxalic acid (OA) as a method for controlling the mites in colonies with and without brood (Imdorf et al. 1996; Brødsgaard et al. 1999; Nanetti and Stradi 1997; Nanetti 1999). During broodless periods, Radetzki (1994), Nanetti et al. (1995) and Imdorf et al. (1997) found it to be highly effective in killing the mites and they estimated the elimination level at 97.3 %, 98.3 % and 99.5 %, respectively. When a capped brood was present, Mutinelli et al. (1997) achieved 95 % efficacy after three

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treatments of 5 % OA and Brødsgaard et al. (1999) reported a 24 % efficacy of one spring treatment administered by trickling. Approximately 50 millilitres of OA solution was used to treat one normally developed colony. Three OA treatments had an efficacy of 39.2 % when a brood was present and 99.4 % when there was no brood (Gregorc and Planinc 2001).

This paper presents data from the periodic checking of the number of mites that had fallen onto the bottom of hives to determine the natural mite-fall. The aim was to establish the effectiveness of OA as a single substance for controlling varroa in honeybee colonies by using a 2.9 %OA/31.95 % sucrose-in-water solution (Gregorc and Planinc 2002) throughout 2001. We also aimed to establish the dynamics of the fallen mites after treating colonies with capped broods and to establish a strategy for administering oxalic acid during the bee-keeping season in order to reduce varroa populations to tolerable levels.

Materials and Methods

Twenty-two *Apis mellifera* honeybee colonies, populated in national standard AŽ "back load" hives (Zdešar 1998) with nine combs (41 × 26 cm) in each brood and honey compartment, were located at one site near Vipava, Slovenia, which has a mild Mediterranean climate. In the spring of 2001, 38 cm × 29.8 cm metal sheets were placed on the floor of each of the hives in order to record the hives' natural mite mortality. Wire screens above the sheets prevented the bees from coming into contact with the debris. Before the experiment, the colonies were equalised to occupy from 5 to 7 brood combs. On the sampling dates, the numbers of mites were recorded and the inserts emptied. The levels of natural mite "drop-down" in the colonies for periods before (April 21 to May 5 and June 23 to August 8) and after the OA treatments were recorded three, four and up to eleven times, respectively.

The first treatment of the twenty-two colonies took place on August 8, after harvesting the honey. The colonies received 50 millilitres of a 2.9% OA and 31.9% sucrose-in-water solution (w/w), using oxalic acid dihydrate (Riedel-de Haën), sucrose (sugar) and de-mineralised water (Gregorc and Planinc 2001). OA solution was trickled over the combs, *in situ*, and bees in the brood compartment using a syringe. The same treatment was repeated on August 16 and 23, October 9 and November 11, with a final treatment of the broodless colonies on December 29. All colonies received the same OA/sugar-concentration solution.

The outside temperatures during the experimental treatments were 30 °C on August 8, 34 °C on August 16, 32 °C on August 23, 25 °C on October 9, 13 °C on November 11 and 5 °C on December 29.

The percentages of mites killed by the experimental treatments (FTB) were estimated using the formula: FTB = FOA1 / (FOA1 + FOA2) X 100 (Gregorc and Planinc 2001). FOA1 is the total number of mites that dropped during the treatments of colonies with capped broods and FOA2 is the number of mites that fell during the December 29 treatment of the broodless colonies.

The efficacy of the treatments was also estimated by comparing the numbers of mites that fell before and after the treatments and the mite mortality between the consecutive OA treatments. The data analyses were performed by ANOVA (analysis of variance) with the aid of the Statgraphic (1991) programme.

Results

During the pre-treatment period of 55 days from April 21 to August 8, 2001, the average natural mite-death per day was estimated at 1.25 (± 1.37) and the mortality was estimated at 68.72 (± 75.20) mites. In this period 3.60 % (± 2.04%) of the total varroa mite population died naturally. The first OA treatment resulted in a significant increase ($p < 0.01$) in mite mortality. While the numbers of dead mites recorded between the consecutive brood-period treatments were not statistically different, the December 29 treatment of the broodless colonies resulted in a reduction in the mite mortality ($p < 0.001$) in comparison with the previous treatments (Fig. 1).

Two days after the August 8, 16 and 23 OA treatments, the mite mortality was estimated at 68.62 ± 12.29%, 65.31 ± 10.61%, and 33.35 ± 13.99 %, respectively. The mite mortality between the second and fourth day after each of these treatments was estimated at 18.69 ± 7.43%, 22.98 ± 7.69 %, and 14.06 ± 6.75 %), respectively. Between the 2nd and 9th days after each OA application, a highly significant ($p < 0.001$) reduction in mite mortality was observed and between the 9th and 11th days after the August 23 application the reduction in mite mortality was also significant ($p < 0.05$). After September 3, the mite mortality in colonies with capped broods was constant at 6.25 ± 1.97% during the five weeks leading up to October 9 (Fig. 2).

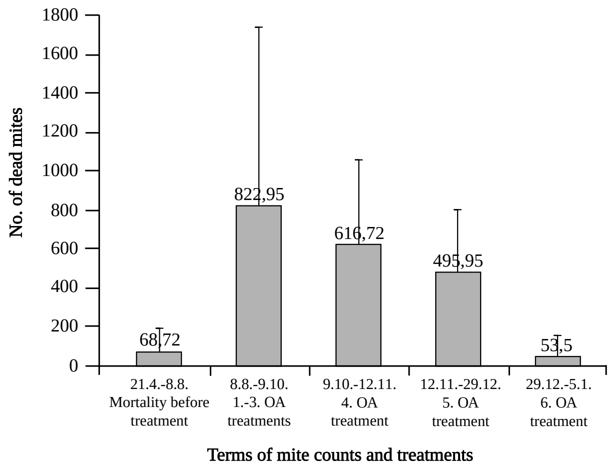

Fig. 1. Mite mortality in the pre-treatment periods and following the oxalic acid applications. Between August 8 and October 8, three OA applications were performed. The sixth OA treatment was conducted on broodless colonies. Bars indicate standard deviations.

The average mite mortality from the three OA applications on August 8, 16 and 23 was 822.95 ± 783.72. After these three treatments, the colonies were grouped in accordance with their fallen-mite count. The colonies of group A (Nos.: 2, 5, 8, 10, 14, 15, 18, 20, 21, 24), which each had up to 500 dropped mites, averaged 303.8 ± 123.17 mites. In the pre-treatment period, the natural mite-mortality per day for these colonies was estimated at 0.65 ± 0.35. The colonies of group B (Nos.: 1, 3, 4, 7, 13, 16, 19) with mite mortalities after OA treatments ranging from 500 to 1,000, averaged 748.14 ± 171.92, and had pre-treatment natural mite-mortality rates averaging 0.94 ± 0.31 mites per day. The colonies of group C (Nos.: 6, 9, 12, 17, 23), which had more than 1,000 dead mites after three OA treatments at an average of 1,966 ± 910.69 per colony, had 2.89 ± 2.23 dead mites per day in the pre-treatment period (Fig. 3). There was a high correlation (R = 0.9896) between the natural mite-mortality and the number of mites that fell during three August OA treatments (Fig. 4).

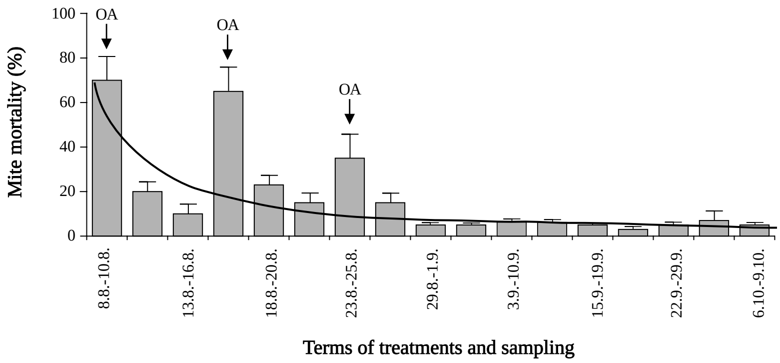

Fig. 2. Mite-mortality dynamics after three consecutive OA treatments of colonies with capped broods. The treatments were conducted on August 8, 16 and 23. The majority of mites fell within two days of each treatment. Bars indicate standard deviations.

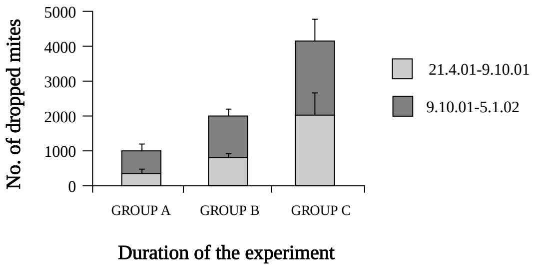

Fig. 3. The average mite mortality of the colonies grouped after three oxalic acid treatments performed in August 2001. The number of mites that fell after the three OA treatments in August correlated with the mite mortality after the autumn OA treatments. Bars indicate standard deviations.

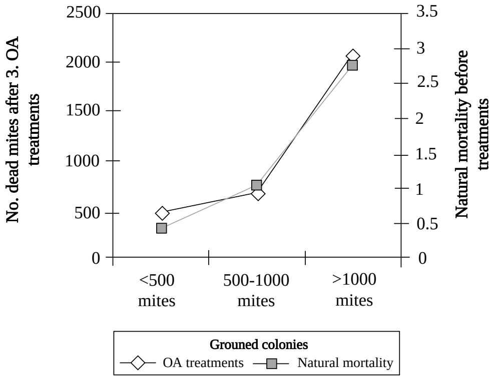

Fig. 4. The correlation between the number of dead mites after three OA treatments in August and the natural mite-mortality per day in the pre-treatment period. The colonies were grouped according to their fallen-mite total after these treatments.

Within two days of the October 9 autumn treatment there was a high level of mite mortality. However, over the next seven days there was a significant reduction ($p < 0.001$) in mite mortality, which preceded a further reduction ($p < 0.001$). From October 27 the mite mortality did not change significantly until the November 11 and December 29 OA treatments. The relative mite drops after each of the autumn OA treatments is shown in Fig. 5.

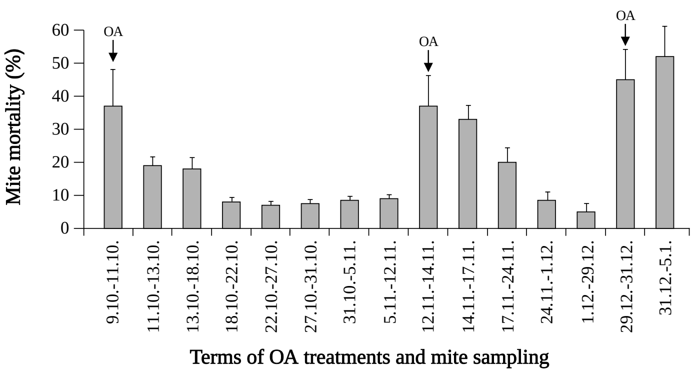

Fig. 5. The relative mite drop (%) after each OA treatment. The autumn OA treatments, conducted on October 9 and November 11, resulted in prolonged mite-drop which is indicative of a reduction in a colony’s capped brood. The mite drop after each OA treatment is calculated as 100%. Bars indicate standard deviations.

Each of the four treatments conducted in colonies with capped broods had an average efficacy of 23.82 ± 1.52%. The efficacy of the treatments increased during the experiment. The analyses of variance showed that the effectiveness of the October 9 OA treatment was significantly higher in comparison with the previous treatments ($p < 0.001$) and that the efficacy of December 29 treatment of the broodless colonies (89.21 ± 7.93%) was significantly higher ($p < 0.01$) than the previous OA treatments. The efficacy of the consecutive OA treatments is shown in Figure 6.

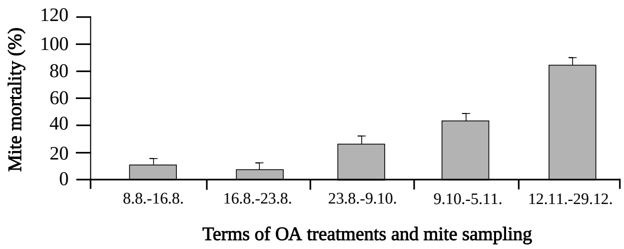

Fig. 6. The relative efficacy of OA after each of the five consecutive treatments expressed as a percentage of dead mites. The OA treatments in August were conducted on colonies with capped broods and the October and November treatments on colonies with significantly reduced capped broods. Bars indicate standard deviations.

Discussion

The natural mite-mortality per day, which until the August OA treatments was estimated at an average of 1.25, increased up to a maximum of 9.57 mites per day. Within two days of the OA treatments in the capped-brood period approximately 70% of the mites had died and the remaining 30% died within next eight days. When there were longer periods between treatments the numbers of mites that fell also dropped to lower levels. Repeated OA treatments have similar mite-drop dynamics and can be used effectively in colonies after the harvesting season. It seems that an OA application in a colony with a brood effects the mites on the adult bees but not those under the brood cappings. When compared to a summer OA treatment an autumn treatment of a colony with a reduced capped-brood results in a higher efficacy due to the prolonged mite-drop.

The consecutive treatments of the colonies initially had an efficacy of 12% and 23% (August) which rose to 51% after the November treatment. The increase in efficacy indicates that the treatments have a higher impact in late autumn when the colonies are preparing for winter and have less brood present. Reducing a colony's mite population by approximately 60%, with three OA treatments in August, after the honey extraction, is essential to ensure its normal development. Gregorc and Planinc (2001) and Brødsgaard et al. (1999) previously established similar levels of OA efficacy against the mites and Gregorc and Poklukar (2003) established a level of approximately 21%.

The number of mites killed in the colonies, which were grouped into three categories after the three August OA treatments, correlated highly with the number of mites that died naturally in the same colonies before the treatments. The natural mortality rates of 0.65, 0.94 and 2.89 mites per day observed during the pre-treatment period correspond to 303, 748 and 1,966 dead mites post-treatment. The natural mite-mortality, which is indicative of the mite population (Liebig et al. 1984), could be effectively reduced during OA treatments. The high correlation between the natural mite-mortality and the mite mortality induced after the three OA applications during August, indicates that the continual monitoring of mites dropping in bee colonies is important. Summertime treatments against the mites should be conducted in colonies when the natural mite-mortality and the threshold level of their mite infestation have been determined. In summertime it seems that colonies with less than 1 natural mite-death per day should have three OA treatments, which will reduce their mite population by approximately 40%. Mutinelli et al. (1997) reduced the mite population in colonies with capped broods by 95% with three OA treatments and after trickling OA, when a brood was present, Brødsgaard *et al.* (1999) recorded a lower efficacy of 24%. Reducing the mite population ensures a colony's normal development and wintertime survival. Further experiments must be conducted to establish how a colony develops when more than one mite per day is found. Treating colonies without capped brood using OA is highly effective (Nanetti et al. 1995; Gregorc and Planinc 2001).

Dynamika spadu roztočů Varroa (Jacobsoni) ve včelstvech medonosné (*Apis mellifera*) po ošetření kyselinou šťavelovou

Cílem studie bylo zjistit dynamiku množství roztočů spadených na dno úlu po ošetření včelstev včely medonosné a zjistit účinnost kyseliny šťavelové při regulaci výskytu varroázy během včelařské sezóny, za účelem redukce populace roztočů na přijatelnou mez. Prezentovány jsou data z pravidelného počítání roztočů spadlých na dno úlu v době před ošetřením a po něm. Aplikace kyseliny šťavelové (2,9 %) a vodného roztoku sacharózy (31,9 %) (w/w) (OA) ve včelstvech způsobily signifikantně vyšší mortalitu roztočů ($p < 0,01$). Dva dny po OA ošetření provedeném 8.,16. a 23. 8., byla mortalita roztočů odhadována na 68,62 % (± 12,29 %), 65,31 % (± 10,61 %) a 33,35 % (± 13,99 %). Mortalita mezi 2. a 4. dnem byla odhadována na 18,69 % (± 7,43 %), 22,98 % (± 7,69 %) a 14,06 % (± 6,75 %). Mezi 2. a 9. dnem po aplikacích byla redukce vysoce signifikantní ($p < 0,001$) a mezi 9. a 11. dnem po aplikaci ze dne 23. 8. byla redukce roztočů signifikantní ($p < 0,05$). Vysoká korelace (R = 0,9896) byla nalezena mezi přirozenou mortalitou roztočů a počty roztočů spadlých během tří srpnových ošetření OA. Účinnost ošetření provedeného ve včelstvech se zavíčkovanými larvami byla v průměru 23,82 % (± 1,52 %). Výsledky pokusu ukázaly, že ve včelstvech s méně než 1 mrtvým roztočem za den může následující ošetření snížit populaci roztočů přibližně o 40 %. Výsledky lze využít k vytvoření programu léčby varroázy ve včelstvech a při vyhodnocování budoucích výzkumů.

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