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Original article

Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials

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Abstract – Direct and air-assisted dusting of fine confectioner sugar (25–40 μm mean particle size) with and without pre-anesthesia of honey bees by CO_2 were studied as a physical control method of *Varroa destructor* under laboratory conditions on samples of 78 bees (range 49–107). CO_2 anesthesia alone had no effect, while sugar dusting resulted in significant mite knock down. CO_2 anesthesia did not affect the effectiveness of sugar dusting, and mean mite fall over 2 days resulting from direct dusting with 5 g sugar and from air-assisted dusting with 0.5 g sugar per sample was 91% and 62%, respectively, and this difference was significant ($P = 0.001$). Ninety-nine percent of the mites in the sugar treatment fell within 18 h of treatment. As a possible side-effect of the dusting, the presence of sugar particles in the T2 spiracles and their tracheal ducts from treated honey bees was investigated under scanning electron microscope. No sugar particles were found in them.

***Varroa destructor* / varroosis control / dusting / CO_2 / tracheal ducts**

1. INTRODUCTION

On a worldwide basis, hundreds of materials have been tested to control the parasitic honey bee mite (Shimanuki et al., 1992), *Varroa destructor*, formerly referred to as *Varroa jacobsoni* Oudemans, (Anderson, 2000; Anderson and Trueman, 2000). In view of the sucking structure of the ambulacrum of these mites (Ritter, 1981; Liu and Peng, 1990), dusts of various materials such as finely ground glucose, pollen or wheat flour sprinkled onto the bees on the combs have been tried as controls for the mite (Shah and Shah, 1988; Ramirez 1989; Loglio and Pinessi, 1991; 1992; 1993;

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Loglio, 1996). Dust particles adhere to the ambulacrum of *V. destructor* and prevent the mites from adhering to the bees or other surfaces (Ramirez, 1989). The mites then fall down to the bottom of the hive where they starve to death, as they are unable to move on the dusty surface (Ramirez and Malavasi, 1991; Ramirez, 1994). Encouraging results were obtained in these trials, especially in the absence of brood, but the particle sizes of the dusts used were not reported. Fakhimzadeh (2000) tested the effect on *V. destructor* of dusting with an air sprayer 15 and 20 g of confectioner sugar with particle sizes mostly below 40 μm onto one and two-story colonies, respectively, and he found significant mite fall after the treatments. Similarly, by dusting adult bees (ca. 350 individuals) with confectioner sugar in a wide mouth jar, Macedo and Ellis (2000) found that a high number of mites were dislodged from the bees.

The impact of such dusting on honey bees is not well known. The breathing apertures of the honey bee consist of ten pairs of spiracles in both larva and adult, from T2 to A8 (Dade, 1977; Erickson et al., 1986; Snodgrass and Erickson, 1992). All spiracles have muscle-operated valves, except for the T3 spiracles which are minute and lie between the upper ends of the mesepimeron and the metapleuron. Both active and inactive honey bees inhale through the T2 spiracles by the pumping movements of the abdomen (Bailey, 1954; Snodgrass, 1956). The valve of T2 spiracles can not be completely closed, hence the tracheal mite *Acarapis woodi* Rennie is able to enter this spiracle, but none of the others (Dade, 1977). Similarly, confectioner sugar particles used for the control of *V. destructor* could hypothetically penetrate through T2 spiracle and interfere with the breathing of the bees.

Two laboratory trials were conducted to quantify the effectiveness of confectioner sugar dusting to knock down *V. destructor* and to determine whether the sugar particles could penetrate into the respiratory system of honey bees through T2 spiracles. Another objective was to study the impact of CO_2 on *V. destructor*, as it had not been investigated previously. CO_2 anaesthesia was used with the assumption that it might drive the mites out from between the segments on the body of the bees and also in order to anaesthetise the bees so that they would not inhale excess confectioner sugar during the dusting treatments.

2. MATERIALS AND METHODS

The studies were conducted in the summer 1997 and 1998 at the Viikki experimental apiary (60° 13' N, 25° 02' E) of the University of Helsinki. Bees were brushed from brood combs directly into glass containers of ca. 430 cm^3, covered by a net on the top. A piece of bee candy was provided for food, and a strip of wax foundation was also provided inside the jar to facilitate bee movements.

The confectioner sugar used in all experiments, including preliminary ones since 1990 (Fakhimzadeh, 2000), consisted of finely ground pure white sucrose without starch or other additive with a nominal mean particle size of 25–40 μm according to the manufacturer (Finnsugar Ltd.; Helsinki, Finland). This size distribution was confirmed by examining the sugar particles with scanning electron microscopy (SEM; Fig. 1).

2.1. Experiment 1

Samples of 49 to 107 honey bees (mean 78) were taken from 6 colonies. The following 7 treatments were used with a completely randomised design and 5 replicates each:

A: Direct dusting of 5 g of confectioner sugar poured directly onto the sample of bees through the net.

B: Dusting of 0.5 g of confectioner sugar onto the sample of bees through the net using the airflow of a simple blower as a

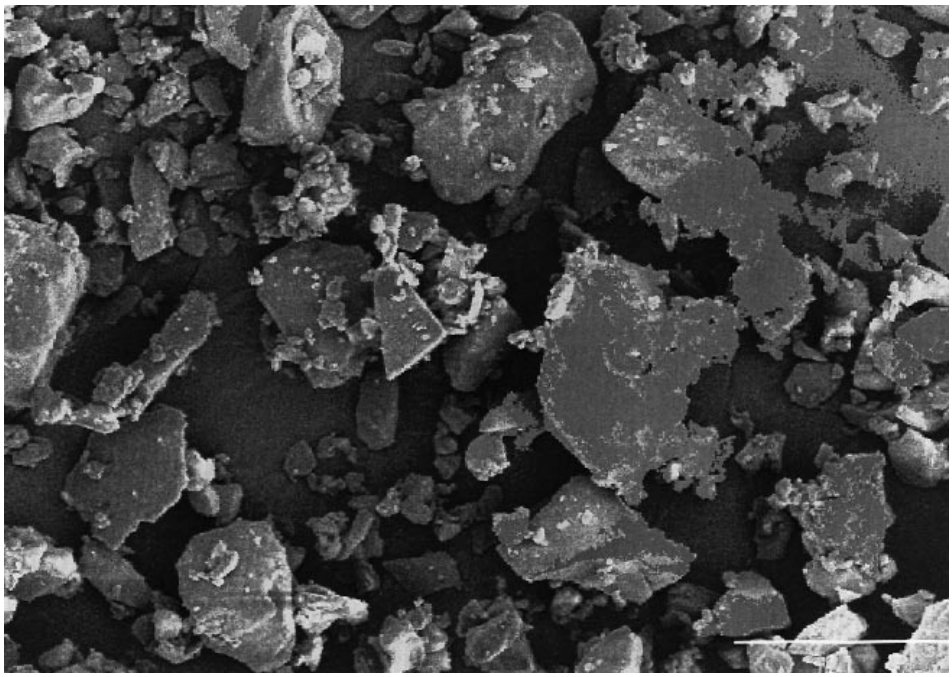

Figure 1. Scanning electron micrograph of the finely ground confectioner sugar used in the study showing most particles less than 40 μm in diameter (bar = 30 μm).

carrier. This air blower consisted on a 600 cm^3 vacuum jar, in which the sugar was placed, connected to a foot air pump (Fakhimzadeh, 2000).

CA: Sample of bees anaesthetised by CO_2 and immediately treated with direct dusting as in A.

CB: Sample of bees anaesthetised by CO_2 and immediately treated as in B.

C: Sample of bees only anaesthetised by CO_2.

D1: Sample of bees shaken and rolled in the jar for a few seconds (shaken control).

D2: Samples of bees kept alongside the others without any treatment (untreated control).

Except for the untreated control, all samples were shaken immediately after treatment and rolled gently for a few seconds so that the sugar particles covered them evenly. The anesthesia was administered so as to knock down all the bees in a sample. All the jars were then placed in darkness and upside down on top of larger, wide-mouth jars, and they remained in this position during the assay to catch any fallen mites and/or sugar particles.

Dead bees and fallen mites were counted daily for the next 2 days. The counting of dead bees was improved by moving each glass jar several times from side to side. On the third day, the rest of the bees were killed and shaken mechanically for 30 min in 70% ethanol to remove the remaining mites as this method removes 100% of the mites in a sample (De Jong et al., 1982; Shimanuki and Knox, 1991). For each replicate, the effectiveness of the treatment was calculated by dividing the number mites knocked down over 2 days by the total number of mites in the sample. Total number of mites

and bees in each sample determined the mite infestation level which was found to range from 1 to 24 mites per 100 honey bees (mean 10 mites/100 bees).

The data consisted of two-way and multi-way tables with the numbers of mites knocked-down or remaining on the bees and the number of bees dead or alive for each test and each treatment after 18 and 42 h. I analysed these tables with G tests whenever there were 5 or more observations per cell (Sokal and Rohlf, 1995). Whenever there was a cell with a smaller frequency, the significant probability (*P*) was calculated using 10 000 Monte-Carlo simulation of the loglikelihood ratio (Engels, 1988). Overall, a total of 252 mites and 2,736 bees were used in the analysis.

2.2. Experiment 2

Samples were prepared as in Experiment 1, but from 3 other colonies with a lower infestation level, to assess the impact of confectioner sugar dusting on healthy and/or relatively mildly infested bees. The mite numbers in these samples were very low, with a maximum of 2.5 mites per 100 bees (2 mites in a sample which occurred in only 4 samples). The experimental design was again completely randomised with four replicates for each treatment. The treatments in this experiment were A, B, CA and D2, as described in Experiment 1, and we also used treatment C3A which was similar to treatment CA, but with 3 minutes of CO_2 anaesthesia. The respiration pattern of active and inactive bees changes due to the extent of CO_2 (Bailey, 1954; Snodgrass, 1956). This treatment was intended to show the impact of relatively high amount of CO_2 on the inhalation of sugar particles. The rolling of the samples and their follow-up after treatment were as in Experiment 1.

As T2 spiracles and their ducts are involved in the inhalation of both active and inactive bees, I investigated only these parts for possible effects of the sugar dusting. The first examination of the tracheal ducts was done on the day following treatment, ca. 18 h after treatment, and the second one was done on the following day. I used this schedule because after 2 days, the bees may have groomed away the external sugar particles so that the effect of sugar dusting may no longer be visible. Ten bees per treatment were taken daily at random, and examined for the presence of sugar particles (total of 200 ducts).

Bees were killed by dissecting and the tracheal ducts were removed with forceps and dissected lengthwise. In order not to wash the possible sugar particles from the tracheal ducts, specimens were air dried at room temperature for 45 min. They were coated with platinum in a sputter coater (Agar Scientific Ltd, Stamsted, UK), and observed with a Jeol JSM-820 SEM (Jeol Ltd., Tokyo, Japan) at an accelerating voltage of 10 kV. The specimens were scanned at 500× and 4 000× magnifications. After sugar dusting, the ambulacrum of female *V. destructor* were also examined under SEM to confirm the presence of sugar particles on them.

3. RESULTS

3.1. Experiment 1

The mite fall results were homogeneous among the 5 replicates for each treatment and they were therefore pooled to compare treatments. There were significant differences among the treatments ($G = 197.1$, $df = 6$, $P < 0.001$), and also among the four treatments with confectioner sugar dusting ($G = 16.63$, $df = 3$, $P < 0.001$; Tab. I). The controls with and without shaking and rolling of the bees and the CO_2 anaesthesia followed by shaking and rolling gave similar results with a low mite fall of 0% and 3% after 18 h and 42 h, respectively (Tab. I). The CO_2 anaesthesia did not affect significantly the mite fall that resulted from the sugar dusting (Mantel-Haenszel chi-square

Table I. Effectiveness of *V. destructor* mites knock down and bee mortality after 42 h following dusting of honey bee samples with 25–40 μm confectioner sugar with or without prior CO_2 anaesthesia (results from experiment 1 pooled over the 5 replicates).

Treatment	Number of mites		Number of bees	
	knocked down	that remained on the bees	dead	alive
A (direct dusting with 5 g sugar)†	32	4	28	349
B (air-assisted dusting with 0.5 g sugar)†	20	11	35	311
CA (CO_2 anaesthesia + A)†	32	2	2	382
CB (CO_2 anaesthesia + B)†	15	10	1	427
C (CO_2 anaesthesia)†	1	43	1	388
D1 (control with bees shaken and rolled)	0	50	5	427
D2 (control)	3	29	1	379

† All bee samples were shaken and rolled after dusting (A, B, CA, CB) or anesthesia (C).

for odds ratio homogeneity = 0.0178, $P = 0.893$) and so the values of the treatments with and without anaesthesia were pooled to compare the dusting treatments. The direct dusting of 5 g of confectioner sugar resulted in 91% mite fall compared to only 62% for the air-assisted dusting of 0.5 g and this difference was significant ($G = 15.89$, $P = 0.001$).

The confectioner sugar dusting acted fairly quickly as almost all the mites that fell were found 18 h after treatment: of 99 fallen mites in the sugar treatment, only 1 mite in A fell between 18 h and 42 h. No mites fell in C, D1 and D2 within 18 h after treatment, and between 18 h and 42 h only 1, 0 and 3 mites had fallen, respectively.

For the 7 treatments except A and B, bee mortality was homogeneous among the 5 replicates of each treatment with an average mortality of 0.5% (Tab. I). In treatments A and B, bee mortality varied among replicates ($P \leq 0.001$). In 3 replicates of treatment A and one replicate of B, mortality was similar to that recorded in the other treatments, but in the 6 remaining replicates, it was much higher and ranged from 8% to 26%.

3.2. Experiment 2

No sugar contamination was found inside the tracheal ducts following any of the treatments. No obstruction of the spiracles T2 with sugar particles was observed, so this was not the reason for the cleanliness of the examined middle parts of the trachea. Figure 2 shows the ambulacrum of a female mite covered by many particles, which are likely sugar particles resulting from the sugar dusting.

4. DISCUSSION

The mean effectiveness of mite knock down with confectioner sugar was 91% with direct dusting and 62% with air-assisted dusting (Tab. I). The former value is consistent with the 80% effectiveness reported by Macedo and Ellis (2000) and also with the results of previous studies on dusting with confectioner sugar (Fakhimzadeh 2000), and with fine glucose and wheat flour that gave good results for mite control in the absence of brood (Shah and Shah, 1988; Ramirez 1989; Loglio and Penessi 1992), and for the detection of live *V. destructor* mites (Loglio and Penessi 1993; Loglio,

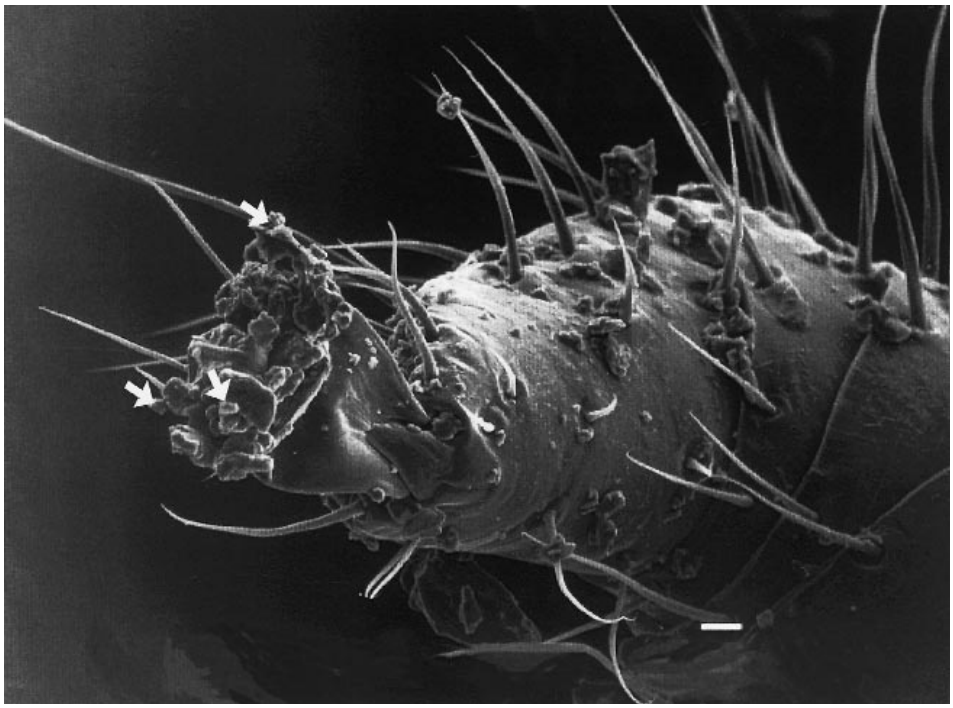

Figure 2. Scanning electron micrograph of the ambulacrum of a female *V. destructor* after confectioner sugar dusting of a sample of honey bees. The arrows indicate particles which are likely sugar ones (bar = 10 μm).

1996). The effectiveness of the direct dusting with 5 g confectioner sugar on mite knock down was also similar to that reported for mite kill in studies using chemical applications, since the effectiveness of Amitraz and fluvalinate on *V. destructor* mites in package bees was found to be 83% and 87%, respectively, following treatment as recommended by the manufacturers (Henderson, 1988).

The significant difference in the efficiency of mite fall with 5 g and 0.5 g of sugar was perhaps due to the fact that a small amount of confectioner sugar is quickly groomed away by bees. This may result in the reduction of contact between sugar and the mites, when they come out from between the bee segments.

The direct dusting of 5 g of confectioner sugar represented an average of 60 mg of sugar per bee, which is more than half the mass of a honey bee. Nevertheless, the fine particles of sugar (< 40 μm; Fakhimzadeh, 2000) did not enter to the trachea via breathing through T2 spiracles. This suggests that the presence of a dense hair cover surrounding these spiracles prevented their contamination with sugar particles. This may not be so surprising since bees are in contact with pollen grains throughout their adult life, especially when foraging in flowers with powdery pollen, and the pollen of most plant species lies in the 25–40 μm size range (Erdtman, 1952; Thorp, 1979). My results suggest that sugar particles did not penetrate and accumulate in the tracheal ducts regardless of the amount and method of

application, and with or without pre-anaesthesia of the bees. However, the cause of the high bee mortality recorded in some replicates of treatments A and B in experiment 1 remains unknown as this mortality did not occur in any of the replicates of treatments CA or CB in which the bees were dusted with similar quantities of confectioner sugar as used in A and B, respectively.

The dusting method may be applicable to package bees for the control of mites (without anaesthesia, as it had no impact on mite fall). As the shaking and rolling of a whole colony is not practical, further laboratory investigation without shaking and rolling the bees are needed to better simulate a method applicable to whole colonies to assess its effectiveness. The side-effects of confectioner sugar dusting on queens and brood also needs to be measured before being able to include this dusting method in integrated pest management strategies to control *V. destructor* mites.

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Résumé – Efficacité du saupoudrage de sucre de pâtissier pour faire tomber *Varroa destructor* des abeilles domestiques adultes lors d'essais au laboratoire. Parce que l'acarien *Varroa destructor* Anderson and Trueman (anciennement *V. jacobsoni* Oudemans) possède un ambulacre avec une structure absorbante, diverses poudres ont été testées comme moyen de lutte. De la fine poudre de glucose, du pollen broyé ou de la farine de blé ont été saupoudrées sur les abeilles sur les rayons pour faire tomber les acariens des abeilles adultes et des résultats intéressants ont été obtenus principalement en l'absence de couvain. Le but principal de l'étude était de déterminer de façon plus précise l'efficacité du saupoudrage de sucre de pâtissier en conditions de laboratoire pour lutter contre *V. destructor*. On a également étudié la possibilité d'un effet secondaire : la contamination des trachées de l'acarien par les particules de sucre.

Le sucre utilisé était constitué de pur saccharose blanc finement broyé dont les particules mesuraient en moyenne de 25–40 µm (Fig. 1). Le saupoudrage a été fait soit manuellement soit à l'aide d'un appareil soufflant de l'air sur des abeilles ayant ou non été auparavant anesthésiées au CO_2. Des échantillons de 49 à 107 abeilles (moyenne 78) ont été prélevés dans 6 colonies et cinq répétitions ont été faites pour chacun des sept traitements : (A) saupoudrage direct de 5 g de sucre de pâtissier, (B) saupoudrage de 0,5 g de sucre à l'aide d'un appareil souffleur simple (Fakhimzadeh, 2000), (CA) anesthésie des abeilles au CO_2 puis traitement A, (CB) anesthésie au CO_2 puis traitement B, (C) anesthésie au CO_2 seulement, (D1) témoin secoué comportant des échantillons doucement roulés et secoués comme dans tous les traitements précédents, (D2) traitement témoin dans lequel les abeilles ne sont ni secouées ni roulées. Au total 252 acariens ont été suivis et les résultats ont été analysés par des tests G.

Une autre expérience a été faite avec trois colonies ayant un faible taux d'infestation (2,5 acariens/100 abeilles) et quatre répétitions ont été faites pour chacun des cinq traitements suivants : A, B, CA et D2 comme ci-dessus, C3A était semblable à CA mais avec une plus longue exposition des abeilles au CO_2 (3 min). Les premiers stigmates thoraciques (T2) et les conduits des trachées correspondants ont été disséqués et la présence de particules de sucre a été examinée au microscope électronique à balayage.

Rouler ou secouer les abeilles avec ou sans anesthésie n'a pas provoqué une chute

significative des acariens. L'anesthésie au CO_2 n'a pas affecté significativement la chute des acariens résultant du saupoudrage de sucre (P = 0,893 ; Tab. I) ; en conséquence les traitements avec ou sans anesthésie ont été regroupés pour comparer les traitements de saupoudrage. Le saupoudrage direct de 5 g de sucre a provoqué la chute de 91 % des acariens contre 62 % pour le saupoudrage de 0,5 g de sucre avec le souffleur (différence significative P = 0,001). Le saupoudrage a agi rapidement puisque presque tous les acariens tombés ont été trouvés au bout de 18 h de traitement. La mortalité des abeilles a été uniforme avec une moyenne de 0,5 % dans toutes les répétitions de tous les traitements sauf pour A et B où elle a été parfois 0,5 % mais a atteint d'autres fois 8 à 26 %. La cause de la mortalité des abeilles reste inconnue puisqu'aucune particule de sucre n'a été retrouvée dans les conduits des trachées quel que fût le traitement (n = 200 trachées observées). Par contre l'ambulacre de *V. destructor* est apparu bien recouvert de particules après les saupoudrages (Fig. 2). L'efficacité du saupoudrage direct de sucre (91 %) est plus élevé dans cette étude que celle mentionnée pour les traitements à l'amitraz ou au fluvalinate des paquets d'abeilles. Il est pourtant nécessaire de vérifier l'impact du saupoudrage de sucre sur le couvain et la reine avant d'inclure cette méthode dans une stratégie de lutte intégrée pour contrôler *V. destructor* sur les abeilles adultes.

***Varroa destructor* / lutte physique / saupoudrage / anesthésie / trachée**

Zusammenfassung – Wie stark ist der Abfall von *Varroa destructor* von adulten Honigbienen nach Einstäubung mit Puderzucker im Laborversuch. Auf Grund der saugfähigen Struktur der Haftlappen (Ambulakrum) bei *Varroa destructor* (Anderson and Trueman, 2000) waren verschiedene Staubpartikel für eine Behandlung getestet worden, in denen feiner Traubenzucker, gemahlener Pollen oder Weizenmehl auf Bienen auf Waben gestäubt worden war, um die Milben von den Bienen zu entfernen. Die Versuche waren vielversprechend, besonders wenn keine Brut vorhanden war. In den hier dargestellten Laborversuchen sollte die Wirksamkeit von Puderzucker zur Milbenbekämpfung mit höherer Genauigkeit bestimmt werden. Auch der Nebeneffekt, die Verunreinigung der Tracheen der Bienen mit Zuckerpartikeln, wurde untersucht.

Ich benutzte Puderzucker, der nach Angaben des Herstellers aus fein gemahlener Saccharose mit Körnergrößen von 25–40 µm bestand (Abb. 1). Das Einstäuben erfolgte direkt oder wurde mit einem Haarföhn verstärkt, mit oder ohne vorherige Narkotisierung durch CO_2. Proben mit 49 bis 107 Bienen (M = 78) wurden aus 6 Völkern genommen und jeweils folgenden Behandlungen unterzogen:

(A) direktes Einstäuben mit 5 g Puderzukker, (B) Einstäubung mit 0,5 g Puderzucker mit Unterstützung durch einen Föhn (Fakhimzeh 2000), (CA) Methode A mit CO_2 – Narkose, (CB) Methode B mit CO_2 – Narkose, (C) nur eine CO_2 – Narkose, (D1) Kontrollprobe mit Bienen, die sanft gerollt und geschüttelt werden wie in vorherigen Behandlungen, (D2) Kontrolle ohne Schütteln und Rollen der Bienen.

Alle Versuche wurden 5 mal wiederholt. Insgesamt wurden 252 Milben verfolgt, und die Ergebnisse des Abfallens wurde mit G Tests analysiert.

In einem anderen Versuch (4 Wiederholungen) wurden Bienen von 3 gering befallenen Völkern (2,5 Milben/100 Bienen) 5 verschiedenen Behandlungen unterzogen: A, B, CA und D2 wie oben beschrieben, und C3A, die ähnlich wie Behandlung CA war, bei der aber die Narkose länger dauerte (3 Minuten). Die ersten Stigmen des Thorax (T2) mitsamt den Tracheenschläuchen wurden seziert und unter dem Raster Elektronenmikroskop auf Zuckerpartikel untersucht.

Weder Rollen noch Schütteln der Bienen per se, noch die anschließende CO_2 – Narkose führte zu einem signifikanten Milbenabfall. Auch beim Einstäuben mit Zukker hatte die Narkose keinen signifikanten Effekt (P = 0,893, Tab. I). Deshalb wurden diese Werte mit denen ohne Narkose zusammengefasst, um die Wirksamkeit der verschiedenen Einstaubmethoden zu vergleichen. Bei der direkten Einstäubung mit 5 g Puderzucker fielen 91 % der Milben ab, im Vergleich dazu nur 62 % nach der Einstäubung mit 0,5 g Puderzucker, der mit Hilfe des Föhns verteilt wurde. Dieser Unterschied war signifikant (P = 0,001). Die Einstäubung hatte eine schnelle Wirkung, bereits nach 18 Stunden wurden fast alle abgefallenen Milben gefunden.

Die Bienensterblichkeit lag gleichmäßig bei einem Mittelwert von 0,5 % in allen Wiederholungen aller Methoden mit Ausnahme von den Behandlungen A und B, bei denen in einigen Versuchen ein Totenfall von 8 % bis sogar 26 % vorkam. Die Ursache der hohen Mortalität blieb unbekannt, da nach keiner Behandlung eine Verunreinigung durch Zuckerpartikel in den Tracheen festgestellt werden konnte (200 Tracheen wurden untersucht). Die Haftlappen von *Varroa destructor* waren dagegen stark mit Zuckerpartikeln überzogen (Abb. 2).

Die Wirksamkeit der direkten Einstäubung mit Puderzucker war mit 91 % höher als die für die Behandlungen von Paketbienen gegen *Varroa* beschriebene Wirksamkeit von Fluvalinat oder Amitraz. Die Wirkung der Einstäubung mit Zucker auf Brut oder Königinnen muss noch genauer untersucht werden, bevor man diese Methode in ein integriertes Behandlungskonzept gegen *Varroa* auf adulten Bienen einbeziehen kann.

***Varroa destructor* / Varroosis Kontrolle / Einstäubung / CO_2 / Tracheen**

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