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Using inert dusts to detect and assess varroa infestations in honey bee colonies

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SUMMARY

Detection and assessment of varroa infestations in honey bee colonies are important for successful beekeeping. We examined the use of inert dusts to dislodge mites from adult honey bees that were isolated from their nest. Six dusts (powdered sugar, fine sugar, wheat flour, talcum powder, corn starch and baking soda) were evaluated for their ability to dislodge mites from adult bees collected in jars. We obtained the highest recovery rate with powdered sugar (92.9 ± 5.5%) and talcum powder (84.0 ± 5.6%). We also examined mite survival after recovery with inert dusts and compared it to mite survival after recovery from brood. After 24 h mite survival was significantly greater when mites were recovered with corn starch, powdered sugar, and from brood ($F = 22.88$, d.f. = 6,35, $P < 0.0001$). Finally, ether and powdered sugar were compared as tools for detecting and assessing the degree of infestation. Powdered sugar did not differ from ether in detecting or assessing low (1-5 mites per sample) infestation levels ($F = 2.81$, d.f. = 1,49, $P = 0.1$). At medium (6-30 mites per sample) and high (> 30 mites per sample) infestation levels, more mites were recovered with powdered sugar (medium: $F = 14.28$, d.f. = 1,29, $P = 0.008$; high: $F = 6.34$, d.f. = 1,17, $P = 0.023$).

Keywords: varroa, honey bees, mites, *Varroa destructor*, *Varroa jacobsoni, Apis mellifera*, dust, powdered sugar, detection, infestation

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INTRODUCTION

Early detection and accurate assessment of the degree of *Varroa destructor* infestation are necessary to prevent honey bee colony injury or loss. They are also important for reducing control costs by indicating when control measures are warranted and when treatments can be delayed (Bach *et al*., 1998). The recent detection of varroa populations that are resistant to Apistan (tau fluvalinate) also supports the need to minimize the frequency of chemical treatments by only applying acaricides when mite populations reach economic levels (Baxter *et al*., 1998, Elzen *et al*., 1999). Delaplane & Hood (1997) provided a research-based economic threshold for varroa in south-eastern USA. However, many beekeepers still rely on the calendar to determine when to apply treatments, and more research on economic thresholds is needed. For beekeepers to fully utilize economic thresholds, a safe, fast and efficient detection and assessment technique is required.

Commonly used detection and assessment methods for varroa include: (1), debris examination; (2), ether roll; (3), alcohol wash; (4), brood examination; and (5), acaricides with sticky boards (Shimanuki & Knox, 1987). Other methods that have been described include heating adult bees to 46°-47°C to dislodge mites (Crane, 1979) and examining combs for mite faeces (Erickson, 1996).

Ramirez (1987) and Shah & Shah (1988) reported that wheat flour was useful in controlling varroa. In 1991, Loglio & Pinessi reported dislodging mites when bees on the combs were repeatedly dusted with wheat flour. Ritter (1993) suggested that powdered sugar or mineral meal could be used to control varroa alone or as a vehicle to deliver acaricides. Ramirez & Malavasi (1991) speculated that these materials interfere with the ability of the mite's ambulacrum to cling to the bee's body. Dust treatments have not been widely adopted by beekeepers because they require removing combs individually for dusting. Recently, Fakhimzadeh (2000) described a device to blow dust between combs that increases dust distribution without removing combs. Macedo & Ellis (2000) reported that powdered sugar could be used as a varroa detection and assessment tool. Fakhimzadeh (2001) reported using powdered sugar to recover varroa from adult bees in laboratory trials.

We examined the use of dust-like materials to dislodge varroa from bees that were isolated from their nest. The first objective of this study was to evaluate six dusts for dislodging mites from bees in glass jars. Mite detection and assessment with the six dusts were evaluated. The second objective was to compare the survival of mites obtained by dusting adult bees with the survival of mites obtained from brood. The third objective was to compare powdered sugar and ether as tools for detecting varroa and for assessing the degree of infestation.

MATERIALS AND METHODS

Dust evaluation

Materials evaluated were powdered sugar (G & H Sugar Company), fine sugar (Best Yet), talcum powder (Marquee), wheat flour (Rainbow), baking soda (Best Yet), and corn starch (Hodgson Mill). We collected 28 samples of 318 ± 7 adult bees from a single varroa-infested colony into 473 ml (pint) wide-mouth glass canning jars. We covered the jars with a two-piece canning lid in which we had replaced the centre portion of the lid with 2 mm hardware cloth. We included untreated bees as controls, and we replicated each treatment four times. Four jars were randomly assigned for each treatment.

One rounded teaspoon (7 g) of dust was added to each of the four jars in each treatment group, except the control, which received no dust. We then rolled the jars to distribute the dust and let them sit for one minute. After one minute, the jars were inverted and shaken for one minute. We counted the number of mites recovered from each jar and washed the bees with 70% ethanol to recover the remaining mites (Shimanuki & Knox, 2000). The efficiency of the dusts was calculated by dividing the number of mites obtained using dust by the number of mites present (mites obtained using dusts plus mites obtained by alcohol wash). Treatments were arranged in a completely randomized design. Data were analysed by analysis of variance, and means were compared by the least significant difference test, $\alpha = 0.05$ (SAS Institute, 1999).

Mite survival

The survival of mites obtained using inert dusts was compared with the survival of mites obtained from brood cells. We collected mites from brood cells by uncapping the cells and removing mature pupae with forceps. Individual mites were then removed with a soft bristle brush and transferred to borosilicate glass scintillation vials. We placed 10 mites in each vial and six replicates were prepared for each treatment. Mites were obtained from adult bees using the six inert dusts and the procedure described for evaluating dusts. We removed the dust from mites using a moist brush and transferred 10 mites to borosilicate glass scintillation vials. We then placed the vials in a growth chamber at 26 ± 2°C in darkness. Mortality was assessed at 12-h intervals for 108 h. Treatments were arranged in a completely randomized design. Data were analysed by analysis of variance, and means were compared by the least significant difference test, $\alpha = 0.05$ (SAS Institute, 1999).

Powdered sugar and ether comparison

Two samples of approximately 300 adult bees were collected from each of 86 varroa-infested colonies and placed into 473 ml (pint) wide-mouth glass canning jars. The mean number of bees per jar was 324 ± 4. To recover mites with powdered sugar, we used the same

procedure described for evaluating dusts. We collected two samples from each of the 86 colonies in the study. We then added one rounded teaspoon (7 g) of powdered sugar (G & H Sugar Company) to one of the jars from each colony and rolled it to coat the bees with the powdered sugar. After one minute, the jars were inverted and shaken over a 33 x 43 cm card with gridlines at 2.54-cm intervals. We then counted the number of mites recovered with powdered sugar for each sample. The ether roll technique (engine starter fluid - Polar) was used to recover mites from the second jar and the number of mites recovered were counted (Shimanuki & Knox, 2000). After recovering mites using powdered sugar and ether, we placed the bees into containers of 70% ethanol. The alcohol wash technique was used to recover any remaining mites (Shimanuki & Knox, 2000). We calculated the efficiency of the two field-testing techniques by dividing the number of mites obtained with powdered sugar or ether, respectively, by the number of mites present (mites recovered with powdered sugar or ether plus mites recovered by alcohol wash).

The 86 duplicate samples were considered independent, as the probability of recovering mites with one method does not affect the probability of recovering mites with the other technique. From the 172 total samples taken, 98 of them contained mites and were separated into categories or blocks, according to the number of mites present in the sample. When one to five mites were present in a sample, it was considered a low infestation. A total of 50 samples fell into this category, 28 from ether roll and 22 from powdered sugar. When there were between six and 30 mites in a sample, it was considered a medium infestation, with a total of 30 samples in this category, 14 from ether and 16 from powdered sugar. With more than 30 mites per sample, it was considered a high infestation, and 18 samples fell into this category, nine from ether roll and nine from powdered sugar.

The experimental design was a randomized complete block design. Results were analysed by analysis of variance (least significant difference, $\alpha = 0.05$). In addition, we used contingency tables analysis to analyse the results of the lowest infestation level group (1-5 mites per sample) to determine if the two techniques differed in their ability to detect low infestations (SAS Institute, 1999).

RESULTS

Dust materials

The mean infestation level of the source colony was 0.144 ± 0.007 mites per bee. We obtained the highest recovery rate with powdered sugar (92.9 ± 5.5 %), followed by talcum powder (84.0 ± 5.6%). We did not recover any mites in the untreated control group. The treatments were significantly different ($F = 21.64$, d.f. = 6,27, $P = 0.0001$). Figure 1 shows the percentage of mites recovered with each of the dusts evaluated.

Mite survival

After 24 h, mite survival was not significantly different for mites recovered from bees with corn starch, powdered sugar and from brood, but it was significantly higher than for mites recovered with talcum powder, wheat flour, fine sugar and baking soda ($F = 22.88$, d.f. = 6,35, $P < 0.0001$). At 48 h, mites recovered from brood and with powdered sugar survived longer than

FIG. 1. Percent recovery of mites treated with different dust materials and untreated bees. Means with the same letter are not significantly different (LSD, $\alpha = 0.05$ d.f. = 6,27).

Table 1. Mean survival of mites recovered from adult bees using baking soda, corn starch, fine sugar, wheat flour, powdered sugar, talcum powder and from brood in four 24-h intervals. Different letters in the same column represent significant differences (LSD, $\alpha = 0.05$ d.f.=6,41).

Treatment	24 h	48 h	72 h	96 h
Corn starch	91.67 ± 3.07^{a}	43.33 ± 5.58^{b}	21.67 ± 4.77^{b}	6.67 ± 4.94^{b}
Powdered sugar	88.33 ± 1.67^{a}	73.33 ± 8.82^{a}	18.33 ± 7.03^{b}	1.67 ± 1.67^{b}
Brood	85.00 ± 4.28^{a}	81.67 ± 6.0^{a}	65.00 ± 9.92^{a}	53.33 ± 7.60^{a}
Wheat flour	63.33 ± 9.55^{b}	20.00 ± 9.3^{c}	6.67 ± 3.33^{bc}	3.33 ± 2.11^{b}
Talcum	48.33 ± 4.77^{bc}	3.33 ± 3.33^{c}	0^{c}	0^{b}
Fine sugar	33.33 ± 4.94^{c}	6.67 ± 3.33^{c}	0^{c}	0^{b}
Baking soda	33.33 ± 5.58^{c}	15.00 ± 4.28^{c}	6.67 ± 3.33^{bc}	6.67 ± 3.33^{b}

Table 2. Mean percentage of mites (± s.e.) recovered from colonies using powdered sugar and ether. The results are shown separately for low, medium and high levels of infestations. Different letters in the same row represent significant differences (LSD, $\alpha = 0.05$ d.f. = 2).

Infestation level	Percentage of mites recovered with ether	Percentage of mites recovered with powdered sugar
Low (1-5 mites/sample)	76.43 ± 6.68^{a}	90.98 ± 4.82^{a}
Medium (6-30 mites/sample)	68.26 ± 3.93^{a}	87.86 ± 3.42^{b}
High (> 30 mites/sample)	66.83 ± 3.99^{a}	82.16 ± 4.59^{b}

mites recovered by the other methods (F = 26.34, d.f. = 6,35, P <0.0001). At 72 h, mites collected from brood survived longer than mites recovered with all the dusts used (F = 18.88, d.f. = 6,35, P < 0.0001). At 96 h, only mites collected from brood were still alive, and all mites were dead at 108 h. Table 1 shows the survival of the mites in 24-h intervals for 96 h.

Powdered sugar vs. ether

There was no significant difference in the number of mites recovered from bees by the two methods in the low infestation group (F = 2.81, d.f. = 1,49, P = 0.1) (table 2). At medium and high infestation levels mite recovery was greater with powdered sugar (medium: F = 14.28, d.f. = 1,29, P = 0.008; high: F = 6.34, d.f. = 1,17, P = 0.0228).

Both methods were always able to detect mites in the medium and high infestation level groups. We conducted a contingency table analysis to determine if there was a significant difference in the ability of the methods to detect low-level mite infestations (1-5 mites per sample). Our results indicated that powdered sugar and ether roll are not significantly different in their ability to detect mites in low-level infestations (Chi-square test = 2.0673, d.f. = 1, P = 0.1505).

DISCUSSION

When we compared dusts for their ability to dislodge varroa from adult honey bees, powdered sugar and talcum powder were the most efficient, followed by corn starch. Besides the fact that it was the most efficient, powdered sugar does not pose a risk of honey contamination and, therefore, we recommend it as a monitoring tool.

Mites recovered with corn starch, powdered sugar or from brood can be used in bioassays when mortality is evaluated after 24 h. Therefore, it is less labourintensive to collect mites using these dusts than to individually open brood cells to harvest them. We recommend this technique to researchers who need a large number of mites for bioassays.

When we compared the survival of mites that were recovered from bees with dusts and from brood, we noticed that only mites taken from brood were alive after 96 h. These results suggest that inert dusts may interfere with some aspect of the mites' biology over time. Another possible explanation is that when one recovers mites by opening the brood cells, one recovers the mother as well as daughter mites when present. Therefore, the probability that one is getting a mite with a greater life expectancy (i.e. young daughter mites) is higher than when one recovers mites from adult bees.

In comparing the powdered sugar dust with the ether-roll method, we observed that both were equally able to detect mites when present. Moreover, powdered sugar proved to be more accurate when assessing mite infestation levels, especially under medium and high levels. Powdered sugar dust is a reliable tool that beekeepers can use to determine when treatment is necessary and when it can be delayed.

Powdered sugar dust sampling can be performed in the apiary. All materials needed are readily available and inexpensive. Unlike other sampling techniques, the sampled bees can be returned to their colonies unharmed. Wind may cause mites to be lost if beekeepers use the technique in open areas. To avoid loss of mites, beekeepers can shake jars over a white pan filled with water.

There are three possible explanations for the high efficacy of powdered sugar shaking for recovering varroa from adult bees. First, the mites use their ambulacrum to adhere to their host. When bees are dusted with powdered sugar it could make it difficult for the ambulacrum to adhere to the bees. Second, powdered sugar stimulates the bees' grooming behaviour. The Asian honey bee, *Apis cerana*, exhibits grooming behaviours that contribute to their ability to keep varroa infestation levels within tolerable limits. We observed that when European honey bees are dusted with powdered sugar, they begin to groom themselves and that they persist at grooming for an extended period of time. Finally, a third explanation is that powdered sugar on the mite's body stimulates it to release from its host to groom.

We conclude that sugar shaking is an inexpensive, efficient, and rapid technique for detecting and assessing varroa infestations. Furthermore, there is no risk of honey contamination, and the bees can be returned to their hive unharmed after the process.

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REFERENCES

BACH, J C; DANKA, R G; ELLIS, M D; MUSSEN E C; PETTIS, J S (1998) Protecting honey bees from *Varroa jacobsoni. American Association of Professional Apiculturists Technical Bulletin*; 8 pp.

BAXTER, J; EISCHEN, F; PETTIS, J; WILSON, W T; SHIMANUKI, H (1998) Detection of fluvalinate resistant Varroa mites in US honey bees. Proceedings of the American Bee Research Conference. *American Bee Journal* 138(4): 291.

CRANE, E (1979) The Varroa mite. *Bee World* 59:164-167.

DELAPLANE, K S; HOOD, W M (1997) Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the south-eastern USA. *Journal of Apicultural Research* 36(3-4): 125.

ELZEN, P J; EISCHEN, F A; BAXTER, J R; ELZEN, G W ; WILSON, W T (1999) Detection of resistance in US *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate. *Apidologie* 30: 13-17.

ERICKSON, E H (1996) Fecal accumulations deposited by Varroa can be used as a simple field diagnostic for infestations of this honey bee parasite. *American Bee Journal* 136(1): 63-64.

FAKHIMZADEH, K (2000) Potential of super-fine ground plain white sugar dusting as an ecological tool for the control of varroasis in the honey bee (*Apis mellifera*). *American Bee Journal* 140(6): 487-491.

FAKHIMZADEH, K (2001) Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. *Apidologie* 32(2): 138-148.

LOGLIO, G; PINESSI, E (1991) Impiego della farina di frumento per la lotta ecologica contro la Varroasi. *Apicoltore Moderno* 82: 185-192.

MACEDO, P A; ELLIS, M D (2000) Detecting and assessing Varroa mite infestations by using powdered sugar to dislodge mites. Proceedings of the American Bee Research Conference. *American Bee Journal* 140 (11): 906.

RAMIREZ, B W (1987) The latest on *Varroa jacobsoni. Newsletter for beekeepers in tropical and subtropical countries* 10: 11. RAMIREZ, B W; MALAVASI, G J (1991) Conformation of the ambulacrum of *Varroa jacobsoni* Oudemans (Mesostigmata: Varroidae): a grasping structure. *International Journal of Acarology* 17(3): 169-173.

RITTER, W (1993) Chemical Control: options and problems. *In* Matheson, A (ed) *Living with varroa*. International Bee Research Association; Cardiff, UK; pp.17-24.

SAS INSTITUTE (1999) *SAS/STAT user's guide, version 8.0*. SAS Institute; Cary, NC, USA.

SHAH, F A; SHAH, T A (1988) *Tropilaelaps clareae*, a serious pest of honey bees, flour dusting controls for Varroa disease. *American Bee Journal* 128(1): 27.

SHIMANUKI, H; KNOX, D A (1987) *Diagnosis of honey bee diseases*. Agriculture Handbook No. AH690. US Department of Agriculture; Beltsville, MD, USA; 53 pp.

SHIMANUKI, H; KNOX, D A (2000) *Diagnosis of honey bee diseases*. Agriculture Handbook No. AH690. US Department of Agriculture; Beltsville, MD, USA; 53 pp.