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Detection of major mite pests of *Apis mellifera* and development of non-chemical control of varroasis

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ACADEMIC DISSERTATION

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Cover picture: In *Apis mellifera*, severe damage by varroasis is characteristically manifested by malformed wings or legs (photo by Kamran Fakhimzadeh).

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In my hard battle at thy Department
My heart was in two compartments
Thy fine lady in my apartment
Present staff of SEL[1] Department

To the staff of Department of Applied Zoology (SEL)
&
My wife Päivi

[1] *Soveltavan Eläintieteen Laitos*

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ABSTRACT

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Varroasis and acarine honeybee diseases have been a significant factor in the decline of the bee industry around the globe. The presence of these diseases is a practical problem of apiculture, and their limitation pertain to enigmas of the detection and control of the causative mites. The chemical detection and chemotherapy of mites have engendered an inappropriate chemical residue in bee products. This has induced the search for non-chemical methods. The absence of *Acarapis woodi* in Fennoscandia and the probable ecological reasons for its absence has been a controversial subject matter for acarologists around the globe. For this thesis the following studies were carried out:

First the existence of *A. woodi* in Fennoscandia was investigated in two surveys performed in Finland, using the standard slicing technique with incubation in potassium hydroxide (8 % KOH). *A. woodi* was discovered for the first time in Fennoscandia, among Finnish bees in June 1991. The second survey demonstrated that *A. woodi* had been in Finland as early as in 1986.

Second, for the detection of *Varroa destructor* in adult bees, a field method and device (mite detector) was designed and developed. Adult bees were laved in a rotational chamber of the new centrifugal device, using detergent water (1 % and 0.03%) to dislodge the mites. Live or frozen bees were centrifuged at 6342, 5718, 5076, and 4752 rpm for 10, 30 and 60 seconds in two simultaneous factorial experiments. The quantitative (% mite detected in a sample) and qualitative (detection % of infested samples) detection efficiency of *V. destructor* by this method was >90 % and 100 %, respectively, when the mite infestation level was >3 %. The currently used ether roll method with five times larger bee samples possesses 35 % quantitative and 12 % qualitative detection efficiency. Using the mite detector, I found *Varroa* mite for the first time in the Caribbean island, Nevis.

Finally, detailed studies were conducted to further explore the possibility of using non-toxic dusting for the control of *V. destructor*. If the mites have dust on their ambulacra, it is expected to cause them to lose their grip and to fall to the floor of the hive, where the mites will eventually die. It has earlier been reported that dusting with wheat flour and other non-toxic dusts are useful for the control of *V. destructor*. In this study, super-fine ground pure white sugar was used. For the control of the *V. destructor*, adult bees were dusted in four laboratory and in two field studies to investigate the impact of two methods of dusting. Twelve colonies were treated sequentially every 3, 7, and 14 days by aerated *sugar dusting* with ca. 20 g of sugar using a simple blower. The impact of dusting on the colony development and on the queen supersedure was also studied. In the laboratory a sample of 78 bees (on average) was treated by direct (5 g)

and aerated sugar (0.5 g) dusting with and without pre-anaesthetisation of the samples with CO_2. One way ANOVA and t-tests were used to analyse the data of the field trial. The laboratory data generated two-way and multi-way tables with the numbers of mites recovered, and numbers remaining on the bees, for each test and each treatment, and were analysed using G tests. The dusting method was expected to produce an accumulation of sugar in the imagos' respiratory system; this was studied by the dissection of T2 spiracles and their ducts (n = 200 ducts) and by using a scanning electron microscope at 500 × and 4 000 × magnifications. No sugar particles were found in them. Direct and aerated sugar dusting methods were highly efficient for recovering the phoretic *V. destructor* from adult bees. Dusting had no adverse effect on the capped-brood and adult bees in colonies. No queen supersedure occurred in the sugar treated colonies. The CO_2 anaesthesia alone or in combination with the sugar dusting had no impact on mite fall. The CO_2 anaesthesia caused bee mortality starting a few days after the treatment. It was concluded that sugar dusting alone is sufficient and a useful tool, which could be included to integrated mite management programs.

LIST OF PAPERS

This dissertation is based on the following publications which are referred to in the text and tables by their Roman numerals.

I KORPELA, S. & FAKHIMZADEH, K., 1991. Tracheal mites in Finland. *American Bee Journal* 131 (9): 587-588.

II FAKHIMZADEH, K., HOKKANEN, H., SIKKILÄ, J., PIRHONEN, K., & LINTULA, E., 1993. The first survey of *Acarapis woodi* in Finland. *Bee World* 74 (3): 129-132.

III FAKHIMZADEH, K., 2000. A rapid field and laboratory method to detect *Varroa jacobsoni* in the honey bee (*Apis mellifera*). *American Bee Journal* 140 (9): 736-739.

IV FAKHIMZADEH, K, 2000. *Varroa* discovered in St. Kitts and Nevis. *American Bee Journal* 140 (11): 857-858.

V 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.

VI FAKHIMZADEH, K., 2001. Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. *Apidologie* (in press).

VII FAKHIMZADEH, K. Acute impact on the honey bee (*Apis mellifera*) after treatment by confectioner sugar and CO_2 for the control of *Varroa destructor*. *Journal of Applied Entomology* (Submitted).

VIII FAKHIMZADEH, K., Impact on the development of *Apis mellifera* colonies after treatment by powdery sugar for the control of *Varroa destructor* (Submitted to the *Journal of Apicultural Research*).

Papers I, II, III, IV, V, and VI are reproduced by permission of the journals concerned.

1. INTRODUCTION

Apiculture is most probably the least comprehended branch of agriculture. The annual value of enhanced crop (e.g. fruit, berry, vegetable seed etc.) harvests due to honeybee pollination is estimated at ~300 million FIM (~50 million USD) in Finland (Yläoutinen 1994) and ~3.5 billion USD in the USA (Southwick & Southwick 1992). Enhanced value is ~150 times the value of the honey and beeswax products in the USA. One third of our total diet comes directly or indirectly from bee-pollinated crop plants (Hoopingarner & Waller 1992). Due to honey bee pollination, turnip rape *Brassica rapa* spp. *oleifera* seed yield increases by ca. 10–15 % in Finland (Korpela 1988). However, beekeepers do not receive any payment for their pollination service from the farmers. Surprisingly entomophily is not appreciated despite its potentially high financial benefits. The concept of entomophily seems to be as romantic as protection of the environment, both of which in practice tend to be ignored especially in developing countries. In many developing countries beekeepers even have to pay rent for keeping their apiaries next to the farmer's fields. In many countries bee research is even not well supported (Fries 1997), and less than 1 % of the enhanced crop value is expended on bee research annually. Perhaps the lack of appreciation of entomophily stems from the incorrect notion that bees continue to pollinate crops even when no measures are taken to protect them, for example from rapidly spreading diseases and mites.

Furthermore, the sweetness of honey unfortunately tends to mask the poisons used for chemical diagnosis and chemotherapy of bee diseases, as well as the residues of agricultural pesticides. However, non- selective pesticides are comparatively minor apian pollutants. The debilitation of the colony as a consequence of forager casualties due to the use of a pesticide (Atkins 1992) on blooming crops is often the only serious impact (Morse & Hooper 1985). The severity of forager casualities is inversely proportional to the distance of colonies from treated (blooming) fields (Morse & Hooper 1985).

The presence of honey bee mites is a practical problem of apiculture, and their limitation pertains to enigmas of their diagnosis and control. The enigma of the diagnosis of the endoparasitic mite *Acarapis woodi* (Rennie) is not only its habitat and small size [~159 μm (Delfinado-Baker 1984)], but also its similarity to the ectoparasites *Acarapis externus* Morgenthaler and *Acarapis dorsalis* Morgenthaler. Infallible diagnosis of only *A. woodi*, can be made solely on the basis of its habitat. The position of other two *Acarapis* spp. on the host is not authentic (Delfinado-Baker & Baker; 1982; Shimanuki *et al.* 1992). There are anecdotal references made to the diagnostic methods of *A. woodi* (Delfinado-Baker 1984; Peng & Nasr 1985; Smith *et al.* 1987; Ragsdale & Furgala 1987; Ragsdale & Kjer 1989; Fichter 1988; Grant *et al.* 1993). However, routine chemotherapy with the assumption of highly infested colonies, is often more profitable for beekeepers than challenging the laborious and technical diagnosis (Cox 1991; Gary 1991).

In practice, the detection of large (ca. 1.8 mm x 1 mm) ectoparasites is not easier than endoparasitic mites. In spring when colonies are purchased, dealers can only trust

each others word. No field diagnosis of *Varroa destructor* [formerly *Varroa jacobsoni* Oudemans (Anderson & Trueman 2000)] is then possible to help decision making. Diminished population of imagos and brood does not permit large adult bee samples of 500–1000 individuals required for the ether roll method, or detrimental brood examination.

The restriction of varroasis has been a serious challenge since the discovery of *V. destructor* on European bees, *Apis mellifera* Linnaeus, in Hongkong in 1962 (Shimanuki *et al.* 1992). Besides poisonous chemotherapy, many different treatments have been utilised: mustard oil (Ritter & Ruttner 1980a), gamma radiation (Matthes *et al.* 1991), ozone treatments (Sychev *et al.* 1981), fatal high temperature in colonies in summer (Ritter 1981; De Jong *et al.* 1982b) or hyperthermia of adult bees in winter, as broods do not hide the mites in that time (Harbo 1994), acid fumigation (Imdorf *et al.* 1995), or dusting followed by starvation (Ramirez 1987). No physical factors were found which were effective against *V. destructor* but harmless to bees (Sychev *et al.* 1981; Ritter 1981; Harlander & Ruttner 1983; Nasr & Kevan 1999). One approach to resolving the problem of mite enigmas is to encourage innovational investigations instead of meretricious reiterating of literature. This thesis backs rational initiatives that have not yet been adequately explored, for example dusting.

The general objective of this work was to develop a new, more efficient method of detection of the major mite pests in Finland, and to elaborate the principle of dusting as a non-chemical control of *V. destructor*, which would possess the minimum adverse impact on the bees and their products.

The specific aims of the individual papers were:

(1) – to study the incidence of *A. woodi* in Fennoscandia, as well as its incidence both before and after the discovery of *A. woodi* (I; II). Numerous scientific questions of acarologists in literature were regarding the absence of *A. woodi* in Fennoscandia and the reason for this (see Morse 1990). Moreover, acarologists believe that the extent of the distribution of *A. woodi* and the means by which it has achieved this distribution, should be catalogued more exactly (Griffiths & Bowman 1981; Bradbear 1988; Matheson 1993, 1995) in order to solve the *A. woodi* enigma (Morse 1990). A survey that demonstrates the magnitude and extent of the problem (II) may prevent unnecessary chemotherapy becoming a matter of routine in practical apiculture.

(2) – to improve non-chemical detection of *V. destructor* in their phoretic phase from adult bees (III; IV). Optimisation of the method was vital in order to raise the quantitative and qualitative detection efficiency, which results in the reduction of the amount of imagos used in the detection process, and so in turn guarantees the applicability of the device for quick detection of *V. destructor* during spring. The currently used quick detection of *V. destructor* requires 500–1000 adult bees (USDA 1987; Gruszka 1988) and possesses a 12 % and 35 % quantitative and qualitative detection efficiency, respectively (Ellis *et al.* 1988; Herbert *et al.* 1989). In spring when bees are purchased for research or for production, unavailability of an appropriate method for the detection of *V. destructor* is a difficult problem for practical apiculture.

(3) – to evaluate the potential of non toxic dusting to control *V. destructor* in its phoretic phase (V). No scientific reports are available concerning non-toxic dusting procedures (Ritter 1999; Shimanuki 2000). The review of the literature indicates encouraging results from using this approach, reported in extension type of articles or short communications in conference proceedings (Ramirez 1987; Shah & Shah 1988). According to Pettis & Shimanuki (1999) the use of screen bottom board alone reduces mite infestation by 15 %. They proposed the use of dust in conjunction with the screen bottom board for the control of *V. destructor*.

(4) – to study any adverse effect of sugar dusting on both individual adult bees and colony growth (VI ; VII; VIII), as well as the impact of CO_2 on mite fall and bees survival (VI; VII). It was also considered important to investigate blockage of the trachea as a possible side effect of the dusting method. This was achieved by direct observation and through dissection of the first thoracic spiracles (T2) and their tracheal ducts under a scanning electron microscope, SEM (VI). In the synthesis I describe the methods of previously unpublished results carefully enough to allow rigorous evaluation of the arguments that have been made. However, in the cases of the studies presented in the original articles, those articles referred to should be consulted. The results reported in the synthesis are mean ± standard error of mean (SE) if not explained otherwise. The word *Varroa* used in the synthesis refers to *Varroa destructor*. The disease caused by *Varroa* is called with three synonym words varroasis, varroatosis and varroosis. The first word is used in this thesis as it is the most frequently used in the scientific literature.

2. MAIN MITE PESTS OF THE HONEY BEE AND THEIR OCCURRENCE IN EU

More than 40 species of mites have been associated with honey bee (Eickwort 1988). Some are known that parasitize *A. mellifera*. They are *Acarapis woodi*, *A. externus*, *A. dorsalis, A. Vagans, Varroa destructor* and *Tropilaelaps clareae* (Bailey & Ball 1991).

Unfortunately, major mite pests of honey bee are also found in Finland. Two of the economically most important mites are *V. destructor* and *A. woodi*. These acari produce implicit problems for the apicultural industry, especially in Europe and in North America. Their current known occurrence in the EU is given in Table 1.

Table 1. Occurrence of *Varroa destructor* and *Acarapis woodi* in EU

Country	***V. destructor*** **Year recorded**[1]	**Reference**	***A. woodi*** **Year recorded**[1]	**Reference**
Austria	1983	Nixon 1983	1954	Girtler 1954
Belgium	1984	Van Laere *et al.* 1988	1954	Van Laere & Gillard 1954
Denmark	1987	Schousboe 1989	1991	I
Finland	1980	Griffiths & Bowman 1981	1986	II
France	1982	Colin 1982	1980	Nixon 1982
Germany	1977	Ruttner & Ritter 1980	1960	Feiling 1960
Greece	1978	Santas 1989	1964	Liakos *et al.* 1995
Irish Republic	Absent	Hume 1989	1991	Kearns 1992
Italy	1981	Colin 1982	1959	Giavarini 1959
Luxemburg	1986	Van Laere *et al.* 1988	1980	Nixon 1982
Netherlands	1983	Ruijter & Eijnde 1984	1984	Ruijter 1993
Portugal	1988	Gómez Pajuelo 1989	1981	Martins & Rosa 1982
Spain	1985	Gómez Pajuelo 1989	1980	Gomez Pajuelo & Fernandez arroyo 1980
Sweden	1987	Stark & Glinski 1991	Absent	II
United Kingdom	1992	Bew 1993	1921	Rennie *et al.* 1921

[1] Year of detection of mites, or earlier if author(s) provided evidence for earlier existence of mites.

2.1. Assessment of acarine disease of honey bee

2.1.1. *Acarapis woodi* detection techniques

The endoparasitic mite, *A. woodi* was described for the first time in 1921 as *Tarsonemus woodi* (Rennie *et al.* 1921). This mite is also called the tracheal mite, as it dwells mainly in the tracheae that lead from the first pair of thoracic spiracles (T2) of adult bees (Delfinado-Baker & Baker 1982; Bailey & Ball 1991; Shimanuki *et al.* 1992) and feeds from the bee's haemolymph (Örösi-Pal 1934). This mite was blamed to be the causative agent of "Isle of Wight disease", a complex of disease conditions that was first noted about 1905 on the Isle of Wight, in the United Kingdom (Bailey 1981). When tracheal mites were found from the diseased colonies, Rennie made the association between the mites and the disease. However, the etiological agent that was causing Isle of Wight disease remains unknown (Henderson & Morse 1990).

A. woodi occurs universally with some exceptions (Bradbear 1988; Shimanuki *et al.* 1992; Matheson 1995). It was found for the first time in Finland in 1991 (I). It has not been reported from Australia, Hawaii (USA), some countries in the Caribbean (Matheson 1993, 1995), Sweden and Norway (II).

Besides *A. woodi* there are two other *Acarapis* spp. associated with adult honey bees. They are *A. externus* Morgenthaler and *A. dorsalis* Morgenthaler. Due to the similarity and size (female ≈ 143–174 μm) of these three spp., *A. woodi* is identified by its location on the bee, instead of its morphological characteristics. *A. woodi* lives exclusively in the prothoracic tracheae and causes the acarine disease in the honey bee (Delfinado-Baker & Baker 1982; Shimanuki *et al.* 1992). Delfinado-Baker (1984) describes morphological identification keys of these three spp. No external symptoms characterise the acarine disease, even though mite infestations are not randomly distributed in bees tracheae. Significantly, more bilateral than unilateral infestations occurs among worker adult bees (Fries & Morse 1992). The infested bees may have disjointed wings, distended abdomen, or both, and may be unable to fly, but they may also appear normal (Bailey 1958; Shimanuki & Knox 1991; Shimanuki *et al.* 1992). The mortality of infested bees is significantly higher among overwintered bees (Bailey 1961), compared with uninfested individuals after March in the UK (Bailey 1958; Bailey & Ball 1991). However, during summer no significant differences in mortality, in the number of foraging trips, or in pollen or nectar loads were found among hives with 50 % of foragers being infested and uninfested bees in the USA (Gary & Page 1989).

The diagnostic examination of honey bees for tracheal mites is estimated to cost over US $10 million annually, worldwide (Otis 1990). In sampling for *A. woodi* detection, moribund bees that crawl near the hive entrance are preferred (Rennie *et al.* 1921; Shimanuki *et al.* 1992). There are anecdotal references made to the diagnostic methods of *A. woodi* (Delfinado-Baker 1984; Peng & Nasr 1985; Smith *et al.* 1987; Ragsdale & Furgala 1987; Ragsdale & Kjer 1989; Fichter 1988; Grant *et al.* 1993). The diagnosis of existence of *A. woodi* in the bees is a tedious task involving careful

dissection, clearing of tissue in lactic acid (Delfinado-Baker 1984) or potassium hydroxide (KOH), followed by microscopic examination (Shimanuki & Knox 1991). First the dead bee is held between the thumb and forefinger, and the head and first pair of legs are removed. Then with a scalpel or fine pair of scissors, a thin transverse section from the anterior face of the thorax is cut to obtain a disk. The disks are placed in 5 % KOH and incubated at 37°C for 16 to 24 hours. The KOH dissolves the muscle and fat tissue, leaving the trachea exposed. Then the disks are examined under a microscope (Delfinado-Baker 1984; Shimanuki & Knox 1991).

A histopathological method was introduced by Peng & Nasr (1985), in which various anionic, cationic and mordant stains were tested on the thoracic disks. The cationic stains coloured the mites intensively and unsheathe the tracheae weakly (Peng & Nasr 1985). Live mites are obtained for research purposes by the Smith *et al.* (1987) method. An anaesthetised bee is fastened to a surface with its lateral side up. The flat lobe is held with forceps and pulled posteriorly at an angle of 0–45° to the horizontal. The flat lobe, the first thoracic spiracle (T2) and much of the tracheal trunk are removed. The trachea can then be inspected under a hand lens of 15–20 x.

Serodiagnosis of *A. woodi* by enzyme-linked immunosorbent assay (ELISA) was developed by Ragsdale & Furgala (1987). This serological method was further refined by other researchers (Fichter 1988; Ragsdale & Kjer 1989). When whole bees are homogenized in a blender and filtered for analysis, ELISA detected infestation at a level >5 %. Automated serodiagnosis of *A. woodi* by ELISA can analyse bulk samples with 10 % of the labour cost of conventional dissection methods (Grant *et al.* 1993).

Nine hours of microscopic observation involving 52 thoracic disks proved that 32 % of the attendant bees imported from California to Finland via queen importation were infested by *A. woodi* (I). Only a 2 % infestation figure was reported from Denmark when similar bees were imported (II). This difference in the infestation level may be attributed to the fact that the mite is transparent and difficult to detect even when using dissection methods, which are according to some investigators the most reliable methods (Delfinado-Baker 1984, 1988 ; Shimanuki & Knox 1991). Additional factors limiting the ability of beekeepers to detect the infestation include the lack of microscope, and other dissection equipment and materials. The difficulty of diagnosing *A. woodi* leads to unnecessary chemotherapy applications as a matter of routine, in which the beekeeper only assumes the presence of mite in the colony. In general the consequence of chemotherapy for mite control is either brood mortality (Nelson *et al.* 1993) or residues in hive products (Rivera *et al.* 1987) or both, and even, contrary to common belief, significant reduction in honey production (Nelson *et al.* 1993).

2.1.2. Acarapis woodi detection in Finland (I; II; III)

The contradictory absence of *A. woodi* in Fennoscandia (Griffiths & Bowman 1981; Bradbear 1988) was an enigma for acaralogists worldwide (Morse 1990). To

cooperate in rehabilitation or innovation of diagnostic methods of *A. woodi* (Delfinado-Baker 1984; Peng & Nasr 1985; Smith *et al.* 1987; Ragsdale & Furgala 1987; Ragsdale & Kjer 1989; Fichter 1988), the author travelled to USA by the invitation of USDA and Iowa State University in spring 1991 (II) to test *V. destructor* detection device (III) in finding *A. woodi*. Beekeepers suffered from acarophobia owing to 70 % colony loss due to *A. woodi* (Cox 1991) and other huge bee mortality around the world in those years (Benedetti 1990; Khan *et al.* 1987; Morse 1990). The quick field diagnose of *A. woodi* was, and still is valuable. However, The detection method of *A.woodi* by the *V. destructor* detection device (III) is still incomplete and needs further perfection before being able to use the method for the field assessment of *A.woodi*.

The change of Finnish importation policy in 1991 to solely trust on health certificates of the American bees, drove us to check for the *A. woodi* presence among American attendants of imported queen bees. The discovery of *A. woodi* infestation of 32 % among 52 attendant bees of nine imported queens from California provoked and induced us to search for the tracheal mite on the domestic bees (I; II).

The first *A. woodi* among domestic bees in Fennoscandia was discovered on 18 June 1991 in Finland (I). The first survey of *A. woodi* however, demonstrated its existence in Finland already in 1986 (II), and provided a proper analysis of the problem and level of infestations in all southern provinces of Finland. More than 70 % of Finnish colonies are located in these provinces. The discovery of the mite in Fennoscandia solved many complex scientific enigmas about the perplexing absence of *A. woodi* in Fennoscandia as reported by many (Griffiths & Bowman 1981; Bradbear 1988), and its cause- questions, which were repeatedly asked by acarologists (Morse 1990). Some acarologists believed that infested bees can not survive the prolonged winters in the Fenoscandia to infest the next generation of young bees (Bailey & Ball 1991). In general the reason for the sparseness or absence of mites in some geographic area may be one or more of the following factors;

- Low colony density
- Long time absence of young susceptible bees due to climate,
- Naturally resistant bees and such kind
- Lack of proper survey

The first survey demonstrated not only the spread of the mite in Finland at that time, but also at an earlier time, and determined the first record of *A. woodi* in Finland for universal acarological records (see Matheson 1993). As the "newness" of *A. woodi* was shown to be false, this may also have reduced the acarophobia of the Finnish apicultural industry, resulting in avoidance of unnecessary chemical treatments of the colonies as a matter of routine.

The discovery of 32 % *A. woodi* infestation among the attendant bees of Californian queens with a health certificate made clear the invalidity of such certificates, particularly when non-infested areas are concerned. This conclusion may be backed by the comparison of differences in *A. woodi* infestation of American imported bees into Finland (32 %) and into Denmark (2 %): the result for Finland was based on nine hours

of concentrated laboratory examination, while similar samples in Denmark were subjected to routine microscopic observation (I).

The November 1991 survey showed 16 % of the samples were infested with *A. woodi* in Finland (II). In another survey *A. woodi* infestations were found in 10 % of inspected samples during 1991–1997 in Finland with considerable increase of infested samples during recent years of the survey (Korpela 1998). In this survey 21 % and 28 % of samples were found to be infested by *A. woodi* in 1996 and 1997, respectively. The increase of the percentage of infested smples was partially due to the mite infestations in four queen rearing operation that found to be infested by *A. woodi* during the survey. The spread of *A. woodi* via queen bees from infested apiaries is possible. For example, bees of many beekeepers found to be infested with *A. woodi* in 1995–1997. These beekeepers had regularly purchased queens from a breeder who lost 87 colonies in 1996. The mean *A. woodi* infestation level in his dead colonies was 90 % (Korpela 1998).

2.2. *Varroa destructor* and its detection techniques (III; IV)

Varroa destructor Anderson & Trueman is an external parasitic mite of honey bees. *Varroa jacobsoni* Oudemans was found on *Apis cerana* Fabricius, in Java (Oudemans 1904) and until recently was generally believed to be the same specie as *V. destructor* (Anderson & Trueman 2000). Even though *Varroa* from different population are physically alike, their virulence toward *A. mellifera* is not uniform (Camazine 1986; Ritter *et al.* 1990; Moretto *et al.* 1991; Anderson 1994; Eguaras *et al.* 1995; De Jong & Soares 1997).

According to Anderson & Fuchs (1998) the mite that was described in 1904 as *V. jacobsoni* is still restricted to the Asian honey bee, *A. cerana* as a host. Only two of the 18 different haplotypes concealed within the complex of mites infesting *A. cerana* have switched host and have become pest of *A. mellifera* worldwide. Both belong to *V. destructor*, and they are not *V. jacobsoni* (Anderson & Trueman 2000; Anderson 2000). These species differ significantly in size, reproductive characteristics (Anderson 1994) and mitochondrial DNA (mtDNA) cytochrome oxydase I (CO- I) gene sequences (Hunt & Anderson 1999; Anderson & Trueman 2000; Anderson 2000). According to Anderson & Trueman (2000) *V. destructor* is larger than *V. jacobsoni*. Apparently, only *V. destructor* is capable of causing extensive damage to bee colonies (Hunt & Anderson 1999). However, the findings of previous research on *V. jacobsoni* are applicable mostly to *V. destructor* (Anderson & Trueman 2000).

The *Varroa* problem started when beekeepers transported *A. mellifera* to Asia (Goncalves *et al.* 1985). Today *Varroa* is found worldwide, with some exceptions (Bradbear 1988; Matheson 1995). Most recently *Varroa* was found in New Zealand (Matheson 2000), in Panama (Calderon *et al.* 2000) and in St. Kitts & Nevis in the Caribbean (IV). The mites attach to the adult bee between the abdominal segments or between body regions (head, thorax, abdomen) and are therefore difficult to detect

(Ritter 1981; Shimanuki & Knox 1991). The adult bee suffers not only from the loss of hemolymph, but may also be subjected to microbial invasion (De Jong & De Jong 1983), such as acute paralysis virus (Ball 1985; Allen *et al.* 1986) causing a reduced life expectancy (De Jone & De Jong 1983). Emerging infested bees weighed 6.3–25 % less than healthy ones (De Jong *et al.* 1982a). Generally, *Varroa* infestation remains undetected for up to three or four years. Clinical symptoms become apparent when several thousand mites are present in a colony (Ritter 1981; Robaux 1988). *V. destructor* can be found on the brood, in hive debris and on the adult bees (USDA 1987). One of the field methods to detect the mite is to uncap the brood cells and to remove the pupae with forceps, a sharp knife or capping scratcher (De Jong 1979; USDA 1987; Szabo 1989). Brood examination is a tiresome process and is not possible in the absence of a capped brood (Herbert *et al.* 1989).

Hive debris can be collected by placing a strong white paper on the hive floor. A wooden and wired (3 mm mesh) frame on the top of the paper protects the debris from the bees. After some weeks the paper should be visually investigated for any fallen mites (Ritter & Ruttner1980b; Fries *et al.* 1991a). At low level infestation debris examination is more efficient than in other methods (Fries *et al.* 1991a). However, this procedure is very laborious (Peroutka *et al.* 1981). The separation of dried debris with the help of different mash sieves and a rotary shaker may help when searching for mites in the debris (Huttianger 1981).

The debris may also be analysed by a floatation method using 98 % alcohol (Brem 1980; Ritter 1981) or in edible oil (0.91 g/cm^3) (Peroutka *et al.* 1981; Vyslouzil 1984) to separate the mites from wax (0.96 g/cm^3) (Peroutka *et al.* 1981) in the debris. This floatation method, however, is not an accurate method (Ritter 1981).

Various chemicals have been recommended in combination with the paper method to facilitate mite fall, such as fluvalinate plastic strip (Ellis *et al.* 1988; Herbert *et al.* 1989). Various smokes or fumes in hives have been reported for the detection of *Varroa* mite. These include bromopropylate (Kim & Choi 1987), fluvalinate smoke (Witherell & Bruce 1990), amitraz smoke (Kim & Choi 1987; Witherell & Bruce 1990), tobacco smoke (Ruijter 1982; Ruijter & Eijnde 1984; Witherell & Bruce 1990), and formic acid (Ellis & Baxendale 1994). Chemical diagnosis is possible only after all honey has been harvested (Ritter 1981). Fluvalinate at a concentration of 120 ppm is toxic to mixed-age bees and highly toxic to one-day old bees (Henderson 1988). Amitraz smoke strip is highly toxic to young bees (Henderson 1988). Another shortcoming of chemical use in the hive is the residue in honey (Atienza *et al.* 1993), and in bee wax (Wallner 1995). The proportion of bee wax samples contaminated with fluvalinate increased from 25 % in 1989 to 95 % in 1993 in Belgium (Greef *et al.* 1994). Almost all German wax samples on the market contained bromopropylate, coumaphos and fluvalinate but only the last was present in many foreign samples (Wallner 1997). *Varroa* has developed resistance against fluvalinate (Colin *et al.* 1997; Bruneau *et al.* 1997; Milani 1999; Elzen *et al.* 1999) and other acaricides as well such as acrinathrin, amitraz, bromopropylate, chlordimeform, coumaphos, and flumethrin (Milani 1999).

A laboratory diagnosis method is to shake bees in a liquid using a rotary shaker for 30 minutes. Various recommendations have been made for using different liquids, such as lukewarm and hot water of 25°,40°,60° and 100°C (De Jong *et al.* 1982a), 96 % ethanol (De Jong & Goncalves 1981), 70 % alcohol (ethyl or isopropyl) (USDA 1987), 25 % alcohol (De Jong *et al.* 1982a), detergent solution, hexane, gasoline or diesel fuel (De Jong *et al.* 1982a; USDA 1987).

Throughout the year, the ether-roll method (Shabanov *et al.* 1980; Ritter 1981) is used in the quick field diagnosis of *Varroa*. Some 450–1000 live bees are collected into a wide mouthed jar (USDA 1987; Ellis *et al.* 1988; Gruszka 1988; Herbert *et al.* 1989) by using a short burst of ether. The bees are then rotated in a jar for a few seconds and the mites adhere to the surface of the jar. The average detection efficiency of ether roll is 35 % quantitatively (individual mites detected in a sample) (Herbert *et al.* 1989), and 12.5 % qualitatively (detection rate of all infested samples) (Ellis *et al.* 1988). However, the latter value would have been higher in a survey with higher infestation levels. Since smoke is used in the apiary, the high flammability of ether is a safety concern when using the ether roll method.

When colonies are sold in spring, beekeepers and apiary inspectors need an accurate technique for the rapid detection of *Varroa*. The decline of colony population affects the practicality of taking brood and/or substantial adult bee samples for ether rolling. A highly efficient field method is required to determine the mite infestation in a relatively small sample of adult bees.

There is no rational field method of *V. destructor* detection in the spring. Therefore development of a new diagnostic method of *V. destructor* was vital to assist in decision making in spring when new colonies are purchased, as well as its utilization for integrated pest management strategy during the season.

A new electric cage was patented in Finland and reported by Fakhimzadeh (1990; 1998). The cage was initially designed for honey bee venom collection. This cage was utilized as a constitutive step for the non-chemical diagnosis of *V. destructor*. However, notwithstanding different parameters of direct and alternative electric currents, these trials proved to be ineffective for *V. destructor* detection. The strength of *V. destructor* in clinging to the bees in such circumstances of antagonistic extravagant movements of bees astounded us, especially when 12 V car battery was used. Based on this observation, a new device and method were invented and developed for non-chemical detection of *V. destructor* from the adult bees.

In preliminary investigation bees were centrifuged in the dry container of the device to remove the *V. destructor* from them. Gradual increase in the speed of rotation resulted in aggregations of bees faeces, while a sudden centrifugation of up to 7272 rpm produced faeces and 20 % of the number of V. *destructor* present in the sample. The sudden centrifugation of bees pre-treated with confectioner sugar dusting increased the efficiency of mite detection to 40 %. The reduction of friction between ambulacrum of *V. destructor* and the bee's surfaces by some suitable liquid was expected to be the solution to increasing the detection level. Therefore the efficiency of different liquids such as ethanol 25 %, 50 %, 70 %, detergent solutions 0.03 % and 0.1 % was analysed

separately. This method is based on shaking solutions and incorporates laving and a specially designed centrifuge for separating mites from bees in a closed container. Adult bees were laved in a rotational chamber of a centrifuge device, using detergent water and 6342, 5718, 5076, and 4752 rpm for 10, 30, 60 seconds. Live and frozen adult bees were examined in apiary and laboratory, using two concentrations of detergent solution 1 ml:1 liter and 1 ml:3 liters. After processing the bees were removed from the device. *V. destructor*, if present, sank down and were suspended in the mite compartment of the transparent rotational chamber, from which they were collected into a container.

In two independent, simultaneous sets of factorial trials adult bees were laved mechanically. Several levels of factors influencing the detection of *V. destructor* by the new method were analysed to ameliorate the quantitative and qualitative detection efficiency in an apiary and laboratory. The 0.1 % detergent solution and 6342 rpm of centrifugation were significantly better than the 0.03 % detergent and 5718, 5076 and 4752 rpm speeds, respectively (Table 2).

Table 2. The results of General Linear Modelling (GLM procedure) of tests involving rotational speed and detergent concentration.

Source	**df**	**Type I SS**	**MS**	**F**	**Pr > F**
Soap	1	1336.10	1336.10	5.79	0.0254
Speed	1	2512.74	2512.74	10.89	0.0034
Time	1	0.027	0.027	0.00	0.9914
Soap* Speed	1	663.34	663.34	2.88	0.1047

No significant differences in detection efficiency between frozen and live bees were found. However, the impact of freezing was further analysed by excluding samples with infestation levels <3.0 %. A significant difference ($P<0.05$) was then detected in favour of frozen bees. The duration of centrifugation is not critical; even 10 seconds of rotation seems to be sufficient especially when optimal levels of other factors are chosen. The maximum velocity, causing the highest centrifugal force, was obtained in less than ten seconds. The mites are either dislodged from the bees (e.g. when on top of the bees' thorax) with the maximum centrifugal force, or remain in a safer position between the bees' segments. Since the duration of centrifugation does not increase the force, it is logical that after 10 seconds the duration does not play any significant role.

The qualitative detection efficiency of the method at 1 mite/100 bees is >85 % (13 positive detection out of 15), whereas for ether roll method it is 12.5 % (Ellis *et al.* 1988). The efficiency of the method developed in this study is 100 % (n = 93) when the infestation level is ≥3 mites/100 bees. Its quantitative detection efficiency with 100 bees

in a sample under optimum conditions is >90 % (e.g. >9 mites recovered out of 10 mites in a sample) when the infestation level is >3 mites/100 bees, whereas ether roll method it has been reported to be 35 % for a sample of 500 to 1000 bees (Herbert *et al.* 1989). The high variation in the detection efficiency was due to infestation levels lower than 3 mites per 100 bees (Figure 1). The data of mite detection in low infestation levels #3 mites / 100 bees, using 6342 and 5718 rpm speeds was analyzed by G test after assuring that the data are homogenous among the 5 replications for each treatment (using Linear - by - Linear association). The mean quantitative detection efficiency of the speeds was 96 and 57 %, respectively, and this difference was significant ($G = 6.92$, $df = 1$, $P < 0.009$).

For the detection of *V. destructor* in the laboratory, among all the recommendations made for laving bees for 30 minutes in different liquids (De Jong *et al.* 1982a; De Jong & Goncalves 1981; USDA 1987), the simple detergent solution proved to be the most economic and convenient to use. The high efficiency of this method with its ten second processing time suggests that it may supersede all other laboratory methods of *V. destructor* detection. It is easy to apply at the apiary or laboratory, especially in the spring when bees are vulnerable to sampling. It is not necessary to examine the bees immediately as in the ether roll method (USDA 1987), and the efficiency of using the stored sample in the freezer is even higher. The impact of immediate freezing on mite detection still needs further research. Using live bees in this method, at least with the low detergent concentration, even after 60 seconds of rotation, more than half of the bees will revive if placed in the sunshine or in a warm place. It may be better to rinse the detergent off the bees first with water. This is true for mites as well, so they must be collected and frozen to ensure death.

The practical efficiency of this device was demonstrated through the first discovery of *Varroa* on the Caribbean island Nevis (IV). The apiary inspectors from the USA have regularly sampled bee colonies in Nevis in search for bee pests and diseases, but have never found any *Varroa* on the island.

This mite detection device was also used in practice in spring 1996 in search of naturally highly infested colonies by *V. destructor* in Southeast of Finland. Contrary to the expectations of many beekeepers who claimed to have a high level of *V. destructor* in their colonies, the method demonstrated rapidly that this was not the case and that their colony was not an appropriate object for *V. destructor* non-chemical control studies (V; VI; VII; VIII) due to a low level of infestation.

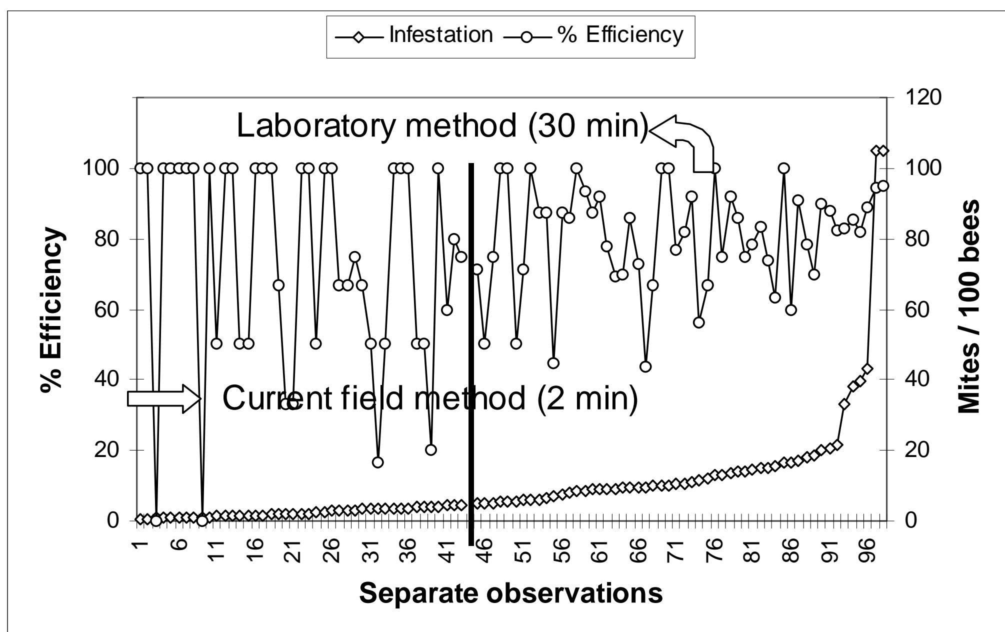

Figure 1. Samples of ca. 100 adult bees were laved and centrifuged with 4752, 5076 and 5718 rpm speeds in 0.03 % detergent solution for 10, 30 and 60 seconds for the detection of *Varroa* mite. The quantitative detection efficiency (mites detected vs total mites in a sample) of the developed method is very high. Notice, variation in the detection efficiency is inversely proportional to the mite infestation level and unequivocally due to lower infestation levels. On the left of the vertical solid line the mite infestation in samples are <5 mites per 100 bees and on the right >5 mites per 100 bees. Currently used Ether- roll method possesses 35 % efficiency with five time larger bee sample than our method (Herbert *et al.* 1989). Alternatively, mechanical shaking in laboratory for 30 minutes detects on the average 97 % of the mites present in a bee sample (De Jong *et al.* 1982a).

3. THERAPEUSES OF VARROASIS

V. destructor is the causative agent of varroasis, in which bee larvae and imagos suffer not only the loss of hemolymph, but are subjected to bacterial and viral invasion (Koch & Ritter 1989; Shimanuki *et al.* 1992). The excellent microclimate of bee colonies protects *V. destructor* from all external limiting factors (Ritter 1981), hence it survives in a wide range of climatic zones and exists in every continent except Australia (Bradbear 1988; Matheson 1993, 1995).

The clinical symptoms of dead brood by Acute paralysis virus (APV) are similar to that of European foulbrood disease, EFB (Koch & Ritter 1989). Heavy mortality in colonies that were highly infested with *V. destructor* was mainly due to APV, Egypt bee virus (EBV) and other bee viruses (Allen *et al.* 1986; Ball 1985; Koch & Ritter 1989; Ball 1988) rather than bacterial infections (Koch & Ritter 1989). In areas where *V. destructor* does not occur, APV persists as dormant or latent infection of adult bees and has never been found to be responsible for bee mortality (Ball 1988). *V. destructor* initiates the viral infection and operates as a vector among imago bees, and between adults and larvae. Wiegers (1988) demonstrated the transmission of APV by mites from pupa to pupa, but no multiplication of virus occurred in the mites.

The minimal affliction of varroasis are both the reduction of imago's life expectancy (De Jong & De Jong 1983) and up to 25 % loss of pupal body weight (De Jong *et al.* 1982b). Honey bee queen broods are relatively safe from varroasis (De Jong 1990), but not in heavily infested colonies that contain no worker, or drone brood (Harizanis 1991). A simulation population dynamic model reveals an intrinsic rate of daily increase in mite number to be 0.21 during brood rearing time (Martin 1998). An entire colony may collapse due to a few pregnant mites within few years (Johnson 1988; Shimanuki *et al.* 1992).

3.1. Current methods

- **Chemotherapy**

According to De Jong (1990) honey production is not possible without the suppressing of *V. destructor* by annual chemical treatment. However, chemotherapy is not safe to use. A repercussion of chemotherapy due to wrong method or timing is the poisoning of hive products (Ritter 1981; Nowottnick 1988). Sometime acaricide strips can be left in the hive based on the false assumption that they will eradicate the mites and therefore lead to better honey production. Contrary to expectation long-term treatment with fluvalinate (½ year) results in lower honey production (Lensky *et al.* 1996). Highly toxic substances like phenothiazine have been mistakenly applied by one third of Japanese beekeepers up to six times per year (Smirnov 1978; Ritter 1981). Yet, even properly prescribed chemical control presents a practical dilemma. Chemical residue in wax (Greff *et al.* 1994; Wallner 1995) and honey (Atienza *et al.* 1993) were

repeatedly reported. Residual persistence in wax is due to the lipophilic properties of chemicals (Vesely *et al.* 1994). Almost all German wax samples in the market contained bromopropylate, coumaphos and fluvalinate (Wallner 1997). The evolution of mites resistant to chemicals is another shortcoming of chemotherapy (Colin *et al.* 1997; Bruneau *et al.* 1997; Baxter *et al.* 1998; Pettis *et al.* 1998; Elzen *et al.* 1999; Milani 1999). In addition chemical control may have detrimental effects on unsealed broods (Choi 1985; De Jong & De Jong 1983) and bees. Henderson (1988) showed that fluvalinate and amitraz are highly toxic to young bees.

Soft-chemicals are used for the control of *V. destructor*. However, sole reliance on autumn treatment of lactic acid for four years expedited the establishment of mite population above the hazardous threshold after three years (Brødsgaard *et al.* 1997). Formic acid treated bees have lower honey production and a lower area of sealed brood (Westcott & Winston 1999). Another handicap is the safety concerns of these acids for users.

More than 150 essential oils have been evaluated in laboratory screening tests. However only a few were successful when tested in field trials (Imdorf *et al.* 1999). Origanum, lemon, thymol, cineole and lemon-grass oils have high acaricidal effect (Lensky *et al.* 1996; Calderone *et al.* 1997; Imdorf *et al.* 1999). But one or two applications of essential oil are not sufficient to maintain a proper control of varroasis. Two sequential applications of (1:1) blend of thymol with cineole, citonellal or linalool left in place for 14 days each, reduced at most only 56 % of mites. Still more efforts are necessary for optimisation and incorporation of essential oils (Calderone *et al.* 1997; Imdorf *et al.* 1999). Another rational approach is to incorporate biological methods along with other measures for limiting mite populations, into an integrated pest management strategy for control of *V. destructor*.

- **Biotechnical campaign against varroasis**

A number of biological methods have been tried to combat *V. destructor*. Microorganisms have been tested in Romania without success (Smirnov & Voronkov 1979). Antiparasitic agents derived from *Bacillus thuringiensis* were used against *V. destructor* (Koromyslov 1981). Biotechnical methods include:

- Hyperthermia
- Brood removal
- The trap-comb method
- The trap comb method in the intermediate nucleus
- Nucleus formation
- Integrated control using a combination of biotechnical methods and the minimal application of chemicals (Ritter 1993). The details of the three main biothechnical methods are described below.

- **Hyperthermia**

According to Choi (1985), several attempts have been made to control *V. destructor* by hyperthermia since its introduction by Chung and also by Lee in 1970. Although the

number of reportedly effective hyperthermia is large (Ruttner 1977; Khrust 1978; Karpov & Zabelin 1978; Ritter & Ruttner 1980b; Solov-eva 1983; Komissar 1985; Hoppe & Ritter 1987; 1989; Rosenkranz 1987; Ahmad 1988; Engels & Rosenkranz 1992; Harbo 1994; Engels 1994), the method has not been unequivocally established.

The optimum and fatal high temperatures of each organism involved in the treatment have been studied by researchers around the globe. Optimum temperature of honey bee workers range 15–36°C but their queens prefer 26–34°C (Komissar 1991). *V. destructor* chose 32.6°C on a temperature gradient, as drone brood temperature (30–34°C). However, as the ambient temperature rose from 21 to 34.5°C , the temperature preferred by mites also rose to 34.2°C (Conte *et al.* 1988). What if the ambient temperature were to rise further? A version of heat treatment in Pakistan is to replace the top covers of the infested colonies with a glass slab covered with black cloth. The colonies' temperature rose to 45–47°C within 15–20 minutes when the ambient temperature was 41–43°C. Encouraging results were obtained when the colonies were treated three times within ten days for 30 min. at 45–47°C (Ahmad 1988).

Several attempts have been made to control *V. destructor* using hyperthermia by heating the adult bees outside the hive in summer (Ritter 1981; De Jong et al. 1982c) Hyperthermia has also been attempted in winter (Harbo 1994). Swarms or package bees may be treated at the time of acquisition (Karpov & Zabelin 1978). Bees are confined and rolled in a cylindrical wire mesh chamber with 2.5 x 3mm apertures, and treated with hot air at 43–48°C circulating for 5–15 minutes (Grobov 1977; Smirnov 1978; Karpov & Zabelin 1978; Komissar 1985).

The duration of hyperthermia is important. Bees that were exposed to high temperatures (42–47°C) evaporate up to 0.5 mg water/min by active body hyperventilation, increasing the frequency and amplitude of abdominal pumping movements (Komissar 1991). In Hyperthermia, control of the temperature is very difficult and directly affect the efficiency of the mite fall. For example, when 1,500 bees were exposed to temperature from 42 to 51°C with RH <20 %, at the time of bee destruction by heat only 6 to 74 % of the mites were killed. In an exposure of 15,000 bees to 48°C for 20 minutes or 50°C for 6 minutes only 23 % and 38 % of mites were killed, respectively (Hoppe & Ritter 1986). This may be due to the clustering and thermoregulatory behaviour of honey bees that neutralise the effect of heat treatment in the hive (Hoppe & Ritter 1987).

Hyperthermia alone is not effective enough to be valuable and has often caused high bee and queen losses due to difficulties in controlling the temperature (Choi 1985; 1986; Hoppe & Ritter 1986; De Jong 1990). Hence, combining hyperthermia with other methods seems essential for the control of *V. destructor*. If exposed to heat of 45 and 48°C and thyme oil, individual mites between the abdominal sclerites will emerge after a mean time of 331 and 152 s and leave the bee within 35 and 5 s, respectively (Hoppe & Ritter 1987).

These methods, however are a labour-intensive task and takes about 30 minutes per colony (Komissar 1985). For example, hyperthermia of confined bees in a cylindrical wire mesh chamber seems less technical and easy, while in practice the opposite is true.

According to Solov-eva (1983) two heat treatments in spring could retard and even kill the colony, so autumn treatment with multi space heat chamber is recommended.

Another alternative is hyperthermia of sealed brood combs outside the hive (Engels & Rosenkranz 1992; Engels 1994). Various temperatures (40 to 45°C) and times (12 h–4 h) were used to heat the worker brood. Total damage to the brood was <5 %, but 5– 40 % of the brood just before and after pupal ecdysis was damaged (Rosenkranz 1987). Heating the brood for 4 hours at 44°C had no apparent effect on emergence rate but the survival rate was significantly lower than in control treatments. Increasing the temperature to 45°C had a negative and frequently significant effect on emergence rate and lifespan. Younger brood (9–10 days) was the most sensitive (Appel & Buchler 1991). Using an Apitherm heater, an average of 7.5 % of brood was damaged (all stages) and only 35 to 50 % of mites were killed (Marien 1995). This procedure can be difficult even for skilful beekeepers (Brødsgaard & Hansen 1994). As the tolerance of both *V. destructor* and *A. mellifera* for extreme high temperatures are very close to each other, and bees neutralise the effect of heat treatments, it can be concluded that in general the establishment of hyperthermia as a control method may not be easy.

• **Brood removal**

As the mite reproduces only in the capped brood cells, removal of the brood is another control measure of *V. destructor. Varroa* parasitise more severely drone brood and at a greater frequency. Drone brood cells are preferred to worker cells (Ritter & Ruttner 1980b; Ritter 1981), as are protrude cells (De Jong & Morse 1988). Hence, some investigators recommend drone brood removal (Brødsgaard *et al.* 1994; Calis *et al.* 1999). Drone brood was removed at regular intervals in spring and summer during six years trials. In treated colonies *V. destructor* population was lower than in untreated colonies at the end of the season (Manino *et al.* 1996).

The strong attraction of *Varroa* to drone larvae led to the production of a commercial product called Varoutest, containing an extract of drone honeybee larvae. It was sprayed before sealing on worker brood , which was later removed. During the treatment the queen is caged for 14 days (Guermant *et al.* 1990). Trapping with worker brood is another possibility but is labour intensive, as additionally requires subsequent treatment of the capped brood to save the brood (Calis *et al.* 1999). Trapping with drone brood demands fewer brood and can be integrated into swarm-prevention techniques (Calis *et al.* 1999). While methods of removing the drone brood (Mel'nik & Muravskaya 1981) reduce mite population, some investigators believe it does not give sufficient control of the mite (Rosenkranz & Engels 1985).

• **Trap-comb method**

An integrated control method for *V. destructor* is to confine the queen by physical barriers for 9 days on a comb and move the sealed brood to other hives, or to cage the queen for 21 days followed by chemical control of the colony. This method prevents egg laying, interrupts the development of brood and disrupts the life cycle of *V. destructor* (Tangkanasing *et al.* 1988). Other similar treatments are the trap-comb method in the

intermediate nucleus and nucleus formation (Ritter 1993). Fries & Hansen (1993) reported the results of five years trials with the trap-comb method. They believe that the trap-comb method alone may not exert sufficient control for *V. destructor,* hence in the fifth year they combined the method with additional removal of one drone brood in spring.

However, these biotechnical methods are not efficient where there is high re-invasion of mites due to high density of bees and highly infested colonies in the vicinity. In such a case a dangerously high mite population can develop in a very short time (Ritter 1993). As a general conclusion, biotechnical methods are labour intensive and should be combined with other methods to raise their efficiency for *V. destructor* control.

- **Radiation and ozone**

In a trial, *V. destructor* was exposed to 20–640 Gy of gamma radiation- it was shown to be sensitive to gamma radiation of ≥160 Gy. However, as already 15 Gy has lethal effects on bees this method is not applicable for mite control (Matthes *et al.* 1991).

Ozone at a concentration of 1–2 g/m^3 weakened and killed bees without any apparent effect on *V. destructor* (Sychev *et al.* 1981). No physical factors were found which were more effective against *V. destructor* than they were harmful to bees (Sychev *et al.* 1981; Ritter 1981; Harlander & Ruttner 1983; Nasr & Kevan 1999).

- **Resistant bees**

A tolerance to *Varroa* is exhibited by *A. cerana*, which consists of several factors (Shimanuki *et al.* 1992). A grooming behaviour followed by puncturing and subsequent killing of the mite by *A. cerana* (Peng *et al.* 1987) is one of the main resistance factors (Lensky *et al.* 1996). *A. cerana* removes mites more frequently and removes more mites which are found to be dead or abnormal, whereas those removed by *A. mellifera* workers were alive (Wongsiri *et al.* 1990). Another behavioural resistance mechanism of *A. cerana* to *Varroa*, is hygienic behaviour (Peng *et al.* 1987). On the other hand, *Varroa* reproduce only in the drone brood cells of *A. cerana* (Koeniger *et al.* 1983) as the developmental time of other casts from egg to emergence is insufficient for the mite development (Shimanuki *et al.* 1992). Using sibling analysis for heritability in *A. mellifera* showed the possibility to enhance the expression of the four characteristics for selective breeding of resistant bees to *V. destructor*. They are as follows: proportion of mites in brood, duration of the capped period, hygienic behaviour and suppression of mite reproduction, but not physical damage (Harbo & Harris 1999). This study reinforces confidence in the ability to breed resistant strains of bees to mites. However, when in practice US Department of Agriculture (USDA) released the *Varroa* resistant stock of bees for sale to beekeepers, the acceptance of the stock by beekeepers was a great disappointment, as the bees were not good honey producers (Danka *et al.* 1995; Delaplane 1997).

3.2. Dusting

Non-toxic dusts have been tested on a small scale, but the scientific literature does not report results from any of them. According to Ritter (1999) and Shimanuki (2000), anecdotal references to dusting procedure exist for the control of *V. destructor* but are not published. Only some notes have been published (Shah & Shah 1988) in extension type articles in Italian (Loglio & Pinessi 1991, 1993; Loglio 1996) or in the proceedings of some apicultural conferences (Ramirez 1987; 1994). Their reports of ”encouraging results” are obtained by combatting *V. destructor* with a non-toxic dust in the hive. A fine powder of glucose or ground pollen (Ramirez 1987) or wheat flour was dusted onto the bees to control (e.g., Shah & Shah 1988; Loglio & Pinessi 1991; 1992) or detect (Loglio & Pinessi 1993) *V. destructor* in bee colonies. Loglio (1996) reports the collection of living mites with the use of wheat flour on adult bees.

The hypothesis explaining why dusting might help to control the mite is based on the fact that *V. destructor*, like other mites, has ambulacra which allow it to adhere to a substrate. The dust on the bees adheres to the ambulacra of *V. destructor*, and this prevents the mite from attaching to the bees’ surfaces (Ramirez 1989). It loses its grip and equilibrium and falls on the floor of the hive, where it dies of starvation (Ramirez 1987; 1994) as it is unable to move on the dusty surface (Ramirez & Malavasi 1991). Additionally, dust may cover important sensory organs of mites. The mite has no known optical system. It has appropriate sensory setae located on the first pair of legs (tarsus I) for the realisation of high body temperature (35–40°C) of its host (Bruce 1997). Encouraging results were obtained by using a blowing equipment to dust the flour onto the bees in colonies (Loglio & Pinessi 1992). Unfortunately the particle size used in dusting the bees is not reported in any of the above studies. In a preliminary investigation wheat flour was replaced by finely ground pure white sugar and 35 g were dusted per colony. As many as 750 *Varroa* mites were collected below a hive after a single dust application. Confectioner sugar application seemed to have no obvious negative side effect. Earlier observations indicated that bees with misshapen wings and malformed body walked out and fell down from flight board after dusting. It seems that other bees reject them by choosing not to clean them.

Overall, an economic analysis of integrated *Varroa* control programme showed both an increase in profitability of beekeeping, and in the production of honey and beeswax (Kirilov *et al.* 1994). On the other hand, none of the present control methods is considered completely satisfactory (Choi 1986; Delaplane 1997).

3.3. *Varroa destructor* non-chemical control by sugar dusting (V; VI)

3.3.1. General methodology

The new method for the detection of *V. destructor* (III; IV) created possibilities to find highly infested colonies in spring 1996 and to start this sugar dusting project. These studies were carried out to determine how to combat *V. destructor* with sugar. Notwithstanding the fact that the development of highly efficient method to control V. destructor (V; VI) was not the only outline of these trials. Safety of individual adult worker bees undergoing such sugar treatments (VI; VII) as well as their colony development (VIII) were equally as important. As a possible side-effect of the sugar dusting, the accumulation of sugar particles in the T2 spiracles and their ducts from treated bees was studied under scanning electron microscope. (VI: Experiment 2) due to some unexplained mortality in the former experiment (VI: Experiment 1).

Super-fine ground pure white sugar was implemented in all trials (including preliminary since 1990) and are referred to as "confectioner sugar" with a nominal mean particle size of 25–40 µm according to the manufacturer (Finnsugar Ltd.: Helsinki, Finland). However, a separate study under SEM showed that *V. destructor* control was due to particulate matters ≤5µm (V; VI). A simple apparatus assisted in air dusting the bees (V; VI; VII; VIII).

Two method of sugar application were studied: direct dusting and air assisted dusting with 5 g and 0.5 g of confectioner sugar, respectively. Both application methods were examined with and without pre-anaesthetisation of the bees by CO_2 (VI; VII). CO_2 anaesthesia was implemented with the assumption that it might drive the mites out from between the bees' segments and also in order to anaesthetise the bees so that they would not inhale the confectioner sugar during treatments. The efficiency of mites recovered with two methods of sugar dusting were studied. The impact of CO_2 alone and in combination with sugar dusting methods in accelerating the mite fall was investigated (VI). The impact of CO_2 anaesthesia and sugar dusting on bees survival were studied in highly mite infested bees (VII) as well as on bees with low mite infestation (VII). The impact of sugar dusting every 3, 7 and 14 days on the colony development and the queen bee supersedure were studied (VIII). The total honey galned by each colony were recorded.

3.3.2. Dusting in laboratory (VI)

Understanding the details of the utilisation of white sugar in defeating *V. destructor* was only conceivable through a laboratory analysis. Laboratory trials were conducted during 1997–1998 in Helsinki to clarify the absolute efficiency (VI) and a possible side effect of sugar dusting (VI: Experiment 2), respectively. The latter effort was due to

unexplained slight mortality of bees in a former study. The mean number of *V. destructor* per sample was 10 mites per 100 bees (range 1 to 24 mites) in VI: Experiment 1, and very low (range 0–2.5 mites /100 bees) in VI: Experiment 2, trial. Completely randomized design was used as an experimental design of the trials.

CO_2 anaesthetisation was administered sufficiently to knock all the bees down in a sample. The daily addition of a drop of water adjusted the humidity of sample, as changes in water balance has been shown to affect the spiracular control (Richards & Davies 1977). Jars were kept upside down to catch any fallen mites and/or sugar after the treatments. Casualties of both organisms were recorded daily. Then all the remaining bees were killed and mechanically shaken for 30 minutes with ethanol 70 % (De Jong *et al.* 1982a; Shimanuki & Knox 1991); this removes almost 100 % of *V. destructor* in a sample (De Jong *et al.* 1982a). Bees and mites were then laved under a two sieve screen to expedite their separation and counting.

The values of treatments with and without anaesthesia were pooled to compare the dusting treatments, since the CO_2 anaesthesia did not affect the mite fall significantly (Mantel-Haenszel chi-square for odds ration homogeneity = 0.018, $P = 0.89$). Direct dusting by 5 g of confectioner sugar resulted in 91 % mite fall compared to only 62 % for the air dusting of 0.5 g sugar, and this difference was significant ($G = 15.89$, $P < 0.001$). The CO_2 alone (treatment C) had no impact on mite fall. D1 (Shake control) demonstrates that shaking and rolling bees gently had no impact on the mite control either.

In the trial (VI: Experiment 2) of probable accumulation of sugar in imagos' respiratory system, five treatments similar to the (VI: Experiment 1) trial were assessed; these were followed by SEM observation of imagos' first thoracic spiracles and their ducts executed 24 h after treatment. The main thoracic tracheal ducts leading from T2 spiracles of 100 bees (n = 200 ducts) were dissected lengthwise and observed under SEM, chiefly at magnifications of 500 and 4000 x. The ambulacrum of female *V. destructor* was also examined. Investigation of particulate matters in the ducts, both immediately after the treatments, and also 48 h from performance of grooming behaviour of bees seemed both difficult and less trustworthy. However, no sugar contamination was found inside the tracheal ducts (Figure 2) in any of the treatments (n = 200 ducts).

Sugar dusting efficiency in knocking down the *Varroa* in some cases was equal to chemical control. For example the efficiency of amitraz and fluvalinate on package bees were 83 % and 87 %, respectively, at the concentration indicated by the manufacturers (Henderson 1988).

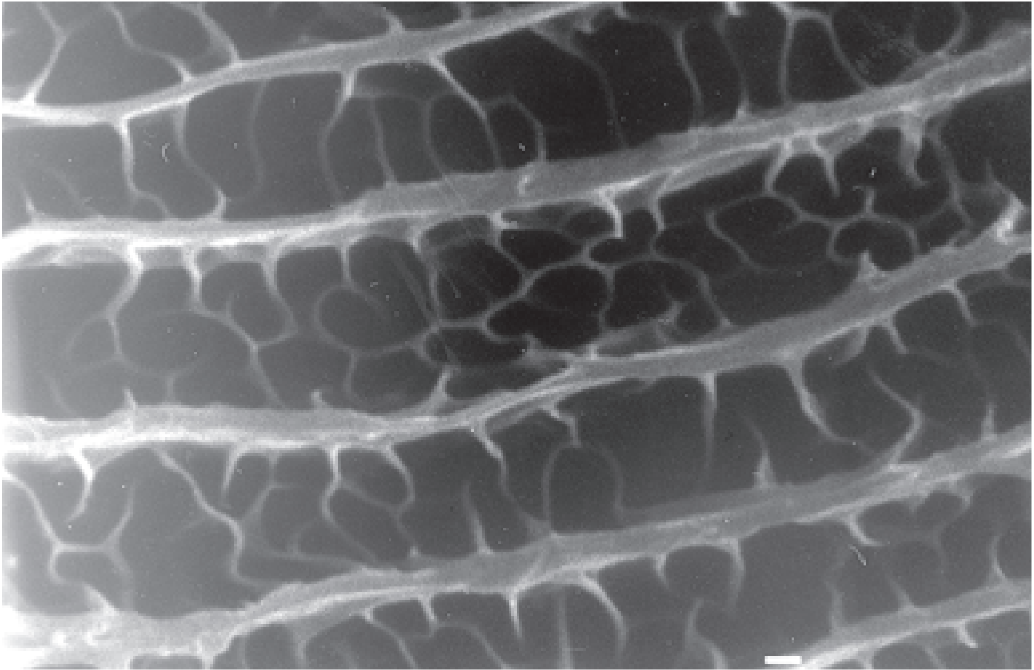

Figure 2. Scanning electron micrograph of the inner surface of the tracheal ducts of honey bee after sugar treatment, examined at magnification 4000 x (bar = 1 µm).

These results reported above are in agreement with those obtained by glucose and wheat flourdusting for which good results have been reported in the absence of brood (Ramirez 1987; Shah & Shah 1988; Loglio & Pinessi 1992), and also for the detection of live *Varroa* mites for further research (Loglio & Pinessi 1993; Loglio 1996).

Mortality of non anaesthetised sugar treated bees was significantly higher than anaesthetised treatments. However at low infestation opposite was true (Figure 3). In pre- anaesthetised bees, no significant impact was found by using either method of sugar application (VII).

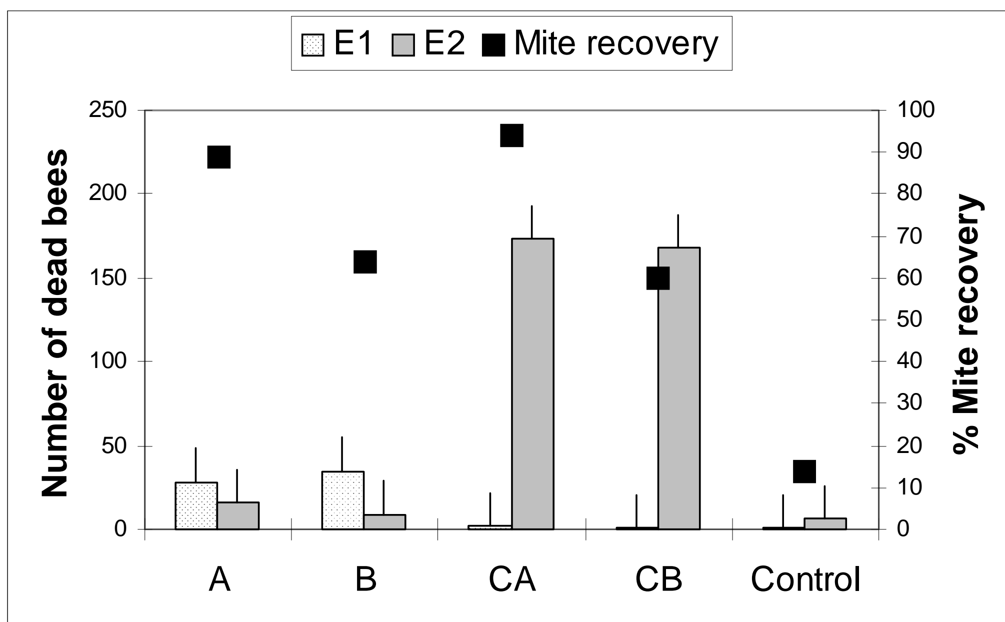

Figure 3. Number of dead bees in high and low infested bees (by *V. destructor*) in laboratory experiment 1 (E1) and experiment 2 (E2), respectively. The treatments were: A; direct dusting with 5 g sugar, B; air-assisted dusting with 0.5 g sugar, CA; CO_2 anesthesia + A, CB; CO_2 anesthesia + B, and control. All bee samples were shaken and rolled after dusting (A, B, CA, CB). The second Y axis shows the percentage of mite recovery in each treatment in the experiment 1. Vertical lines represent SE.

3.3.3. Dusting in apiary (V; VIII)

In a Finnish apiary at Viikki in Helsinki (60°13´N, 25°02´E), 24 naturally infested colonies of *A. mellifera* were assigned randomly to five treatments (groups) with 4 and 6 replicates in treated and control groups, respectively. Similar sugar treatment was applied by aerating confectioner sugar (with the simple apparatus) sequentially at intervals of three, seven and 14 days in July 1996.

Debris samples were secured under a wired frame (USDA 1987; Herbert *et al.* 1989; Pettis & Shimanuki 1999). The whole debris sampled prior to treatment was termed as BT, and samples after treatment as SD (6 h) and ND (18 h). AT (24 h) is a summational value, and its subdivision to SD and ND alleviated the vulnerability of the experiment to missing data (V).

Under field conditions, the mite fall per day after treatment in all three sugar-treated groups was statistically significantly higher than the levels measured before treatment, and that in the control colonies. For the first sugar treatment (n = 12), the mean mite fall per day was 23 times greater in AT samples, in contrast to BT samples of

the same treatments. The dusting treatment significantly accelerated mite fall. The $\bar{x} \pm$ S.E. for BT were 0.17 ± 0.06 (n = 12), and for same day (SD) samples 5.8 ±1.7 and for ND 3.3 ± 2.5 mites per hour. The mite fall of SD and ND were combined to form the AT, which was 3.8 ± 1.8 mites per h.

In order to quantify the pattern of colony growth (VIII), the amount of capped brood cells and a census of a colony's population of adult worker bees in each colony were recorded before, during and after the treatment period.

No statistical differences were found among the experimental treatments in the total number of capped brood cells in colonies, before (F= 1.515, df = 4, P= 0.237), and after (F = 0.481, df = 4, P = 0.749) the experimental period (Figure 4). Adult bee population of chemical control group was significantly higher than mite infested groups at the start of experiment (F = 7.629, df = 4, P < 0.001). However, no statistical differences were found among treatments in bee population growth, which was estimated 20 days after the start of the experiment (F = 0.553, df = 4, P= 0.7) (Figure 5). No queen bee supersedure occurred in sugar treated colonies during the treatment period.

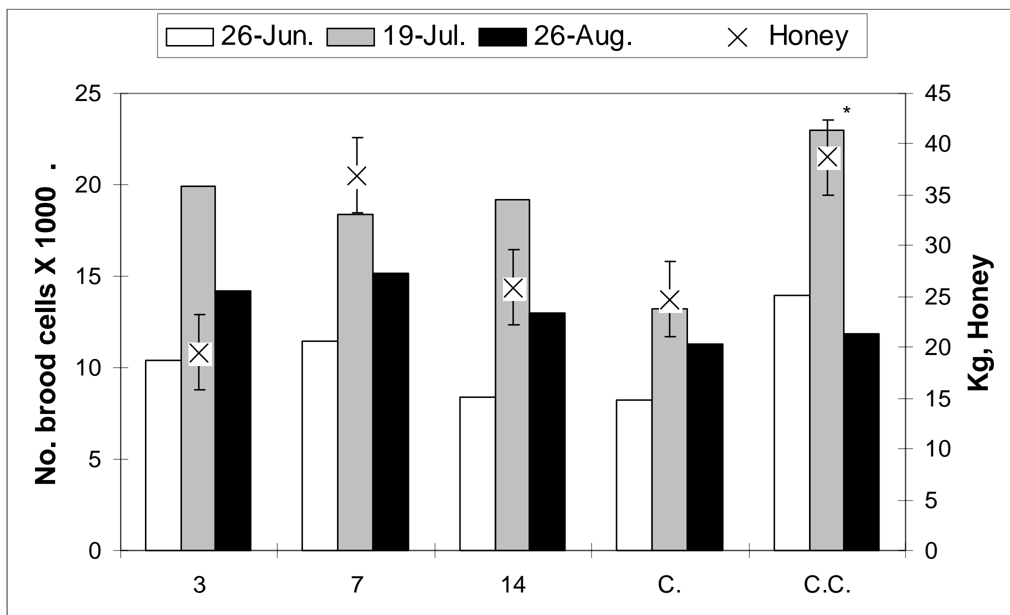

Figure 4. The development of brood in colonies dusted by sugar during July every 3, 7, and 14 days, control (C) and chemical control (C. C.). The dates show mean number of capped- brood cells in each experimental treatment during the same summer. A rough estimation of the honey yield ($\bar{x}$ ± SE) is shown in kg. *, different at P < 0.05.

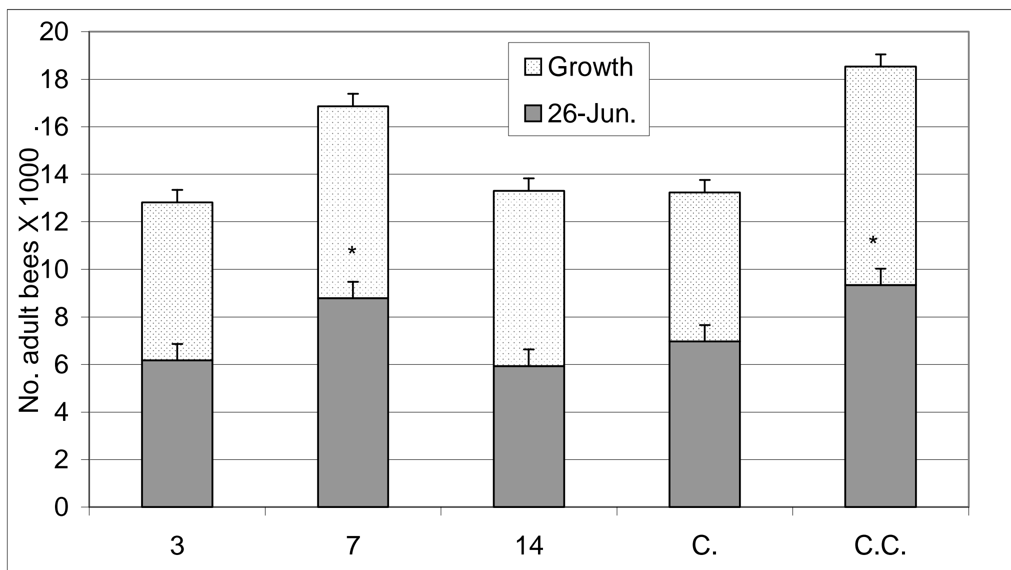

Figure 5. Number of adult bees ($\overline{x} \pm$ SE) in colonies treated by powdery sugar every 3, 7, and 14 days (n = 4, in each group), control (C) (n = 5) and chemical control (C. C.) (n = 6). The 26th June shows mean number of adult bees in each experimental treatment on that date. The growth = number of adult bees on 19th July minus the number of adult bees on 26th June in the same summer. *, different at $P < 0.05$.

The true estimation of mite population in the experimental colonies after the treatment period was not possible. Fries *et al.* (1991b) studied the population dynamic of *Varroa* in cold climate. However, it could be that the high variation between the colonies that they reported as unknown variation was due to interaction of *A. woodi* infestation (II). High infestation of up to 93 % was reported by Korpela *et al.* (1992). According to Furgala *et al.* (1989), when 30 % of the bees in a colony become infested by *A. woodi* the likelihood of winter survival decreased with a corresponding increase in infestation. The mortality of infested bees is significantly higher than uninfested bees (Bailey 1961). Individual bees are believed to die because of the disruption to respiration and damage caused by *A. woodi* to the tracheae, microorganisms entering the hemolymph through the damaged trachea (Shimanuki *et al.* 1992). In addition *Varroa* population does not remain intact. According to Müller (1987) and Fries *et al.* (1991b) the *Varroa* mite does not leave its host if the host dies during broodless periods. No other appropriate studies was known to the author to compare the mite downfall in order to estimate total mite population in colonies. A complimentary chemical treatment after the dust treatment period to determine the number of remaining *Varroa* in the colony was not also possible as the mites in control treatments were needed for the study of the following years. The exact efficiency of dusting to control *V. destructor* under field conditions needs further research.

4. SUMMARY

– *A. woodi* was discovered for the first time in Fennoscandia, among Finnish bees in June 1991. A second survey demonstrated that the mite had been in Finland as early as in 1986. This ended the controversies between acarologists regarding the absence of *A. woodi* in Fennoscandia. Before the mite discovery in Fennoscandia, some acarologists believed that the infested bees in prolonged winters do not survive long enough to infest young susceptible bees the next spring.

– The quantitative (% of mite individuals detected in a sample) and qualitative (detection % of infested samples) detection efficiency of *Varroa destructor* by the method developed in this study was >90 % and 100 %, respectively, when the infestation level was >3 mites/100 bees. These values for the currently used ether roll method with five times larger bee samples are 35 % and 12 %, respectively. Applying this new method I detected the mite for the first time in St. Kitts and Nevis (in the Caribbean) in August 2000, and found that the mean mite infestation level was only 4 %.

– Throughout the experiment, aerated dusting at the colony level demonstrated significant acceleration of mite fall in all sugar treatments. In the first sugar treatment (n = 4) the mean number of recovered mites was up to 56-fold compared with the level before the treatment. In laboratory trials, the mean rate of recovery of the total number of mites with direct and aerated sugar dusting was 91 % and 62 %, respectively, and this deference was statistically highly significant. No sugar particles were found in the T2 spiracles and their ducts indicating that the bees do not inhale the fine dust. Anaesthetisation had no significant impact on either the mite fall or the inhalation of sugar by the bees. Sugar dusting had neither side- effect on capped brood nor caused any supersedure of queen bees.

These results are important because:

– The results of the two surveys of *A. woodi* in Fennoscandia were fundamental for the understanding of its distribution in Finland, as well as for defining a rational reason for its long time absence in Fennoscandia.

– The quick field detection of the *V. destructor* is now possible during the spring time when the colonies are purchased. This method is also useful in finding *V. destructor* in areas where the mite has not been found before, as was demonstrated in St. Kitts and Nevis. Due to the high quantitative and qualitative efficiency, and the availability of the result in less than 11 s, it may supercede all currently available laboratory and field methods, in which sub-sample of imagos is the basis of the assessment.

– Another important result of the study was the successful development of the dusting method for the control of the *V. destructor*. Both methods of using confectioner sugar dusting were highly efficient for recovering the phoretic *V. destructor* from imagos of the bees in the laboratory trials. No adverse effect on the bees, capped brood and queen bees were found in the field studies with sugar dusting.

– The CO_2 anaesthesia caused bee mortality starting a few days after the treatment.

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