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WINTER MORTALITY OF HONEY BEE (*APIS MELLIFERA* L.) COLONIES: REDUCING THE IMPACT OF DIFFERENT FACTORS

MESILASPEREDE TALVISE HUKKUMISE VÄHENDAMISE VÕIMALUSED

KALLE TOOMEMAA

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Opponent: Dr. Otto Boecking, PhD
Institute for Apiculture Celle
Lower Saxony State Institute for Consumer Protection and Food Safety

Supervisor: Prof. Marika Mänd, PhD
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals in the text.

I **Toomemaa, K.**, Martin A.-J., Williams, I.H. 2010. The effect of different concentrations of oxalic acid in aqueous and sucrose solution on *Varroa* mites and honey bees. Apidologie 41: 643-653.

II **Toomemaa, K.**, Martin, A.J., Mänd, M., Williams, I.H. 2010. Using oxalic acid in water solution in control of *Varroa* mites and its influence on honey bees. Agronomy Research 8 (Special Issue II): 345-350.

III **Toomemaa, K.**, Martin, A.-J., Mänd, M., Williams, I.H. 2013. Determining the amount of water condensed above and below the winter cluster of honey bees in a North-European climate. 2013. Journal of Apicultural Research 52(2):81-87.

IV **Toomemaa, K.**, Mänd, M., Williams, I.H. Wintering of honey bee colonies in cylindrical nest cavities versus oblong box-hives in a North-European climate. Journal of Apicultural Research 54 (published online: 11 May 2016).

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Paper	Idea and study design	Data collection	Analysis of data	Manuscript preparation
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III	**KT**	**KT**	**KT,** MM	**KT**, IW, AM, MM
IV	**KT**	**KT**	**KT,** MM	**KT**, IW, MM

AM – Ants-Johannes Martin; IW – Ingrid H. Williams; MM – Marika Mänd; KT - Kalle Toomemaa

1. INTRODUCTION

The European honey bee, *Apis mellifera* L., has developed into many different geographical subspecies and various ecotypes, adapted to survive in a wide range of climatic and vegetation zones. Bees' basic production – honey – was and is an important international commodity. The global production of honey was estimated at 1.07 million tonnes in 2007 (FAO, 2009). Using the average 2006 US price for honey, $1168 per a tonne the global value of honey production in 2007 had an estimated worth of US$1.25 billion (vanEngelsdorp and Meixner, 2010). Important are also other bee-products (beeswax, propolis, bee pollen, royal jelly, bee venom) used mainly in the developing alternative apitherapy for the people.

Honey bees are the most important pollinators of wild and agricultural plants.The global plant production volumes show that 60% of global production comes from crops that do not depend on animal pollination, 35% from the crops that depend on pollinators and 5% are unevaluated (Klein *et al.*, 2007). The mean forage distance of honey bees is found to be 5.5 km in mid-August and only 1 km in May, when there are plenty of flowers available (Beekman and Ratnieks, 2000).The other data note the average distance of 2.2 km and the maximum distance of 10.9 km (Visscher and Seeley, 1982). It has been mentioned that, however, bees can travel more than 10 km in search of desirable floral rewards, they tend to forage within 2.0 km of their hive if there are attractive floral resources in the vicinity (Osborne *et al.*, 2001). The total economic value of pollination worldwide was estimated €153 billion, which represented 9.5% of the value of the world agricultural production used for human food in 2005 (Gallai *et al.*, 2009).

However, beekeeping is in decline. The consistent declines in colony numbers in central European countries and significant reduction in the numbers of beekeepers across Europe between 1985 and 2005 are found (Potts et al, 2010). Data from the USA show a pattern of steady decline in the numbers of managed colonies from a peak nearly 6 million in the 1940s and 4.5 million in 1980s to 2.4 million in 2005 and 2.3 million in 2008 (vanEngelsdorp *et al.*, 2007; Pettis and Delaplane, 2010; Ellis *et al.*, 2010). In European countries colony numbers decreased from over 21 million in 1970 to about 15.5 million in 2007 (FAO, 2009; vanEngelsdorp and Meixner, 2010). Twenty years ago, it was acceptable

to have 5 to 10% winter colony losses (Le Conte *et al.*, 2010). Since the autumn of 2006 disastrous colony losses have been reported in Europe and Northern America. The American beekeepers have reported the total losses on average of 30% in 2006/7 to 2012/13 (vanEngelsdorp *et al.*, 2007, 2010, 2011) and 23.7% in 2013/14 (Lee *et al.*, 2015). The average mortality calculation includes the colonies that died with the Colony Collapse Disorder (CCD) symptom "no dead bees in the hive or apiary", which percentage has ranged from 36 to 60% in the winters of 2007/8 and 2008/9 (vanEngelsdorp *et al.*, 2010) and 34.5% in 2013/14 (Lee *et al.*, 2015). In European countries losses have ranged from 6.3 to 22.0% in 2008/9 and from 8.0 to 37.8% in 2009/10 (van der Zee *et al.*, 2012). Periodic colony winter losses of 30% and more have been reported in Germany already for more than 50 years (Grädinger, 1984; Genersch *et al.*, 2010).

It was noted that even summer losses may be noticeable in some regions and if losses were not assessed over the full year, the winter losses would have grossly been underestimated in the total yearly mortality (Lee *et al.*, 2015).Thus, for example, in the United States the total winter losses were reported to be 30.6% and the annual total loss of 45.2% in 2012/13 (Lee *et al.*, 2015) and 28.1% and 44.1%, respectively, in 2015/16 (Steinhauer *et al.*, 2016)

This is not the first time beekeepers have suffered large regional colony losses without any known reasons. The periodical colony losses ("disappearing disease") have been registrered since 1869 in Europe, America and Australia and the cause of the losses was generally unknown (Underwood and vanEngelsdorp, 2007). In 1960's it was noted that sometimes colony populations literally disappeared within a short time; only a "handful" of bees was left; honey stores were present; small amounts of pollen were sometimes present although pollen was generally absent; brood rearing was almost non existent; and cheks for pesticide residues were negative (Oertel, 1965).

Beekeepers have reported various causes of winter losses: poor wintering conditions, starvation, weak in the fall, queen failure, *Varroa destructor,* pesticides, Colony Collapse Disorder, *Nosema* spp., disaster, unknown and other reasons. Common causes of death written in the "other"category were wasps, ventilation/moisture, wax moth, swarming and robbing (Lee *et al.*, 2015). VanEngelsdorp *et al.* (2008) adds to this list

management mistakes, nutrition, stress and viruses as “other” factors. It was noted that the colony losses did not differ between the beekeepers classified by the operation size (managed less or more colonies)(Lee *et al.*, 2015)or larger operations (150 colonies and more) had significantly lower losses than the smaller operations (van der Zee *et al.*, 2014). No differences were found between the beekeepers who moved or did not move colonies into the almond groves for pollination (vanEngelsdorp *et al.*, 2008; Lee *et al.*, 2015). But regions with lower average temperatures experienced higher losses (vanEngelsdorp *et al.*, 2008). Both of these studies refer strongly to the threats originating from poor management practices. Among the top five reasons vanEngelsdorp *et al.* (2008) named two and Lee *et al.* (2015) even four mistakes of the beekeepers as courses of colony deaths.

Winter is the greatest challenge to the honey bee colonies in the northern climatic conditions. Poor overwintering leading to weakening and dying of bee colonies is a well known problem in beekeeping, especially in the northern countries. Colonies usually perish either in winter, before wintering or after it in spring. In case of bad overwintering the metabolic rate of bees is increased, which, in turn, resulting in a higher temperature in the winter cluster, increased food consumption, exhaustion of the bees, shortening of their lifespan, overfilling of their hindgut, diarrhoea, nosemosis and excessive moisture in the hive. This indicates the need to investigate more profoundly the bees' wintering biology, more suitable vitality sustainable wintering conditions, as well as soft varroosis treatment methods, substances and their different treatment regimens. Little is known about how to achieve minimally onerous metabolic rate for the bees in winter and which internal and external factors are working against it and which winter conditions would be more suitable for wintering bees, especially in northern countries. The present thesis examines the ways to reduce the overwintering bees' exhaustion through varroosis as well as varroosis control and adverse wintering conditions.

2. REVIEW OF THE LITERATURE

2.1. Essential factors influencing the survival of honeybees in winter

2.1.1. Varroosis

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000) is the most detrimental honey bee parasite in the world today (Rosenkranz *et al.*, 2010). It is noted that regions with established mite populations had consistently higher losses than those without (Dahle, 2010; Neumann and Carreck, 2010). So *Varroa* mite has been reported by many of researchers as the leading cause for the current colony losses (Topolska *et al.*, 2008; vanEngelsdorp *et al.*, 2008; Dahle, 2010; Genersch *et al.*, 2010; Guzmán-Novoa *et al.*, 2010; Le Conte *et al.*, 2010; Neumann and Carreck, 2010).

The reproductive rate of *Varroa* mites (the number of viable offsprings per mother mite) has been calculated of 1.3-1.45 in single infested worker brood and 2.2-2.6 in drone brood (Martin, 1995). *Varroa* female can perform under field conditions 2-3 reproductive cycles during her life time (Fries and Rosenkranz, 1996; Martin and Kemp, 1997). During the summer, up to 90% of the mite population can be within the brood (Rosenkranz and Renz, 2003). The population of mites in the colony can increase during a summer up to 100 times in a climate with a brood-rearing period of less than five months (Liebig *et al.*, 1984; Fries *et al.*, 1991) and sometimes even up to 300 times (Kraus and Page, 1995). It was found that the amount of mites in the colony can double within each 20 days (Fries *et al.*, 1994) and a large variation in mite population increase existed between colonies (Fries *et al.*, 1991). Conditions of weather and foraging may also influence the number of mites. During the periods with a low nectar flow and, therefore, due to high robbing activities, strong colonies may significantly increase their mite population (Rosenkranz *et al.*, 2010). It has been experimentally shown that highly infested honey bee colonies present a substantial risk to already treated colonies up to distance of 1.5 km away (Frey *et al.*, 2011). Global warming can induce longer spring and fall periods resulting in the longer brood rearing periods and more drone brood, both of which lead to the significantly larger mite population at the end of the year (Le Conte and Navajas, 2008). Prediction to that extent of a starting mite population in the spring will increase until autumn is still not possible (Rosenkranz *et al.*, 2010).

Under the temperate conditions untreated colonies may collapse due to varroosis 3-4 years after the initial infestation by a few mites (Korpela *et al.*, 1992; Büchler, 1994). In the initial phase of *Varroa* establishment symptoms in the 1980s it was often found 7000-11 000 mites in colonies without a dramatic outcome (Ritter and Perschil, 1982; Fries *et al.*, 1994). Today even about one thousand mites in a colony of 10 000 bees is considered to be a danger for the winter survival of the colony (De Jong, 1997; Siede *et al.*, 2008). The bees' tolerance is reduced and it is associated mainly with the viruses distributed by the *Varroa* mites. Damage at the colony level mainly appears during the autumn and the winter, when the host population declines, the relative parasitization increases and the long-living winter bees are damaged (Amdam *et al.*, 2004).The loss of bees starts already during the early autumn when individual bees from highly infested colonies need longer time to return or do not return at all, their loss per flight is higher compared to a less infested colony (Kralj and Fuchs, 2006). Low*V. destructor* infestation levels before and during the transition to winter bees result in an increase in lifespan of bees and a higher colony survival compared to the colonies that were not treated and that had higher infestation levels (vanDooremalen *et al.*, 2012). In the pupae phase of developing bees the parasitizing mites cause the loss of hemolymph volume and the protein content and the significant decrease of the weight of the hatching bees. Even single infestation may be caused in an average loss of the body weight of 7% for the hatching bee (De Jong *et al.*, 1982). Adult bees, infested as pupae, do not fully develop the physiological features of long-lived wintering bees, their vitellogenin titer, the total protein stores in the hemolymph, the hemocyte characteristics and the ectysteroid titer are changed, thus they have a significantly reduced life span and many of them do not survive until spring (Amdam *et al.*, 2004).

Varroa mite has an ability to transmit various honeybee viruses. The co-infections *V. destructor* with viruses has been shown to be a major role in the *Varroa*-induced honeybee colony collapse (Martin, 2001). Best known is the Deformed Wing Virus (DWV) infection causing the typical symptoms of crippled wings and the shortened abdomen in heavily infested colonies (Boecking and Genersch, 2008). The high viral titre of this virus causes fatality in around 21% of the infested developing brood, and the bees with wing deformity die within 48 hours of emergence (Martin *et al.*, 2013). Acute Bee Paralysis Virus (ABPV)

infection also shows a strong correlation between high fall mite loads, viral loads and increased winter mortality (Siede et al, 2008). It was found that colonies continue to collapse in winter with high DWV or ABPV titres even though *V. destructor* had been successfully eliminated during late summer and autumn treatments (Higfield *et al.*, 2009; Genersch *et al.*, 2010). So, both DWV (Highfield *et al.*, 2009) and ABPV (Genersch *et al.*, 2010) can potentially act independently of *Varroa* mites and may be a major factor in overwintering colony losses. Thus, both DWV and ABPV are clearly related to the colony losses in autumn and winter (Genersch *et al.*, 2010). According to some sources slow paralysis virus (SPV) is also associated with the loss of infested colonies (Carreck *et al.*, 2010). Israeli acute paralysis virus of bees (IAPV) was found to be strongly correlated with CCD (Cox-Foster *et al.*, 2007). It has been mentioned that the immunity of winter bees is poorly understood and higher colony losses in winter are associated with decreased cellular immune that may have increased honey bees' susceptibility to viruses (Steinmann *et al.*, 2015).

2.1.2. Nosemosis

Nosemosis, a disease of the digestive tract of honey bees, caused by the spore-forming intracellular parasite *Nosema apis* Z. It is correlated with the winter loss of the queens and colonies, poor spring build-up and reduced honey yield (Fries, 1993). The infection has a low level during the summer, a small peak in the autumn and a slow rise during the winter. In the spring the level of infection increases rapidly as brood rearing starts while flight possibilities are still limited (Fries, 1993, 1988). *Nosema* spores, ingested by bees, germinate in the ventriculus, causing inflammation and damage to the gut epithelial cells. The digestive proteolytic activity of the midgut is deceased, showing impaired protein metabolism (Malone and Gatehouse, 1998). Infected bees have a significantly reduced lifespan and the development of their hypopharyngeal glands is affected (Wang and Moeller, 1971), they have lower levels of proteins in the fat bodies (Lotmar, 1939) and decreased levels of proteins and fatty acids in the haemolymph (Tomaszewska, 1979). When the queen becomes infected, her ovaries degenerate and her egg laying capacity is reduced (Liu, 1992). Such queens are superseded by the bees during brood rearing season, but in winter they often perish (Fries, 1993). The resistance of infected bees to various infections is lowered; several types of viral infections are correlated with *N. apis* (Bailey, 1982). Dysentery,

that often occurs, is an important factor in spreading the disease in the colony, but it was found that *N. apis* is not the natural cause of dysentery or the prime cause of death of colonies (Bailey, 1967). No differences in *N. apis* infection levels were found between the colonies that died and those that survived (Bailey, 1967; Guzmán-Novoa *et al.*, 2010). Two natural factors are found to reduce the level of *Nosema* infection: the emergency of brood allows the replacement of infected bees with healthy young bees; winter flights reduce the amount of defaecation in the hive, and help to eliminate diseased bees by chilling them in flight (Moeller, 1972).

Another species of the genus *Nosema* – *N. ceranae* as a pathogen of the Asian bee *Apis ceranae* has been described (Fries *et al.*, 1996). *N. ceranae* has been present in the US since at least 1995 (Chen *et al.*, 2007) and in Europe (Finland) since 1998 (Paxton *et al.*, 2007). However, in 2005 *N. ceranae* was isolated in European worker honey bees from colonies suffered unexpected population loss in Spain (Martin-Hernándes *et al.*, 2005; Higes *et al.*, 2006). The detection of the parasite in the European honey bee *A. mellifera* may not have been related to its jump to *A. mellifera* but rather to the development of new highly sensitive and specific molecular tools that allow distinguish the two species from each other (Higes *et al.*, 2010). Today *N. ceranae* is widespread in most European countries (Higes *et al.*, 2010; Klee *et al.*, 2007). It has been reported to cause severe colony losses, especially in Southern Europe (Higes *et al.*, 2007). However, in other studies the infection by *N. ceranae* does not seem to be so harmful (Gómez *et al.*, 2008; Aronstein *et al.*, 2011). It has been mentioned that if *N. ceranae* is so virulent as to kill colonies in 18 months, then it is surprising that greater colony losses have not been reported from across Europe, given that *N. ceranae* has been in the continent since at least from 1998 (Paxton, 2010). This disease has a long asymptomatic incubation period, some clinical features are: a longer breeding period during cold months; a higher proportion of frames containing the brood relative to nurse bees during the warm months, and diminished honey production. Finally, infected colonies become clearly weakened and depleted of adult bees and they collapse in a period of 1.5-2 years (Martin-Hernándes, 2007; Higes *et al.*, 2010). It was shown that the heavily infected bees (foragers preferably) do not return to the colony and it is interpreted as a general response of honey bees to diseases to decrease the pathogen load within the colonies (Kralj and Fuchs, 2009).

2.1.3. Pesticides in the honey bee hive

Beekeepers use a wide range of different chemical substances and application methods to reduce mite populations in colonies. For controlling of varroosis synthetic acaricides (miticides, varroacides) like organophosphate coumaphos, the pyrethroids tau-fluvalinate and flumethrin and also formamidine amitraz are widely used. Organic acids and essential oils represent the natural compounds used for the control of varroosis and the use of them as an alternative varroa control is usually less toxic to bees and doesn't lead to increased residues in beeswax and honey. Also, it is generally thought to have a low risk of genetic pesticide resistance (Dietemann *et al.*, 2012). *Varroa* mites have developed resistance towards the synthetic acaricides (Milani, 1995, 1999; Elzen *et al.*, 2000) and this favours the escalation of chemical applications leading to increased doses and the residues of miticides in the hive (Le Conte *et al.*, 2010). The situation is aggravated by synergistic interactions between various in-hive miticides. It was shown that a large increase in the toxicity of tau-fluvalinate to young bees that had been treated previously with coumaphos had taken place. So the observed synergism may lead to bees' mortality with the application of sublethal doses of miticide when both miticides are simultaneously present in the hive (Johnson *et al.*, 2009). Miticide residues can become even more toxic when combined with agricultural crop pesticide residues when the contaminated pollen loads are brought back to the hive by the foragers (Pilling *et al.*. 1995; Chauzat et al, 2006; Mao *et al.*. 2011; Johnson *et al.*, 2013).

Different beehive matrixes have been analysed for discovering pesticide residues. The amount of pesticides contained in beeswax, pollen samples, nectar, honey and bees themselves has been studied. In various studies 36 -121 different pesticides and metabolites were found (Chauzat *et al.*, 2009; Generch *et al.*, 2010; Mullin *et al.*, 2010; Pohorecka *et al.*, 2012; David *et al*., 2015), but no statistical relationship between colony mortality and pesticide residues was found (Chauzat *et al*., 2009). Many laboratory studies described the lethal and sublethal effects of neonicotinoids on the foraging behavior and the learning and memory abilities of bees, while no effects were observed on the bee mortality, brood development, strength and honey yield in the field studies at field-realistic dosages (Blacquière *et al.*, 2012; Pohorecka *et al.*, 2012). Pistorius *et al* (2015) found the increase in honey bee mortality in field realistic dose of clothianidin containing dust. Still too little work has been done to examine the pesticide effect on

the overwintering success (Döke *et al.*, 2015). It has been demonstrated that chronic exposure during the 33 summer days to imidacloprid in the sugar syrup did not affect the overwintering of colonies (Faucon *et al.*, 2005) and the assessment of overwintered colonies in spring found no differences in bee mortality, worker longevity and brood development in the colonies that foraged on flowering canola (*Brassica napus*) grown from the seed treated with clothianidin (Cutler and Scott-Dupree, 2007). Dively *et al* (2015) has demonstrated the potential of higher field realistic doses to affect long time colony survival, however in commonly found weaker doses no effet was observed. Among all these studies most often found pesticides in bee-products, especially in the comb and foundation wax samples, were various acaricides used for the control of *V. destructor*: tau-fluvalinate, coumaphos, amitraz, in some cases chlorfenvinphos (Mullin *et al.*, 2010; Pohorecka *et al.*, 2012; Genersch *et al.*, 2010; Orantes-Bermejo *et al.*, 2010). However, synergism of insecticide toxicity by fungicides, yet the combinations of herbicides with fungicides and insecticides in 3 or more component mixtures was not studied for long time (Mullin *et al.*, 2010). For now, there is at least one study analysing the effects of four pesticides together (Zhu et al., 2014).

Interactions between pesticides and pathogens could be a major contributor to the increased mortality of honey bee colonies (Pettis *et al.*, 2012). However, it was found that the honey bee colonies, located in the neonicotinoid treated cornfields, expressed significantly higher pathogen infection than those located in the untreated cornfields (Alburaki *et al.*, 2015). *Nosema* infections increased significantly in the bees from the colonies fed with imidacloprid in dosages below the harmful levels to bees (Pettis *et al.*, 2012). Exposure to sublethal doses of fipronil and thiacloprid highly increased the mortality of *Nosema ceranae*-infected honey bees, but had no effect on the mortality of uninfected honey bees (Vidau *et al.*, 2011). It has been mentioned that neonicotinoids indirectly weaken the honey bee health by inducing physiological stress and increasing pathogen loads (Alburaki *et al.*, 2015).

2.1.4. Decreasing genetic variability and hybridization

High genetic variability within the colony is known to be important for resistance to many diseases (Tarpy, 2002), for stable nest thermoregulation (Jones *et al.*, 2004) and for overall colony fitness. It was found that

swarms from genetically diverse colonies, in comparison with swarms from genetically uniform colonies, founded new colonies faster, had greater foraging rates, food storages, population growth and winter survival (Mattila and Seeley, 2007). However, professional specialists in queen breeding produce and distribute a large number of progeny from few queen mothers, which inevitably reduces genetic variability in honey bee populations (vanEngelsdorp and Meixner, 2010). Genetic similarity among colonies in wide areas increases the chances of successful disease transmission, and therefore the risk of the colony losses (vanEngelsdorp and Meixner, 2010).

The distribution of the preferred commercial subspecies as more productive stocks outside their native range is widely used in the practice of modern beekeeping. It has been mentioned that genetic diversity can be lost rapidly as native populations are threatened by newly introduced parasites or replaced by the imported stock (vanEngelsdorp and Meixner, 2010). Large parts of the original European dark honey bee (*A. m. mellifera*) areas in Western Europe are today occupied by the introduced stock with more desirable apicultural traits (vanEngelsdorp and Meixner, 2010).

The southern subspecies, introduced into northern regions, are suffering from the stress which is linked to the acclimatization to the new ecological-climatic conditions (Benkovskaya, 2008). The European dark honey bee (*A. m. mellifera*) has a specific level of various metabolic processes, which are justified in these natural climatic conditions, since a higher level of the protective system response is necessary in the case of the threatened breach of homeostasis, ensuring response adequacy. The activity of catalase was significantly higher in the local northern subspecies than in the native bees from southern regions (Zherebkin, 1979). The higher metabolic rate that is constantly observed in introduced southern subspecies will contribute to the emergency of stronger pathogenetic processes during the development of stress reaction and reduce viability under the natural climatic conditions of the northern part of the species' area (Farooqui and Farooqui, 2011). The colonies with local queens survived on average 83 days longer without any therapeutic treatment against diseases than those with non-local queens (Büchler *et al.*, 2014). Introgressive hybridization modifies the genetic pool of the local honey bee populations, leading to the loss of their genetic identity, but the mentioned study has shown that the use of local honey bee populations provides a higher chance of colony survival compared to the maladapted bees (Büchler *et al.*, 2014).

In the case of dark honey bee cross-breeding with southern subspecies the historically developed genetic structures of geographically isolated wild populations are broken. Such hybrids constitute a set of endlessly diverse genetic structures' fragments. Upon further crossing of the hybrids, breaking of the initial structures grows several times (Gubin, 2002). In other words, the coadapted genetic complexes of the parents' genotypes are disrupted which results in the decreased viability and reduced resistance to unfavourable influences (Benkovskaya, 2008). The productivity of bee colonies falls sharply. It is noted that such hybrid colonies have low winter hardiness and reduced resilience to nosemosis, foulbrood diseases and other diseases that provoke the death of colonies in winter and even in summer. They build more swarm cells, swarm more often and the prime swarms often do it repeatedly. Anti-swarming methods are ineffective even in good harvest (Shunk, 2005). Hybrid bees are often with heightened aggressiveness (Gubin, 2002). The only bred ecotype (actually a hybrid) – the Buckfast bees – often becomes very aggressive in the case of natural mating with local bees after two or three generations. They are able to be worked with only after replacing their queens with the original ones every year (Galkina and Kadora, 2014).

2.2. Biology of wintering of honeybees

2.2.1. Physiological changes

The body weight of winter bees born in autumn is 16-26% bigger compared to that of summer bees. It is noted that at the time, when there is no brood rearing in the colony, the young bees feed intensively on the stored pollen and it contributes to the accumulation of nutrient reserves in the body (Zherebkin, 1979). Autumn bees have more proteins, fats, carbohydrates in the head, thorax and abdomen which raise the bees' winter security. It has been found that in strong colonies the body-weight of autumn bees is 5.5% higher and there are more nutrient reserves in their bodies than in weak colonies (Ulanovski, 1991). The hypopharyngeal glands, the fat body and the ovaries of autumn bees are noticeably more advanced which shows the accumulation of nutrient reserves in them (Zherebkin, 1979). Most nutrients accumulate in the fat body which reaches its maximum development in late autumn when permanent frosts arrive and decline to minimal by the end of the winter season. In weak colonies or in the colonies with less favourable conditions the nutrient reserves shrink to the almost zero-level by spring (Moskalenko, 1982).

The longevity of the individual workers varies with the season. The life of summer bees lasts only about 30 days, but winter bees are characterized by a long life of 6 to 8 months (Fluri *et al.*, 1982; Mattila *et al.*, 2001) thus up to 10 times longer (Münch *et al.*, 2013). When brood rearing ceases, and with adequate food supplies available, the protein reserves in the bees' bodies rise, the hypopharyngeal glands and fat bodies attain full development, and the length of life increases; they become, in fact, long-lived winter bees (Maurizio, 1950). So a lifespan is strongly dependent on the nurse load. The lack of brood can induce colonies to transition to their wintering physiological state. The glyko-lipoprotein vitellogenin extends the worker bee lifespan by functioning in free radical defense, immunity and behavioral control. Brood rearing or pre-wintering exposure to the brood pheromone produced by developing larvae, reduces worker vitellogenin stores and colony's long-term survival (Smedal *et al.*, 2009). The colonies in which brood rearing ceased earlier showed earlier shifts in worker survival, but all winter bees died at around the same time in the next spring, regardless of the time of emergence (Mattila *et al.*, 2001). The colonies with extended pollen supply produced more workers throughout autumn than the colonies with less pollen, but the development of the population of long-lived winter bees was delayed until relatively later in autumn (Mattila and Otis, 2007a). Similar numbers of winter bees survived until spring in the colonies and bees had similar physiology and brood-rearing efficiencies (Mattila and Otis, 2007b).

The dry weight of the hypopharyngeal glands of summer bees showed distinct changes in the ageing bee: 3-21 day- old nurse bees had significantly heavier glands than the newly emerged bees and 26-40 day-old forager bees. The hypopharyngeal-gland dry weights of winter bees remained in a well-developed condition and the bees were slightly lighter than those of nurse bees in summer and heavier than those of forager bees (Fluri *et al.*, 1982). It was suggested that the glands are involved in protein storage during winter while in summer they secrete proteins for feeding larvae and the queen, as well as enzymes for the preparation of the food storage of the colony (Fluri *et al.*, 1982).

The level of juvenile hormone and vitellogenin in the hemolymph of summer and winter bees is significantly different. It was found that the juvenile hormone titre increases continuously in the hemolymph of the ageing summer bees, but winter bees keep a constant low level of this

hormone, however, at the end of winter it rose from a low to a high level, comparable with the high titre of 20-40 day-old summer bees (Fluri *et al.*, 1982).The content of total haemolymph proteins, as well as vitellogenin, is clearly higher in winter bees than in summer bees. At the end of winter, when colonies begin to rear brood, the titres of total haemolymph protein and of vitellogenin fall, and the dry weight of the hypopharyngeal glands diminishes, but the titre of juvenile hormone increases. These physiological variables of overwintered bees are similar to those observed in the 24-40 day-old summer forager bees (Fluri *et al.*, 1982). Vitellogenin is the most abundant haemolymph protein (32-40% of the total proteins) and its level shows the protein status in the bee's organism. However, very large differences in the amounts of vitellogenin within the bees of a colony and between the colonies were found (Otis *et al.*, 2004). It was suggested that protein nutrition may affect the colony health quite considerably and good protein nutrition may enable bees to tolerate the effects of haemolymph loss because of varroa mites and also good functioning of the bee's immune system, enabling it to resist microbial infections (Otis *et al.*, 2004).

The activity of enzymes (peroxydase, dehydrogenase and catalase) in honey bee remains high in winter. Catalase has an important role in the body's oxydizing processes. It protects the organism from the putrefaction processes in the hindgut and reacts with any toxic hydrogen peroxide that is formed, producing water and oxygen. As digestion remainders gather in the hindgut, ferment's activity increases during the winter but it drops rapidly in the case of the nosemosis onset or on diet of honeydew (Zherebkin, 1979). The bees, who have a high catalase activity in autumn, winter well without diarrhea and there would be low number of dead bees in the hive during the end of wintering. The catalase activity in the intestine is found to be highest in the northern countries with cold winter, where the bees form a tight winter cluster and it has found to be about 2 times weaker in the southern regions, where bees do not form any lasting close cluster and have a chance to make cleansing flights in winter (Zherebkin, 1979). If the southern domestic bees are taken into the northern regions with harsh climate, then in the new ecological conditions they retain the inherent physiological parametres, including the lower catalase activity and the associated weak winter hardiness (Zherebkin, 1979). So some honey bee races with a greater level of catalase are better adapted to withstand the rigours of winter.

The bees' autumn transition from the active state to the passive state is accompanied by a sharp decline in the intensity of respiration.The resting metabolic rate (Kovac et al., 2007)and similarly oxygen consumption (Eskov, 1995) of honey bees increase steeply with ambient temperature. The rate of CO_2 production increases ten times from 5°C to 38°C (Kovac *et al.*, 2007). The oxygen consumption of bees from average and elder age groups was found to increase 67 times from 10°C to 30°C and 218 times from 0°C to 30°C (Eskov, 1995). Low temperatures reduce the enzyme activity connected with aerobic respiration and against the background of the sharp drop in the metabolic rate dehydrogenases take the lead (Zherebkin, 1979). However it is hard to compare the metabolic chracteristics of individual bees measured one-by one with those staying in the middle of winter cluster with very different conditions. It is noticed that in the central part of the cluster during the first half of the winter the concentration of CO_2 in air may be on the level of 3.5-4.5% for a longer period (a few weeks), rarely for a short time (a few hours) up to 6-7% while it is accompanied by O_2 drop of about 3-4% (Eskov, 1995). In another study the 5-6% level of CO_2 in small colonies has been reported to maintain uninterrupted throughout the winter (vanNerum and Buelens, 1997). The atmosphere usually contains 21% of O_2 and 0.03% CO_2. Studies have shown that the elevated CO_2 concentration in bees' winter cluster encourages metabolic processes to slow down, which finds its expression in less food consumed in winter, decreased amount of faeces in the hindgut and the bees' better viability and work fitness in spring (Zherebkin, 1979; Eskov, 1995).

In the preparation for wintering, the water content in the honeybee body is reduced on average by 2.2% (Zherebkin, 1979). It is noted that in autumn bees the amount of free water in the frontal part of the body decreases 9.2% and in abdomen 11.6% but the amount of bound water in the cytoplasm of the cell grows 5.3% and 8.8%, respectievely (Martynov and Haritonov, 1985).

Various physiological modifications enable bees to recycle water in the individual bees and within the cluster. When honey is ingested and digested, its water content may be absorbed from the midgut by the haemolymph which surrounds the digestive tract. This may result in the loss of water through the walls of tracheae into the air within the cluster (transpiration)(Johannson and Johannson, 1979). The water, not absorbed in the midgut, is retained in the hindgut, where it forms a re-

serve that is utilized by osmotic diffusion when the water content of the haemolymph becomes too low. The water, used by the bees for diluting honey, producing digestive secretions, and flushing waste nitrogenous products from the Malpighian tubules into the hindgut, can also be re-used by reabsorption (Altmann and Gontarski, 1961; Johannson and Johannson, 1979). It is noted that in the mantle of the cluster where bees are tightly pressed against each other and, therefore the ventilation is aggravated, the humidity is likely to be very high. Such conditions are unfavorable for the water to evaporate from the bees' tracheas (Komissar, 1981). The evaporation within the winter cluster decreases very rapidly from the centre towards the periphery, so the bees in the core will be dehydrated, while the bees in the shell will be hydrated (Omholt, 1987). It has been suggested that with food transfer from the periphery, where honey is usually stored, to the centre, simultaneous transport of the water contained in honey may occur from hydrated bees to dehydrated bees (Omholt, 1987).

Bees have different strategies to cope with excessive water. It is noted that to get rid of excess water, the bees are able to raise the temperature of the exhaled air, i.e. – to produce additional heat (Komissar, 1981). The most intensive water removal takes place in the case of active ventilation seen in poor wintering when the majority of bees are in the active state and release heat but the cluster shell is thin, consisting of a small number of less active bees (Komissar, 1981). Activation is always accompanied by significant energy losses because any air movement in the hive increases the heat loss and too strong ventilation removes heat from the hive and forces bees to increase their metabolic rate to compensate for it. Another way of getting rid of excess water is brood rearing in winter. It has been suggested that brood rearing in the winter cluster is likely to be a strategy which the bees have adopted in order to reduce their own water content when it has reached a specific level (Omholt, 1987). Partially it is confirmed by the experiment where brood rearing was initiated at 11°C by supplying additional humidity to the indoor air and keeping a relative humidity at 85% level (Pirker, 1987).

Theoretically the consumption of 1 g of honey produces 0.68 g metabolic water (Cheshire, 1879; Frisch, 1921; Wedmore,1947; reviewed by Johannson and Johannson, 1979). The absolute air humidity in a honey bee winter cluster has been reported to be 11.5-17.0 g/m^3 in the first half of the winter and 13.0-18.5 g/m^3 in the second half of the winter (Simp-

son, 1950). The relative air humidity in the unoccupied by bees areas inside the hive reduces with the decreasing of ambient temperature. It was measured 79% at -10°C and 67% at -22°C above the cluster of bees (Eskov, 1995). Air humidity in the hive varies highly in the area close to the entrance where it depends directly on the ambient air humidity. The same has been observed on the wall opposite to the entrance, on the floor and on the combs close to them (Eskov, 1983).

2.2.2. Behavioural changes

Low temperatures in the periphery of the hive in autumn force the bees to move inwards to the space between the combs where they start clustering as well as entering the empty comb cells. The bees' aggregation density is the highest at the lowest part of the cluster because it is coldest there and lowest in the centre of the cluster. The abundance of bees is the greatest in the central comb streets and it decreases in the direction of the hive's peripheral comb streets. Thus the shape of the formed cluster of bees is approaching an ellipsis in shape, having shifted towards the front wall (entrance) of the hive (Eskov, 1995).

In the winter cluster we can distinguish the centre or core of the cluster according to the temperature in it, where the temperatures stay between 14-27°C and with its diurnal fluctuations up to 10°C (Zhdanova, 1962) and the periphery or the mantle of the cluster with the temperatures of 6.5-12.0°C (Owens, 1971) or 6.5-14°C (Zhdanova, 1962). The thickness of the mantle is 2.5-7.5 cm depending on the outside temperature and the bees are packed there thorax-to-thorax with a multitude of interlacing thoracic hairs, and with heads pointed inwards to the centre (Southwick, 1983). A large part of them is located, if possible, in empty comb cells. In the case of the falling temperature the amount of comb cells, filled with bees, increases (Lvov, 1954).

A low mantle temperature also reduces the loss of water from all of the bees in the cluster (Omholt, 1987). The bees on the periphery of a cluster are older individuals with a lower temperature preference and a greater thermoregulatory ability than the younger bees placing in core (Allen, 1955). Unusual cluster movements sidewards or downwards and back on several occasions were registrered and it was suggested that it had been the transfer of honey into clustering area or with the expansion of the cluster (Owens, 1971; Zhdanova, 1962; Harchenko, 1980). It

was noted that bees can maintain cluster temperatures more easily if the colony is well insulated (Owens, 1971; Szabo, 1985) and that the colonies with large entrances are damaged more by the external environment (Szabo, 1985).

The bees in the interior of the cluster produce the majority of the heat and the bees on the periphery of the cluster act as an insulating layer. Bees actively produce heat by shivering their flight muscles without moving their wings (Moritz and Southwick, 1992). It was found that the abundance of endothermic bees is highest in the core and decreases towards the surface, however at least 8% of weakly endothermic bees were found on the surfaces of winter clusters (Strabentheiner *et al.*, 2003). The study of the swarm clusters has shown that the bees on the swarm mantle regulate their own, and hence swarm mantle temperature, affecting the swarm size, porosity and internal convection currents (Heinrich, 1981). In the other study it was suggested that the insulating mass of the winter cluster is not passive but thermogenetic (vanNerum and Buelens, 1997). It was mentioned that in order to optimize the energetic investment, the core bees should know about the thermal needs of the bees sitting closer to the surface and the heat produced by endothermic core bees is of benefit for the thermal comfort of themselves and their sisters on the cluster periphery (Strabentheiner *et al.*, 2003). It is in accordance with the superorganism model of the winter cluster thermoregulation which says that the active thermoregulatory behaviour is performed by the bees on the mantle (Moritz and Southwick, 1992).

Heat loss is minimized during colder ambient temperatures by reductions in the cluster size. Tight clustering can reduce the effective area of heat exchange by as much as 88% (Southwick, 1983). After maximal cluster compaction, when temperature continues to fall, the bees increase metabolic heat production in order to maintain a reasonable cluster core and the surface temperature (Southwick and Heldmaier, 1987). The measurements of the cluster volumes at various temperatures has shown the decreases of about 55% in the cluster volume and 40% in cluster surface area as ambient temperature decreased from 4°C to -23°C (Severson and Erickson, 1990).

The minimal metabolic rate of bees in the winter cluster has been found to be 4-8W/kg at the ambient temperature of 5-10°C. It rises below 5°C and above 10°C (Southwick, 1983). Similar optimally suitable

wintering temperatures are provided by other investigators (McCutcheon, 1984; Eskov, 1981; Free and Simpson, 1963). In ideal conditions a wintering honey bee consumes 2-2.1 mg of food daily. Practically, this is rarely achieved and mainly because the wintering conditions are not ideal. Therefore, the consumption of food per bee is usually about 3 mg a day, or 8-10 kg of honey per colony weighing 2kg in the period of 150 days (Komissar, 1994).

Under deeper ambient hypoxia (15% and less of oxygen) the bees in a small cluster (300 g) switched to the ultra-low metabolic rate with a low cluster temperature. This status persisted at least several days, and was terminated under normal oxygen at 15°C (vanNerum and Buelens, 1997). It was suggested that the conditions triggering this collective phenomenon remain as yet unknown but it ables bees to reconcile warm wintering in an alert state, with water and energy saving and a prolonged lifespan (vanNerum and Buelens,1997).

2.2.3. Attempts to improve overwintering by beekeeping management

Farrar has said that the cluster does not heat the unoccupied space in the hive. During a protracted cold period the temperature in this space will become almost as low in a well insulated hive as in one with no protection (Farrar, 1973). A number of comparative studies, however, recognize the need of insulation, especially in northern conditions. It is noted that in Norway the average food consumption in 60 mm-walled beehives was 38% less in winter than in the hives 22mm thick walls (Villumstadt, 1974). In Poland the food consumption in cold hives was 0.5-1.1 kg higher than in warm hives (Bornus and Nowakowski, 1974). The food consumption was found to be 0.5 kg per comb in Langstroth insulated hives and 0.65 kg in the hives not insulated (Zmarlicki, 1978). In the experimental cold chamber at the temperature of -18°C, 2-3 times more frost and ice was formed on the inner walls of the thin-walled hives (Villumstadt, 1974). There was also more moisture on the honeycombs and on the walls of cold hives (Bornus and Nowakowski, 1974; Zmarlicki, 1978). The weakening of colonies in winter was usually bigger in cold hives and their development in spring was significantly slower than in the warm ones (Villumstadt, 1974). But there was no difference in the bee-production in summer (Villumstadt, 1974; Bornus and Nowakowski, 1974). It is also noted that in the hives with two entrances

(upper and lower), as well as in the areas not protected from winds, the bees used a bit more feed in winter (Bornus and Nowakowski, 1974). Air flow permeable roofs bring the advantages of thermalizing the hives to nil (Bielby, 1974). Wintering in the hives with a screened bottom the food consumption was 10-15% higher, there was 50% less brood in autumn as well as in spring and there were 1.8-2.0 times less dead bees after wintering than in the hives with a wooden bottom (Horn, 1988).The bees wintering in plastic hives are reported to use 1.6 kg more food and contained more moisture than in the wooden hives (Detroy and Owens, 1971). Colonies, housed in wooden hives, achieved superior performance over polystyrene hives as measured by overwintering colony survival, winter population loss, brood area, colony strength and lower aggressiveness (Dodologlu *et al.*, 2004). However no significant difference of the colony winter losses was found in wooden vs. styrofoam hives in another study (Genersch *et al.*, 2010).

The hives' winter ventilation has risen opposite views. Widely spread recommendations are for good ventilation when both upper and lower entrances are opened and extra openings are made in the top part of the hive, thereby creating a constant airflow through the hive. For indoor wintering in the beehouse it has been recommended to create the airflow by an air compressor as minimal of 0.1 l/sec per hive, but often more, up to 1.6-2.3 l/sec per hive (McCutcheon, 1984). Later Underwood and Currie (2004) have suggested the standard ventilation of 0.24 l/sec per hive. On the other hand, successful wintering has been notified in the conditions of limited ventilation. The colonies with closed entrances had decreased food consumption, bees' mortality, the amount of faeces in the hindgut, but they reared more brood in spring (Perepelova, 1947; Michailov, 1964). Very successful experiments with wintering colonies in very limited ventilation conditions (underground and in pits dug into the soil) have been described (Naumovich, 1891; Ivanov, 1893; Lukin-Jerzhov, 1889; Tsvetkov, 1945; Gurijev, 1949; Starkov, 1956, reviewed by Michailov, 1963). They had almost no dead bees on the bottom and the food consumption was nearly two times lower than in the colonies wintering in beehouses (Pavlov, 1947).

Sokolov (1900) suggested that in nature a honey bee colony will seek a nest site in a tree cavity where it can fill up the entire cross section of the cavity, and at the first frosts seals any space with a large number of bees forming a living cork, thus blocking access by cold air to the stored hon-

ey. The bees press themselves against the cavity walls protecting themselves from the cold air coming up from below (Sokolov, 1900). The bee colonies wintering in tree cavities are reported to consume as much as 3 times less honey than those in a hive (Nesterov, 2003). It was reported that a colony weighing 2.5 kg which filled the entire cross-section of the nest cavity where the combs were fixed to the walls, consumed not more than 3 kg of food during winter (Soklakov, 2003).

3. AIMS OF THE STUDY

Among the causes of deaths of bee colonies in winter are, according to beekeepers, the reasons associated with the poor preparation of colonies for wintering or poor management: starvation, poor quality queens, management, weak colonies in the autumn (vanEngelsdorp *et al.*, 2008). In another study, however, five reported top causes were poor wintering conditions, starvation, weakness in the autumn, queen failure and *Varroa*-mites (Lee *et al.*, 2015).

1) Varroosis has been proved to be the main culprit of large colony losses, and at the same time the lack of efficient control methods currently available against the parasite has been highlighted (Dietemann *et al.*, 2012). So an insufficient or delayed autumn treatment of *Varroa* often leads to the destruction of bee colonies before or during the winter. On the other hand, the treatment itself comprizes a toxic influence on the bees and when performed in late autumn aggravates greatly the wintering of bee colonies. This is an additional stress factor in winter to the colonies harmed or weakened by *Varroa*. Among the organic acids, oxalic acid (OA) has shown the greatest effect against *Varroa*. Oxalic acid is generally used in the concentrations of 2-3% and the solutions containing less than 2% OA have been poorly studied so far.

The aim: To determine the concentration of the OA soluted either in water or sugar solution that would combine good control of the *Varroa* mite with low bee mortality (**Papers I and II**).

We hypothesized that the weaker solutions of OA combined with sugar solution may have the same effectiveness than the concentration commonly used in practice, being at the same time considerably less toxic to bees.

2) It is widely known that the lower the metabolic rate of wintering bees and the associated food consumption, as well as the temperature of the winter cluster, the deeper is the bees' dormancy status, the smaller is the exhaustion of the organism and accordingly the more successful is the overwintering. Increased food consumption is accompanied by the increased excretion of metabolic water. To avoid excess moisture in the hive, intensive ventilation is often applied. In contemporary beekeeping thin-walled hives are widely used. In winter they have a completely open

entrance and in addition either a screened bottom or sometimes even ventilation tunnel on the floor of the hive causing draught. This is done in high latitudes for the removal of moisture, at the northern natural border of the spread of honey bees. The situation is often made more difficult by the southern subspecies introduced into the northern regions. Recently little attention has been paid to the heat losses and the higher level of metabolism. Also, little is known about water vapour movement in the hive after leaving the winter cluster and the conditions in the hive which would allow the water vapour to leave the hive in a natural way. The factors, which would allow the bees to keep the water balance of the colony during the winter, are still unclear.

The aim: To show experimentally water vapour movement, condensing place and the actual amount of condensed water in the hives without top ventilation. In other words, the possibility to export the excessive metabolic water from the nest of the bees without increased ventilation is investigated (**Paper III**).

We hypothesized that although the vapour leaving the cluster rises upwards it can, if not finding the exit, descend and leave the hive or condense in the coldest place beneath the nest.

3) It is a widely spread opinion that honey bees winter ideally in tree cavities, because a round cavity corresponds more to the shape of the cluster than the quadrangular nest space in the hive. The winter cluster cannot occupy the entire nest space in the hive, especially its corners and therefore the heat losses are higher which, in turn, increases the metabolic rate of wintering bees.

The aim: To establish whether wintering in a cylindrical cavity is more effective than wintering in an oblong cavity (hive). Temperatures by the inner sides of the walls, the food consumption and the bee mortality of colonies during the winter in hives and cylinders were compared (**Paper IV**).

We hypothesized that in a cylindrical cavity there are more favourable conditions for wintering bees and therefore both food consumption and mortality are smaller.

4. MATERIALS AND METHODS

4.1. Study region, colonies and hives

This study was carried out in the County of Lääne-Virumaa in Estonia (26°29'E, 59°10'N) in the years 2002-2007 (**I,II**), in 2000-2005 (**III**) and in 2001-2004 (**IV**). Experimental and control colonies were housed in the Estonian standard wooden one-box long hives with a lower hive entrance and a frame size of 414×277mm (1000 cm^2 per comb side). Colonies were headed with the queens of the local strain, which were reared and mated naturally. The strength of the colonies was estimated by the Liebefeld method (Gerig, 1983) and they were selected for wintering experiments with the strength of six frames covered by bees (**III**) or seven frames covered by bees (**IV**).

4.2. Equipment and design of experiments

4.2.1. Experiments on varroosis

4.2.1.1. Equipment of experiments

Stainless wire-mesh cages (100×80×100mm) for submersion tests and a hand-operated atomizer for spraying experiments were used. Experimental and control hives were provided with the screened bottom boards in field experiments. Apistan® strips were used after the treatments to determine the number of mites that had survived the test (**I,II**).

4.2.1.2. Experimental design

Two methods of the application of the oxalic acid (OA) solutions were used: 1) submersion tests of 100 g of bees from the infested hives enclosed in cages; 2) spraying bees on combs in field conditions. The submerging technique was used for experimental assessment of the toxicity of different oxalic acid concentrations to both mites and bees in the laboratory conditions.

In the **submersion** tests caged bees were submerged into one of a range of solutions at 25°C, and spun around the horizontal axis for 8 seconds to let air bubbles out before being removed from the solution. Each cage was submerged twice and thereafter kept for 48 hours at 25°C and fed

with honey. The control cage with bees was submerged in water in the same manner. Dead mites were counted periodically, and dead bees on the bottom of cages were counted at the end of the experiment. Thereafter, half a strip of Apistan® was placed in each cage to determine the numbers of mites survived in the treatment. There were water solutions with 0.5, 1.0, 1.5 and 2.0% OA in the first experiment (Exp 1) and water versus sucrose solutions (50%) with 0.1, 0.2, 0.3, 0.4 and 0.5% OA in the second experiment (Exp 2). The Exp 1 was replicated ten times and Exp 2 five times.

In the **spraying** tests the solutions were sprayed directly onto bees on the combs removed from the hives, at the dosage of 25 mL per comb. The strength of colonies was estimated before and after the spraying experiments. In spring experiments little brood was cut out of a brood comb and the queens were caged to prevent egg-laying prior to treatment. Test colonies were sprayed with the different concentrations of OA water solutions at 3-6 day intervals, control colonies were sprayed with same amount of water. Seven days after the last spraying, dead mites were counted and Apistan® strips were placed into each comb space, but not in the utmost spaces, for 6 days. The tested concentrations of OA were 0.5, 1.0 and 1.5% four times in spring (Exp 3), 0.5% five times in spring (Exp 4), 0.5% five times in spring , 0.5% one and two times in autumn (Exp 6) and 0.3% once in autumn (Exp 7). Various experiments were replicated 3 (Exp3), 6 (Exp 4), 11 (Exp 5), 15 (Exp 6) and 8 times (Exp 7)(**I**).

4.2.2. Experiments on determining the amount of condensed water

4.2.2.1. Equipment of experiments

Condensers were constructed of thin sheet metal, onto which water vapour from the winter cluster could condense and flow into a plastic bottle for measurement. Upper condensers were roof-shaped and placed above the frames, fully covering the entire 6-frame nest. The water condensing on the inner surface of the condensers could flow into the gutter and from there into the collection bottle.

The lower condensers were of two designs. The lower 'wall' condenser was installed into the base of the hive to collect condensed water near the winter cluster in the lower half of the height of the nest. The lower 'gutter'

condenser was installed inside the hive floor to collect moisture gathered under the frames. It was similar to the upper roof-shaped condenser but inverted and covered with wire mesh to avoid bees from falling into it. The upper condenser created an empty space with the volume of 7200 cm^3 above the nest and the lower 'gutter' condenser increased the space under the frames to the same extent, but the lower 'wall' condenser did not (**III**).

4.2.2.2. Experimental design

The experiments were conducted in two variants. The first variant included two test groups (n=3 each) and one control group (n=3) in both of the winters. The hives of one test group were fitted with an upper condenser, while those of the second test group were fitted with a lower 'wall' condenser. The second variant had one test group and one control group (n=3 for each group and each of the three years), test hives were fitted with two condensers, an upper one and a lower 'gutter' condenser. In both variants all the openings above and between side walls, partition boards and floor were sealed with silicone. Control colonies were not hermetically sealed. Both colonies of experimental and control groups had open entrances at 15×30 mm and were equally well insulated above and on the sides. In the spring, the amounts of the condensed water collected by the upper and lower condensers of test colonies were measured separately.

The food consumption of colonies was determined by weighing them in a special weighing box in October and March. Frames with bees were lifted out from their hives and placed into the box for weighing without the hive. In spring, dead bees on the bottom and the number of bees having left the hive during winter and perished outdoors were determined (with the wire mesh cage placed in front of the entrances in autumn). The area of wet surfaces of the hive walls, the bottom and frames was measured in cm^2 (**III**).

4.2.3. Experiments on wintering in the cylindrical nest cavity

4.2.3.1. Equipment of experiments

The five **cylinders** were made of wood, opening vertically in the middle. Their inner height was 450mm, the inner diameter 280mm, and the wall thickness 60 mm. They had a lower entrance measuring 100×15mm in front and 70×15mm lower entrances on their both sides. Seven parallel

comb foundations were fixed at 35mm intervals inside the cylinders. Thermistors (NTC 4.7kΩ) were installed in total of 56 into the cylinders and 32 into the control hives in order to measure the temperatures at different positions on the walls of the nest room. Each cylinder had eight rows of thermistors fixed vertically to their inner walls with a space of 11cm between the rows. Seven thermistors were positioned vertically, with a distance of 7 cm between them. The control hives had eight rows of thermistors installed vertically, four in each row, spaced 8 cm apart. The thermistor rows were located in the middle of the back wall and in the front wall (i.e. by the entrance), in the middle of both partition boards and in all four corners of the nest cavity in the hive.

The colonies in both cylinders and box-hives were insulated at the top and on the sides with 10cm thick rock wool and with an additional 5cm thick cotton wool mat. The cylinders were located in box-hives in order to protect them from the wind and external moisture (**IV**).

4.2.3.2. Experimental design

Swarms of equal weight (2.5 kg) were driven into the cylinders(n = 5) in June. The control hives (n = 3) were selected out of overwintered colonies which were comparable in their strenght. The strength of both control colonies in hives and experimental colonies in cylinders was assessed in October. The cylinders were opened up from below and vertically from the middle and the density of bees was assessed. Temperatures were measured in winter (November-March) at about 1-week intervals to establish extreme ambient temperatures. The food consumption of colonies was determined by weighing them in October and March. The colonies in box-hives (control) were weighed separately from the hives, but the colonies in the cylinders with the cylinders (detailed description in paper IV). Overwintering mortality of bees was assessed as in previous experiments (**IV**).

4.3. Data analysis

Statistical software Statistica 7.0 was used for each analysis. One-way ANOVA and *t*-test was applied at a significance level of $p<0.05$ (**I,II,I-II,IV**),means were compared using the LSD Test (**I,II**). A multiple linear regression was used to determine the effectiveness of spraying the colonies with various little capped brood areas (**I,II**).

5. RESULTS

5.1. Experiments on varroosis

Submersion tests showed that all the tested concentrations of OA in the range of 0.5-2.0% were equally toxic to *Varroa* mites, but the concentrations of 1.5 and 2.0% were highly toxic to bees (**I**, Exp 1, Fig 1). The concentrations of 0.1% water solution of OA had a noticeable effectiveness (59.9±3.7%) and the sugar solution even slightly more (71.1±4.2%). Higher concentrations raised the effectiveness: with 0.2% water solution the varroa mite mortality was 89.8±2.2% and with sugar solution 90.0±1.0%, with 0.3% was 94.9±0.9% and 95.5±0.5% in water and sugar solutions, respectively, and both the 0.4% and 0.5% water and sugar solutions achieved up to 100% effectiveness (**I**, Exp 2, Fig 2). The OA in the sugar solution showed slightly, yet not significantly, a higher mite control than the OA in the water solution, but was more toxic to bees (**I**, Exp 2, Fig 3), The concentrations of OA from 0.3 to 0.5% in the sugar solutions were five times more lethal to bees than in the water solutions ($p<0.05$) (**I**, Exp 2, Fig 3), but sugar solution only did not cause any harm to bees.

Spraying experiments at a dose of 25 mL per comb showed a high effectiveness of 0.5% water solutions against *Varroa.* The solutions of 1.0 and 1.5% OA, sprayed in April, gave similar mite control (98.4±0.1%) and 98.3±0.1%), but the 0.5% solution gave a weaker, but a significant effect (94.9±0.1%) (**I**, Exp 3, Fig 4; **II**, Fig 1). However, the 1.0 and 1.5% concentrations were highly toxic to bees ($p<0.05$) (**I**, Exp 3, Fig 5, **II**, Table 1). Spraying bees five times in May was 99.1±0.2% effective and non toxic to bees ($p=0.68$). However, spraying five times in the next April had a similar effectiveness (99.4±0.1%), but was toxic to bees: 4 of the 11 test colonies weakened considerably. Noticeable weakening occured after the fourth and fifth spraying and was best seen 12 days later (**I**, Exp 5, Fig 7). Spraying colonies with little sealed brood once or twice showed high effectiveness compared to the control: 92.9±0.1% and 91.8±0.1%, ($p<0.001$) (accordingly: **I**, Exp 6, **II**, Fig 2). Treatment effects were smaller in the colonies with more capped brood during spraying ($r= -0.83$) (**I**, Exp 6). Test colonies were not weaker than the control colonies after the autumn spraying or the following spring ($p<0.05$) (**I**, Fig 8). Spraying colonies once with 0.3% OA in September had the effectiveness of 76.4±4.0%, that was significantly lower than 92-93% effectiveness obtained in the previous autumn ($p<0.001$) (**I**, Exp 7).

5.2. Experiments on determining the amount of condensed water

Most of the metabolic water, released during winter, condensed on the lower condensers. Both designs of lower condensers removed significantly more condensed water than the upper ones ($p<0.001$ both for 'wall' condenser and $p<0.001$for 'gutter' condenser)(**III**, Fig 2). In the hives with both upper and lower condensers fitted, the upper condenser helped to remove on average only 2.5±1.3% of water summed over the three winters (**III**). However, in spring the test colonies fitted with upper condensers had considerably larger areas of moist or wet surfaces than the control colonies or those with lower condensers ($p=0.02$)(**III**, Fig 3). The presence of upper condensers simultaneously with the lower ones also increased humidity in the nest in comparison with the control ($p=0.001$)(**III,** Fig 3). The lower condensers did not cause increasing humidity in the hive ($p=0.17$)(**III**, fig 3). Test and control colonies did not differ significantly in food consumption or in total bee mortality (both factors: $p>0.05$)(**III**, Fig 4, Table 2). The food consumption during the five winters was 7.1±0.4 kg on average and the amount of condensed water was 445.3±45.8 g on average per colony (**III**).

5.3. Experiments on wintering in cylindrical nest cavity

It was considerably warmer by the inner walls of the cylinders than in the box-hives, especially in the lower part. Temperatures in the areas unoccupied by bees under the ceiling of the cylinders fell rather low, nearly as low as or even lower than under the cluster in the first half of winter, but not in the second half of winter (**IV**, Fig 1). Both in the hives and the cylinders, the winter cluster touched the front wall but stayed away from the back wall, especially with cold weather. The surface of the warm area decreased with cold weather and increased at around 0°C. In the second half of the winter there was a larger warm area in the cylinders than in the hives. The coolest places in the hives were the corners on the bottom and by the lower entrance, in the cylinders, by the back wall on the bottom and by the entrances on the sides (**IV**, Fig 1). In case of outside temperature of -24°C the lowest temperature in the hind corner of the box-hive was -18.2°C and -6.8°C by the lateral entrance on the floor of the cylinder. Both in box-hives and in cylinders, sidewalls and the front wall were always warmer than the back wall, in addition, no cold corners detected in the box-hives were noted in the cylinders (**IV**, Fig 1).

The food consumption of colonies in cylinders was significantly less (13.5%) than in the box-hives ($p=0.027$): 7.2±0.3 kg in the cylinders and 8.3±0.2 kg in the hives (**IV**, Fig 2).There were significantly fewer dead bees on the floor of the cylinders than on those of the hives ($p=0.01$), but the total number of dead bees (dead bees on bottom+bees flown out in winter) did not differ significantly between the cylinders and the hives ($p=0.25$)(**IV**, Fig 3).

6. DISCUSSION

6.1. Possibilities to improve overwintering using milder *Varroa*-treatments

The results showed that the 0.5% OA in the water solution was as effective as the higher concentrations in both submersion and spraying experiments. Weaker concentrations of OA in water and sugar solutions (0.1, 0.2, 0.3, 0.4%) showed a good effect against the mite in submersion experiments, but 0.3% OA was significantly less effective than 0.5% OA in spraying experiments (**I**). However, OA in the sugar solution was considerably (up to five times) more toxic to bees than OA in the water solution (**I**). It is possible that the sugar solution containing OA stuck to the cuticle and spiracles of bees prolonging the toxic effect, whereas the water from the water solution evaporated and the remaining OA crystals fell from the bees. A role of sugar in the spraying or trickling solution is not clear and needs further research. Some researchers have established that adding sugar to a trickling solution of OA increased the effectiveness of mite control, but decreased bee tolerance (Charrière, 2001; Milani, 2001; Gregorc and Planinc, 2001; Charrière and Imdorf, 2002; Nanetti *et al.*, 2003). In most cases solutions without sugar have been reported to be less effective (Nanetti *et al.*, 2003).

Spraying five times in May with 0.5% OA water solution in 2003 was non-toxic, but in April 2004 it was toxic to bees. A noticeable toxic effect was observed after the third and particularly after further sprays (**I**). It was probably due to the reduced ability of bees to fly from the hive on the cleansing flight due to cold weather in early spring. Furthermore, the bee colonies in early spring were comprised mainly of old overwintered individuals, whereas by May, a new generation of bees had emerged before the treatment. Most of the mites died after the first spraying and less after the second spraying. Following experiments showed that spraying once or twice of colonies with little brood with 0.5% OA water solution in autumn (September) had an equally high efficacy of mite control and little toxicity to bees (**I**, **II**). So a concentration of 0.5% OA in the water solution at a dose of 25 mL per comb should be used. Spraying in late autumn, when there is less brood in the hive, can increase its effectiveness. Spraying bees on combs from different directions, i.e. wetting them as much as possible with an OA solution, can also increase the effectiveness of treatments.

However, the treatment by spraying is very laborious, therefore it is used more in small hobby apiaries. The trickling of OA sugar solution containing 35 g of oxalic acid dihydrate in 1 litre of sugar water 1:1, is more convenient in practice, because it is simple, quick, cheap and very effective (Charrière and Imdorf, 2002). But spraying with a weak solution of OA is less harmful to the honeybees and to the people applying the treatment, therefore it could be recommended to integrate in beekeeping practice when there is no or only small areas of capped brood: after hiving of swarms, in broodless periods of swarmed colonies and nucleus colonies, after honey harvesting with only a small amount of brood in the hive, in broodless periods in late autumn and early spring. If the number of mites is high, the colonies should be treated in early autumn, after honey harvest, using the spraying with 0.5% OA or using other natural substances like formic acid, lactic acid or thymol.

Treatments in late autumn may disturb the preparation of bees for the wintering period. It has been reported that higher bee losses may occur in winter after the trickling treatment in autumn (Charrière and Indorf, 2002; Nanetti *et al.*, 2003) and that multiple (2-3) autumn or summer treatments are poorly tolerated by bees (reviewed by Rademacher and Harz, 2006). Observations showed that the colonies with a little amount of *Varroa* mites, which were not treated in autumn, overwintered always significantly better than the colonies treated with OA in late autumn (the author's observations, unpublished). One possible option is to start the treatments earlier. But the presence of brood in the colonies in autumn is a hindrance for earlier treatments. In Estonian climatic conditions some colonies continue to rear brood in October and even in November, so these colonies become the sources, from which mites will spread into the other colonies in the next year. Brood rearing in autumn, when the bee population decreases, creates good conditions for increasing the mite population that often will lead to disappearing (flying off) heavily infested colonies. Ceasing of the brood rearing also stops the increasing of the mite population. Further research is needed to put an earlier end to brood rearing or suspend it within colonies in autumn.

Compared to synthetic pesticides the alternative methods of *Varroa* control enhance the chances for the colony survival and ensure residue-free bee products (Imdorf *et al.*, 2003; Nanetti *et al.*, 2003). Oxalic acid is the most effective organic acaricide and less dependent on climatic conditions than other soft miticides like formic acid and thymol. Our experiments

showed that 0.5% OA is very effective to kill *Varroa* mites. Probably its efficiency would increase by the development of new application methods and by a possible synergistic effect with various natural compounds.

6.2. Possibilities for reducing the metabolic load of wintering bees

The results of the present work showed that most of the water collected during the winter condensed below the nest on the lower devices. Using of lower condensers decreased the area of moist surfaces in the hive (**III**), so it removed successfully the excessive moisture from the nest. It shows clearly that the place under the frames, where moisture has reached naturally, would be practical for removing it. Another possibility is to direct it into a drinking dish inside the hive for consuming by bees in spring and sometimes also in winter. Water reserves in the hives may be very important in early spring when it is difficult for bees to fly out for water collection (**III**). However, collecting devices probably require change in the hive construction. Removing excessive moisture from nest bottom without using increased ventilation is very important for decreasing heat losses and for the creation of more favourable conditions for wintering bees, which as a result can reduce the metabolism level, food consumption and the mortality of bees.

As mentioned above, any air movement in the hive increases heat losses. That is why it is not right to have strong ventilation in the lower part of the hive by draught corridor or using a screened bottom. The colonies housed in the hives with the screened bottom during the winter period have been reported to consume 10-15% more food than the colonies in the hives with wooden bottom (Horn, 1988). Wintering in the hives with a screened bottom is widely used today, even in northern countries like Estonia, but few comparative studies are carried out to evaluate the wintering success in various hives. Beekeeping is also widely used in the Langstroth and Farrar box hives without insulation, where winter conditions are not favourable for bees, especially for weak colonies. Increased food consumption and excessive humidity would be present in such cold hives as mentioned by earlier researchers (Villumstadt, 1974; Bornus and Nowakowski, 1974; Zmarlicki, 1978). Unfortunately there are no present-day studies available on that topic.

In the present research the amount of metabolic water collected by condensers was substantially less than the production expected from the

amount of food consumed (**III**). As theoretically the consumption of 1 g of honey produces 0.68 g water (Johannson and Johannson, 1979), so 4.43 kg of water remained uncollected. It is important to mention that, however, beekeeping literature talks about more than 5 l water releasing during winter, the actual amount of it has not yet been experimentally determined. It is not clear how much of honeybees' metabolic water returns to the bees' life cycle. The actual amount of water produced in winter by the bee colony varies and depends on several factors such as the metabolic rate of the colony, the crystallisation level of the stores, the brood rearing in winter, the water content of winter reserves, the humidity of the external air (**III**).

Wintering in cylindrical nest cavity was more favourable for bees. Our experiments showed that honey bee colonies wintered in the cylindrical nest cavity were less affected than those in the box-hives by the low outside temperature (**IV**). The winter cluster did not occupy the entire cross section of the round cavity, but stayed away from the back wall. The temperatures under the cylinder ceiling were nearly as low as those under the clusters in the first half of the winter, but not in the second half of the winter, when the clusters had moved upwards (**IV**). Beekeepers have often thought that in two-box hives the warm air, coming from the winter cluster, moves upwards and staying there keeps the upper part of the hive together with honey supplies warmer. Our experiments do not support it, because in the round nest room of the cylinders, where the bees had better conditions than in the box-hives the temperatures under the ceiling dropped very low, especially in the first half of the winter when the cluster was located below (**IV**).

In the second half of the winter, the bee clusters expanded up to the round walls that protected them from cold and thereby they were more able to avoid heat losses. We can say that in the round cavity only a single wall surrounds the cluster, but in the hive four walls, with cold corners are present (**IV**). Food consumption was considerably lower (13.5%) in the cylinders than in the hives (**IV**) and the bees probably had respectively a lower metabolism level. However it should be mentioned that this energy saving was not enough to justify the reconstruction of hives. The hives with a round nest cavity or combs attached to side walls would be costly and would make practical work with bees much harder.

The measurements of temperatures showed that it was always warmer both inside the hive and the cylinder than outside, even when calculation included the areas not covered by bees (**IV**). This warmth is produced by weakly endothermic bees on the surface of the cluster as observed in an earlier study (Strabentheiner *et al.*, 2003). It demonstrates an important principle of wintering of honeybees: although the cluster retains most of the produced heat in its core, some is lost from the surface and the more it does, the greater is the gap between the temperature of the surface and that of the surrounding air (**IV**). Thus, good hive insulation plays a significant role in reducing heat losses.

The other important role of the insulation is the avoiding of excessive moisture inside the hive. It has been shown that under steady outside conditions condensation does not increase even when the ventilation is restricted, so long as the proportion of heat passing through the walls of the nest cavity is negligible (Simpson, 1961). Thus, insulation diminishes the amount of ventilation necessary for decreasing condensation, and when insulation is minimal, strong ventilation is necessary to avoid condensation inside the hive (Simpson, 1961). A nest cavity with walls and the top of low thermal permeability and restricted ventilation ensures differences inside and outside the hive and it generates a passive (natural) replacement of air in the hive. However, in mild winters, when temperature differences are smaller, it works less effectively.

6.3. Unclear wintering aspects needing further research

Farrar (1973) suggested that it is a good practice to take winter losses in the autumn. The colonies which are not strong enough for overwintering should be united with better colonies or killed to prevent needless consumption of stores (Farrar, 1973). The colonies suitable for overwintering in northern regions should rear sufficient brood to provide a cluster of 8 to 10 pounds (3.6-4.5 kg) of young bees that emerge between August 20 and the early part of October (Farrar, 1973). Thus, it has been widely recommended to overwinter only strong colonies with large populations of workers and abundant food stores (Döke *et al.*, 2015). According to Farrar (1973) 3-6 well-filled combs of reserve pollen in the autumn within the cluster should be available for brood rearing (Farrar, 1973). The question about the colony size best suitable for wintering is still unclear and the research in the topic has found

contradictory results. In large colonies (20 000-30 000 and more bees per colony) brood rearing in winter months has been reported to be common in North America. At the same time, despite nonstop or increasing brood rearing during winter, the adult bee population declined and approximately half of the bees died (Avitabile, 1978; McCutcheon, 1984; Szabo, 1993). It was found that stronger colonies (that covered 12.3±5.1 bee spaces) had significantly higher survivorship than weaker colonies (10.0±5.4 bee spaces) in German conditions (Genersch *et al.,* 2010). On the other hand it was found that a total mass of bees, less than 100 gram was a threshold under which mass-specific heat loss rised sharply. But when the cluster masses were increased above 200 g, the rates of mass-specific metabolism declined only slightly (Southwick, 1985). The metabolic rate of small colonies around 300 g of bee mass was found to be 5.7W/kg in broodless winter months with the mean temperature of 3°C (vanNerum and Buelens, 1997). But it was suggested that the clusters of 1.7 kg or larger actually use less energy at the colder temperature. In the bees, the metabolic rate per gram or per individual increases as the cluster mass decreases (Southwick, 1985). However, the comparison of artificially composed bee populations with caged queens of different strength showed that a 9000- bee population consumed significantly less honey per bee during winter than a smaller population (4500 bees), but not significantly more than a larger population of 32 000 bees (Harbo, 1983). Despite of this research, beekeepers have often reported on successful overwintering of small colonies and the failure of strong ones (personal communications). Thus, the optimal colony size for wintering in various climatic conditions needs further research.

Joining colonies has not been studied much. Lebedev (1979) has found that in the case of putting colonies together the bees get excited and stay in the condition of agitation for a long time. Bees in such colonies consume more nutrient reserves in body during the winter and in spring they raise less brood than bees of the control groups. It was also found that adding 50% of extraneous bees increased feed consumption, the fulfilment of hindgut, dysentery, the amount of mould in the nest and the number of *Nosema* spores in the midgut. Therefore weak colonies are recommended to join with average ones in autumn and not to join 2-3 colonies together (Lebedev, 1979). Thus, uniting colonies is not just a simple act of adding homogeneous bee mass but joining of genetically different living organisms.

In modern beekeeping various violent management practices are widely used. The transport of colonies to long distances, crowding a lot of colonies in small areas, removing the brood or young bees from the colony and settling them into other colonies, splitting and uniting colonies, removing the queens (killing them) and their replacement with foreign ones, crushing bees by displacement of boxes etc. are worth mentioning here. The problem may be due to poor workmanship. It is not known how stressful these destroying manipulations are to the whole colony as a superorganism and how it affects the overwintering success. Therefore it is extremely important to work out suggestions for the best management practices for beekeepers.

Malnutrition is an important cause of colony losses. The bees' immunity system is badly affected by the shortage or absence of pollen in the nest, feeding on many different plant species guarantees more diverse protein content in the feed and thus supports the immunity. It was found that the colonies which fed protein supplements had higher levels of *Nosema* and black queen cell virus than the colonies which fed natural pollen (De Grandi-Hoffman *et al.*, 2016). Hence natural forage could improve overwintering.

In autumn beekeepers often take all the honey away from bees and replace it with a large amount of sugar (20 kg or more). So the bees are constrained to live only on the one-sided glucose-containing sugar feed during the whole period of autumn, winter and spring. Such malnutrition results in the protein deficiency in the bees' organism. Little is known about the influence of such malnutrition on bees' health, spring development and forager capability. Feeding sugar in autumn has a negative influence on bees' physiology, especially in weak colonies (Zherebkin, 1979). Inverting sugar the bees add ferments and proteins to the syrup, their metabolic rate rises, they raise the nest temperature and these exhausts their organism decreasing their lifespan (Zherebkin, 1979). Feeding sugar to bees has an especially negative influence in the case there is no bee-bread (pollen) in the nest (Zherebkin, 1979) as well as at the high rate of varroa infestation and associated virus infections. It is not clear how much the mortality of varroa-infested bees' increases after the inversion of big amounts of the sugar syrup and feeding on it during the following winter.

Failing of overwintering often begins with the restlessness of bees and the rise of temperature in the nest as observed by many beekeepers. In-

creased temperature promotes the evaporation of water and it can lead to the disturbance of water balance in the winter cluster. The bees become more active, enforce the ventilation at the entrance, fly out for search of water and even cluster outside the nest in the empty hive space (the author's observations, **III**). Increased temperature can induce brood rearing in winter and it, in turn, aggravates the situation. The bees who participated in brood rearing lose their longevity and they cannot survive the winter. The developing brood also needs water, the lack of which becomes more serious for the colony. Thus both, brood rearing and the bees flying out for search of water would lead to the loss of wintering bees. The increased level of metabolism, associated with increased temperature and food consumption would lead to the exhaustion of food storages at the end of winter or early spring. The colonies of equal strength have different excretion of metabolic water (the author's observations, unpublished) and consequently a different metabolic rate. Thus, any disturbance of water balance in a wintering colony may be the main reason for high winter mortality. However, water balance and water conservation of winter clusters have not been studied enough.

Supplying the bees with water in winter would help them. Bees calm down quickly and decrease the nest temperature when water is given into the nest (**III**, the author's observations). However, water delivery to bees by itself is a troublesome task. Moreover, added water can freeze and turn to be inaccessible for bees. Therefore, it is important to determine the reasons by which the bees cannot stay in a little active dormancy status in late autumn and winter. One possible reason is the treatments against *Varroa* in late autumn, immediately before wintering. Even chemicals with mild effects like organic substances may be toxic to bees.

Some beekeepers have reported that overwintering is more successful in cold hives with little or no insulation, even in northern countries like Estonia. Thus, it seems, science has gone wrong. However, it has not. Wintering in unfavourable damp and cold conditions is always physiologically more costly for bees, especially in weak colonies. But due to global warming winters have become milder and so more tolerable for the bees. It is possible that in less favourable conditions the bees are forced to cluster more tightly and thereby the water balance would be less disturbed. However, it is not a help for bees to create bad conditions for best overwintering. In the case of honeybees not damaged by mites, viruses, control treatments and different stress factors have shown good

overwintering in most cases (the author's observations, unpublished). So, at first, these pathological factors should be eliminated and thereafter we can compare overwintering success in various conditions. It has been observed that even weak colonies (that covered less than 3 frames, i.e. approximately 400-500 g of bees), heavily damaged by *Varroa* mites and DWV infection, can overwinter well after releasing them from *Varroa* mites using milder methods (the author's observations, unpublished). It is possible in good winter conditions, but questionable in unfavourable conditions where bees are forced to maintain the higher metabolic rate. The best overwintering conditions and the influence of different hive constructions for creating them need further research.

The observations made long ago have shown that bees fall into dormancy when the frost has subsided and good quiet weather has arrived. Listening to such a colony not the slightest sound is heard; the bees sit absolutely quietly and do not respond to the first knocking against the hive. Sometimes one needs to knock up to 10 times to get a feeble fizzing response. If such a hive is opened, the bees remain motionless and do not move themselves at first. Such a dormant state must be taken for an ideal calm of bees. The deeper the dormancy, the quieter the buzz (Snezhnevski, 1925).

7. CONCLUSIONS

The present thesis has shown the possibilities to save the energetic costs of bees during overwintering and reduce winter losses associated with several factors damaging the bees before and during the winter. The results showed that the effect of using weaker concentrations and probably also smaller dosages of oxalic acid for antivarroa treatment would be milder and less harmful to the bees preparing for winter. The development of the application methods and possible synergistic effects of various natural compounds will make the treatments less laborious and more effective against mites.

In the control of varroosis the toxicity of the used substances to honey bees is a frequent problem. However the potency of weaker dosages have gained little attention by scientists. The aim of the present research was to determine the efficiency of weaker doses of oxalic acid in the control of *Varroa* mites and their toxicity to honey bees. The experiments revealed:

- The 0.5% oxalic acid (OA) in the water solution was as effective in the *Varroa* control as the higher concentrations. One, two or more times of spraying at the dose of 25mL per comb achieved the effectiveness of 95-99% in the colonies without brood and 92-93% in the colonies with little brood in autumn.

- The OA concentrations of 1.0, 1.5 and 2.0% were highly toxic to bees at the dose of 25mL per comb. The 0.5% OA was not visibly toxic to bees but after five times of spraying in early spring (April) it was toxic to bees but in late spring (May) it was not.

- The OA in the sugar solutions was not significantly more effective against *Varroa* than the OA in the water solution but it was approximately five times more toxic to bees.

  The frequent problem for the winter cluster of honeybees is the excessive moisture appearing which makes the wintering conditions more difficult for the honey bees and conduces the development of diseases. To avoid it, very often strengthened ventilation is used (either from the top or the below of the nest), although it brings along bigger heat losses and makes honey bees raise their metabolic rate

to compensate heat losses. Natural movement of water vapour in the hive has been little studied. The experiments of present study revealed:

- Most of the metabolic water released during winter moved downwards and condensed on the lower condensers below the nest of the bees. The upper condenser removed only 0.6, 0.4 and 3.25% of water in various years.

- The attempts to remove the metabolic water from the top of the nest, using the upper condenser, lead to the increase of moisture in the nest. Using of lower condensers decreased the area of moist surfaces in the hive.

The shape of the honey bees' winter cluster matches better with the round nest space in the cylindrical cavity than in the angular space in the box-hive. Consequently, wintering in such a nest space should promote the lower level of the honey bees' metabolism and save their energy. The research revealed:

- The honey bee colonies which were wintered in the cylindrical nest cavity had more favourable conditions; it was considerably warmer by the inner walls of the cylinder than in the hive. The winter cluster did not occupy the entire cross section of the round cavity but stayed away from the back wall.

- The food consumption of the colonies in the cylinders was significantly smaller (13.5%) than in the box-hives.

- The amount of dead bees on the bottom was significantly lower in the cylinders, however, when including the bees which were lost by flying out, the total number of dead bees did not differ significantly between cylinders and hives.

The results of the current research lead us to the conclusion that it is possible to use successfully the oxalic acid in lower concentrations which are less toxic to bees. We also found that it is suitable to remove the water vapour from lower part of the nest to avoid excessive moisture in the hive. The removal of excessive moisture without increased ventilation enables us to decrease heat losses and thereby the

metabolic rate of wintering bees. It is especially important to create more favourable conditions for bees in the conditions of a high level of varroosis when a lot of bees are damaged by *Varroa* mites and associated viruses. The *Varroa* population should be held at a low level during the whole year to avoid the damage of the last generations of bees in autumn. All the factors that may disturb the colonies in winter and force the bees to become more active and rise the metabolic rate, which in turn, can lead to the disturbance of water balance of the colony, should be eliminated. The low metabolic rate ensures a good overwintering of honeybee colonies.

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SUMMARY IN ESTONIAN

Mesilaste põhiline toode – mesi on alati olnud ja on ka praegu tähtis toiduaine ja kaubandusartikkel. Ka teised mesindussaaded (vaha, õietolm, suir, taruvaik, mesilasema toitepiim, mesilasmürk) omavad kõrget väärtust eelkõige alternatiivse loodusravi – apiteraapia jaoks, ja üha enam tuleks neid väärtustada inimeste pidevalt halveneva tervise taustal. Kõige tähtsam roll on mesilastel aga looduslike ja kultuurtaimede tolmeldamisel, mis ületab mesilastelt saadava otsese tulu kümme või rohkem korda. Mesilasperede hulk on aga Euroopas ja Põhja-Ameerikas langustrendil. Alates 2006. aastast on täheldatud suuri iga-aastaseid mesilasperede kaotusi (30% ja rohkem), mis aastate lõikes küll erinevad, kuid jäävad siiski ebatavaliselt kõrgeks, ületades tunduvalt tavalisi 5-10% piiresse jäävaid talvekaotusi.

Mesilaspered hukkuvad tavaliselt kas enne talve, talvel või talvitumise järel. Ühte kindlat põhjust ei ole leitud ja nii arvatakse sellel olevat palju erinevaid põhjusi. Enamiku uurijate arvates on põhiliseks süüdlaseks mesilaste hemolümfist toituv parasiitne lest *Varroa destructor* koos tema poolt levitatavate viirustega. Sageli esinevateks põhjusteks on peetud ka halbu talvitumistingimusi, mesilasemade sagedast hukkumist ja perede hooldamisel tehtavaid vigu. Varroatoosi tõrjeks kasutatakse sageli erinevaid keemilisi preparaate, mis kahjustavad mesilaste immuunsüsteemi ja eluvõimelisust, ja seda vahetult enne talveperioodi. Seetõttu on väga tähtis tõrjuda varroatoosi selliste vahenditega, mis on mesilastele vähem toksilised. Teiseks oluliseks sammuks on luua mesilastele talveks sellised tingimused, mis neid vähem koormaks ja võimaldaks neil hoida ainevahetus võimalikult madalal tasemel. See kindlustaks hea talvitumise ja vähendaks mesilaste organismi kurnatust ja säästaks nende energiat talvel. Mesilaste väiksem koormus talvel on eriti oluline sellistes tingimustes, kus talvituma minevad mesilased on suuremal või vähemal määral varroalestade poolt kahjustatud.

Lähtudes nendest probleemidest oli käesoleva doktoritöö eesmärkideks:

1. Kindlaks määrata, kas oblikhapet kui lestatõrjel seni teadaolevalt kõige efektiivsemalt mõjuvat orgaanilist hapet on võimalik kasutada ka nõrgemates kontsentratsioonides kui tavaliselt, tagades sellega mesilastele väiksem toksiline toime (I, II).
2. Selgitada, kuidas on otstarbekas viia välja talvel mesilaste elutegevuse käigus eralduv ainevahetusvesi, et vältida liigniiskust pesaruumis ja

vähendada soojuskadusid, mis suurenevad alati koos ventilatsiooni intensiivistumisega (III);
3. Hinnata, kas ümmargune pesaruum pakub mesilastele paremaid talvitumistingimusi kui kandiline taru, kuidas see mõjutab temperatuure talvekobara lähiümbruses ja talvist söödakulu (IV).

Oblikhappe erinevate kontsentratsioonide mõju määramiseks nii mesilastele kui varroalestadele kasutati: 1) puuristatud mesilaste sukeldamiskatseid; 2) katseperede pritsimist välistingimustes. Tõrje üle elanud lestade hulk määrati kindlaks Apistan® ribadega.

Talve jooksul ainevahetuse käigus eraldunud veeauru liikumine pesaruumis ja kondenseerunud vee hulk määrati kindlaks õhukesest plekist valmistatud seadmetega, mis asetsesid mesilaste talvepesa peal ja all. Tehti kindlaks mesilasperede söödakulu talve jooksul, tarus hukkunud ja tarust lahkunud mesilaste hulk ja märgade alade pindala pesaruumis kevadel.

Talvitumise kvaliteedi uurimiseks silindrilises õõnes kasutati puidust valmistatud silindreid kõrgusega 450 mm ja sisemise diameetriga 280 mm, mille siseseintele (samuti ka kontrolltarude pesaruumi seintele) kinnitati temperatuuriandurid (termistorid). Silindrid asustati juunikuus võrdse kaaluga sülemitega. Talvitusperioodil (november-märts) mõõdeti temperatuure pesaruumi seinte ääres nädalase intervalliga, võrreldi mesilasperede söödakulu ja talve jooksul hukkunud mesilaste hulka silindrites ja tarudes.

Uurimistöö tulemused

- Oblikhappe dihüdraadi (OA) 0.5% vesilahus osutus varroalestade tõrjel peaaegu sama efektiivseks kui kõrgemad kontsentratsioonid ja pritsides doosis 25 mL kärje kohta saavutati haudmeta peredel 95-99% efektiivsus ja vähese haudmega peredel 92-93% efektiivsus (**I, II**).
- OA 0.5% vesilahus oli mesilastele vähetoksiline, kuid kõrgemad kontsentratsioonid (1.0, 1.5 ja 2.0%) olid väga toksilised. Oblikhappe suhkrulahus oli ligikaudu viis korda toksilisem kui vesilahus (**I, II**).
- Suurem osa (keskmiselt 97.5%) talve jooksul talvekobarast eraldunud ainevahetusveest liikus pesaruumis allapoole ja kondenseerus pesa all olevale seadmele (**III**).

- Ainevahetusvee väljaviimine pesa alt vähendas niiskete pindade pindala pesaruumis, kuid püüded vett välja viia pesa peal asetseva seadme abil suurendasid niiskust pesas (**III**).
- Silinderjas õõnes olid mesilastele talvitumiseks soodsamad tingimused kui tavatarus, kuna õõne siseseinte ääres ja põrandal olid valdavalt kõrgemad temperatuurid kui tarus. Söödakulu oli keskmiselt 13.5% väiksem kui tarus. Talve jooksul hukkunud mesilaste hulk silindri ja taru võrdlusel oluliselt ei erinenud (**IV**).

Uurimus näitas, et varroatoosi tõrjet on võimalik teha pehmemalt ja seega mesilasi vähem kahjustavalt. Kaasajal eksisteerivaid looduslikke või orgaanilisi tõrjevahendeid tuleb edasi arendada nii, et saavutataks veelgi suurem efektiivsus ja väiksem ajakulu. Varroalestade arvukus tuleks hoida madal kogu aasta vältel, eriti hilissuvel, et vältida sügiseste mesilaste põlvkondade kahjustamist.

Talvitumistingimusi on võimalik paremaks muuta eelkõige soojuskadude vähendamisega. Üleliigse niiskuse väljajuhtimine pesa alt võimaldab vältida soojuskadude suurenemist, mis toimub tugevdatud ventilatsiooni korral. Ümmargune pesaruum on küll mesilastele bioloogiliselt enam sobiv, kuid ei näi olevat majanduslikult perspektiivikas kuna raskendab oluliselt tööd.

Mesilaste kõrgenenud aktiivsus talvel on seotud kõrgema ainevahetustasemega ja võib viia talvekobara veebalansi häirumisele ja lõpuks talvitumise ebaõnnestumisele. Seda vallandavad ja soodustavad tegurid vajavad veel väljaselgitamist ja elimineerimist. Madal ainevahetuse tase kindlustab hea talvitumise.

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I

ORIGINAL PUBLICATIONS

Toomemaa, K, Martin, A-J., Williams, I.h. 2010

THE EFFECT OF DIFFERENT CONCENTRATIONS OF OXALIC ACID IN AQUEOUS AND SUCROSE SOLUTION ON VARROA MITES AND HONEY BEES

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Original article

The effect of different concentrations of oxalic acid in aqueous and sucrose solution on *Varroa* mites and honey bees*

Kalle TOOMEMAA, Ants-Johannes MARTIN, Ingrid H. WILLIAMS

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 1A Kreutzwaldi St, 51014 Tartu, Estonia

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Abstract – The toxicity of various concentrations of oxalic acid dihydrate (OA) in aqueous and sucrose solution to *Varroa destructor* and to honey bees (*Apis mellifera*) was assessed using submersion tests of caged bees and by spraying bees in colonies with and without brood. An aqueous solution of 0.5% OA gave effective control of the mite and was non-toxic to bees whereas higher concentrations of OA (1.0–2.0%) were highly toxic to bees. Submersion tests into solutions with 0.1% OA were acaricidal both in aqueous (59.9 ± 3.7 %) and in 50% sucrose solution (71.1 ± 4.2%) whereas concentrations of 0.2–0.5% OA were highly effective; OA in sucrose solution was more toxic to bees than OA in the aqueous solution. Spraying with 0.5% OA solution at a dose of 25 mL per comb in May 2003 and in April 2004 was 99.01–99.42% effective in mite control in Estonian standard one box long beehives with 22 frames (each 414 × 277 mm, area 1000 cm^2 per comb side). Most mites fell after the first spraying. In autumn, spraying test colonies that had little capped brood once or twice with a 0.5% OA solution gave effective mite control (92.94 ± 0.01% and 91.84 ± 0.02%, respectively) with no noticeable toxicity to bees.

***Apis mellifera* / honey bee / *Varroa destructor* / oxalic acid**

1. INTRODUCTION

Spraying bees with a weak aqueous solution of oxalic acid (OA) has been used to control the mite, *Varroa destructor*, since the 1980's. Takeuchi and Harada (1983a) first reported that 2% OA in aqueous solution, at a dose of 100–150 mL per colony, was very effective against the mite and at the same time harmless to bees. Subsequently it was reported that even spraying four times did not ensure complete control of the mite, and that better results were achieved by spraying once with 300 mL per colony (Takeuchi and Sakai, 1985). More recently, spraying 5–8 mL of 3% OA solution per comb has been recommended (Radetzki et al., 1994). A single spray applied during a brood-free period was on average 94.5–98.8% (Imdorf et al., 1997) and 97.29% (Radetzki et al., 1994) effective against the mite, whereas spraying twice was up to 99.5% effective (Nanetti et al., 1995). Usually, no negative toxic effects have been observed on bees, even when a 5% OA solution was sprayed and higher doses (20 mL per comb) were used (Radetzki et al., 1994). However, some negative after effects have been reported, such as a decrease in brood area after spraying four-times (Higes et al., 1999).

Current practice, widespread throughout the world, involves trickling a sucrose solution containing OA into the spaces between combs covered with bees, as it is highly effective, simple and cheap. In brood-free periods in autumn, the efficacy of a single trickling treatment has exceeded 90% (Nanetti et al., 2003) and, in some cases, even 99% (reviewed by Rademacher and Harz, 2006). Trickling three times in the presence of brood resulted in

Corresponding author: A.-J. Martin,
ants.martin@emu.ee
* Manuscript editor: Peter Rosenkranz

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Corresponding author: A.-J. Martin,
ants.martin@emu.ee
* Manuscript editor: Peter Rosenkranz

95% efficacy in autumn (Mutinelli et al., 1997) and 39.2–52.3% in summer, or 44% on average (Gregorc and Planinc, 2001). However, trickling in summer was highly toxic to bees (Rademacher and Harz, 2006). The lowest effective oxalic acid dihydrate concentration has been reported to be 30 g/L; this is only slightly less effective than higher concentrations. Others have reported that trickling may also cause loss of bees in winter (Charriere and Imdorf, 2002; Nanetti et al., 2003).

The effect of solutions containing less than 2% OA has been little studied so far. Aqueous oxalic acid solutions in concentrations of 1.7 and 1.4% have been reported to kill *Varroa* mites whereas a 0.7% aqueous solution had no effect (Takeuchi and Sakai, 1983b). A 3% aqueous solution (Higes et al., 1999) and sucrose solutions of the same or higher concentrations were reported to be toxic to bees; others found that the 3% concentration weakened colonies in the winter following treatment, particularly after two or three treatments during the summer or autumn (Rademacher and Harz, 2006). Gregorc et al. (2004) reported a level of 82% cell death of midgut epithelium in honey bee larvae 50 h after spraying OA in sucrose solution (2.97%/31.95%, w/v) directly onto uncapped brood. Increased levels of larval mortality were found after two treatments with OA in sucrose solution (3.0%/50%, w/v) in summer; the area of uncapped brood was reduced by 17.5% and remained low for about two months (Hatjiana and Haristos, 2005). Despite extensive recent research, optimum OA concentrations and the amount of OA per comb have not been established. The aim of our present study was to determine the concentration of oxalic acid solution that would combine good control of the mite with low bee mortality.

2. MATERIAL AND METHODS

Seven experiments were conducted to test a range of oxalic acid concentrations for their toxicity to *Varroa* mites and honeybees. Solutions for testing were prepared by dissolving a known weight of oxalic acid dihydrate in either distilled water or a 50% sucrose solution. Two methods of application of the solutions were compared: (1) submersion tests of bees from infested hives enclosed in cages; and (2) spraying tests of bees on combs. In the submersion tests, 100 g of bees in cages were submerged into one of a range of solutions. The spraying tests were carried out at an apiary in West-Viru County, Estonia. The bees were housed in Estonian standard one-box long hives with a frame size of 414 × 277 mm and an area per comb side of 1000 cm^2. Hives were provided with screened bottom boards. In the spraying tests, the solutions were sprayed directly onto bees on combs removed from the hives, using a hand-operated atomizer, and then replaced. The strength of each colony was estimated according to the Liebefeld method (Gerig, 1983; Imdorf et al., 1987) before and after the spraying experiments. Seven days after the last spraying, dead mites were counted and colony strength was estimated again. Apistan® strips were then placed in each test and control colony to determine the number of mites that had survived the test. A strip was placed into each comb space, but not in the end spaces, for 6 days after which mites were counted that had fallen onto the sheet of paper placed under the screened bottom board. In determining the effectiveness of the control, the cumulative mite-fall after oxalic acid and subsequent control treatment was assumed to be 100%.

2.1. Submersion tests in the laboratory

2.1.1. Experiment 1: aqueous solutions with 0.5, 1.0, 1.5 and 2.0% OA (July 2002)

Bees were collected from the brood combs of each experimental colony and 100 g of bees were placed into each of five stainless wire-mesh cages (100 × 80 × 100 mm) (Tab. I). One test cage for each concentration and one control cage was used for each test series. Each test cage with bees was submerged into a solution of OA of known concentration at 25 °C, and spun around the horizontal axis for 8 seconds to let air bubbles out before being removed from the solution. Each cage was then held above the bath for 10 seconds before being resubmerged into the solution for 8 seconds and spun again to wet the bees more thoroughly. The control cage with bees was submerged in water in the same manner. The caged bees were then kept for 48 hours at 25 °C and fed with honey. Fallen and dead mites were counted periodically, and dead and injured bees on the bottom of cages were counted

Table I. Methods of treatments with OA in aqueous- and sucrose solutions.

Variant of experiment	Number of experiment	Time of experiment	Concentration of OA, %	Dose per comb, mL	Number of treatments	Interval treatments, between days	Number of replications	Presence of brood, +, –	Aqueous (A) or sucrose (S) solution
	1	July, 2002	0.5		2		10	–	A
			1.0		2		10	–	A
			1.5		2		10	–	A
			2.0		2		10	–	A
Submersion	2	July, 2007	0.1		2		5	–	A, S
			0.2		2		5	–	A, S
			0.3		2		5	–	A, S
			0.4		2		5	–	A, S
			0.5		2		5	–	A, S
	3	April, 2003	0.5	25	4	3–6	3	–	A
			1.0	25	4	3–6	3	–	A
			1.5	25	4	3–6	3	–	A
Spraying	4	May, 2003	0.5	25	5	1	6	–	A
	5	April, 2004	0.5	25	5	1	11	–	A
	6	Sept., 2005	0.5	25	1 and 2	6	15	+	A
	7	Sept., 2006	0.3	25	1		8	+	A

at the end of the experiment. After two days, half a strip of Apistan was placed in each test and control cage to determine the number of mites that had survived the treatment. As Apistan strips had never been used before in the control of *Varroa* mites in the experimental apiary, the risk of mite resistance to Apistan was low. The experiments were replicated 10 times, with four different concentrations and one control for each test series

2.1.2. Experiment 2: aqueous versus sucrose solutions with 0.1, 0.2, 0.3, 0.4 and 0.5% OA (July 2007)

The submersion method used was as for Experiment 1. The control group was similarly submerged into either an aqueous or a sucrose solution (50%) without any OA. After submersion into the sucrose solutions the cages was washed in hot water (35 °C) to release mites that had stuck to the cages. The experiments with different concentrations were replicated 5 times (Tab. I).

2.2. Spraying tests onto bees in colonies

2.2.1. Experiment 3: aqueous solutions with 0.5, 1.0 and 1.5% OA (April 2003)

A total of 12 colonies were used with three test hives and a control colony in each of three experimental groups (Tab. I). The brood was cut out of the brood comb and the queens were caged to prevent egg-laying prior to treatment. Test colonies were sprayed with different concentrations of OA aqueous solution at 3–6 day intervals depending on the weather; control colonies were sprayed with the same amount of water. The bees were sprayed until wetted. A dosage of 25 mL per comb was used. The bees out of the sprayed combs were given an additional spray with 25 mL OA solution. Thus, for an 8-frame colony, $8 \times 25 + 25 = 225$ mL solution of OA was applied.

2.2.2. Experiment 4: aqueous solution with 0.5% OA (May 2003)

Five test colonies and three control colonies were used (Tab. I). The queens in each colony were caged on 15 April, and the experiment started when all the brood had emerged (three weeks later).

2.2.3. Experiment 5: aqueous solution with 0.5% OA (April 2004)

Eleven test colonies and five control colonies were used (Tab. I). The queens in these colonies were caged on 15 March. Dead mites were counted two days after each spraying. Colony strength was estimated again after the third and fifth spraying and again 12 days later to determine bee tolerance.

2.2.4. Experiment 6: aqueous solution with 0.5% OA (September 2005)

Forty colonies were split into two groups, each having 15 test colonies and five control colonies (Tab. I). The area of capped brood was assessed in each colony. Group 1 test colonies were sprayed once and group 2 twice over a 6-day interval. The following spring (30 March 2006), colony strength was reassessed and mite survival assessed.

2.2.5. Experiment 7: aqueous solution with 0.3% OA (September 2006)

The test group was comprised of eight colonies and the control group five colonies (Tab. I). The area of capped brood in each colony was assessed.

For statistical analyses one-way ANOVA was employed using Statistica 7.0. Means were compared using the LSD Test, and *t*-tests were applied at a significance level of $P < 0.05$. A multiple linear regression was used to determine the effectiveness of spraying the colonies with various capped brood area.

3. RESULTS

3.1. Submersion experiments

Experiment 1 submersion tests showed that all concentrations of OA in the range of 0.5–2% were equally toxic to *Varroa* mites but that 1.0% and stronger OA solutions were toxic to bees (Fig. 1). The 0.5% aqueous OA solution was non-toxic to bees, the 1.0% solution caused the death of a small number, and concentrations of 1.5 and 2.0% were highly toxic to bees.

Experiment 2 submersion tests showed that a 0.1% OA concentration killed mites (Fig. 2). The aqueous OA solution was 59.9 ± 3.72% effective while the sucrose solution was slightly, but not significantly more effective at 71.1 ± 4.20% (*t*-test: $t = -1.885$, $P = 0.096$, df = 8). More concentrated solutions of OA (0.2%) were more effective: 89.77 ± 2.18% in aqueous solution and 90.03 ± 1.03% in sucrose solution, respectively. Effectiveness of the 0.3% concentration in aqueous solution was 94.89 ± 0.93% and in sugar solution

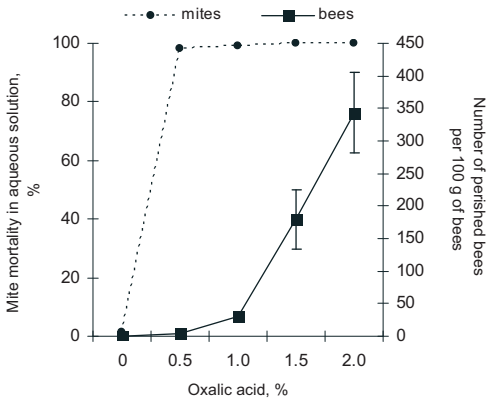

Figure 1. Effect of 0.5, 1.0, 1.5 and 2.0% oxalic acid dihydrate (OA) in aqueous solution on *Varroa* mites and bees in submersion tests (n = 10 experimental series) at the end of July 2002. Data are presented as means ± standard errors. Concentrations of 0.5–2.0% had similar toxicity to the mites. The 0.5% concentration showed little toxicity to bees but toxicity to bees increased considerably with concentrations higher than 1.0% OA.

was 95.51 ± 0.54%, which were statistically similar. Both the 0.4% and 0.5% aqueous and sucrose solutions achieved up to 100% effectiveness (Fig. 2 A, B) (ANOVA, LSD test, for water solution $F_{4;20} = 69.5849$, $P = 0.00$, for sugar solution $F_{4;20} = 38.1801$, $P = 0.00$). The OA in sucrose showed slightly, but not significantly, higher mite control than the OA in water (Figs. 2 A, B), but was considerably more toxic to bees (Fig. 3). The 0.3% OA solution in sucrose was five times more lethal to bees than that in water. The increase in concentration from 0.4% to 0.5% of OA in sucrose solution increased the mortality of bees about five times more than the aqueous solution with the same concentration of OA, a killing significant number of bees (Fig. 3, $P < 0.05$, *t*-test). Aqueous OA solutions were milder; at concentrations of 0.1–0.4% only a few bees died, but the 0.5% concentration caused the death of 35 ± 5 bees on average (ANOVA, LSD test, for aqueous solution $F_{5;24} = 8.287$, $P = 0.00$, for sucrose solution $F_{5;24} = 30.003$, $P = 0.00$). About 90% of all mites were dead within 24 hours after submersion into 0.5% OA solution. Solutions weaker than 0.5% solutions had a slower effect.

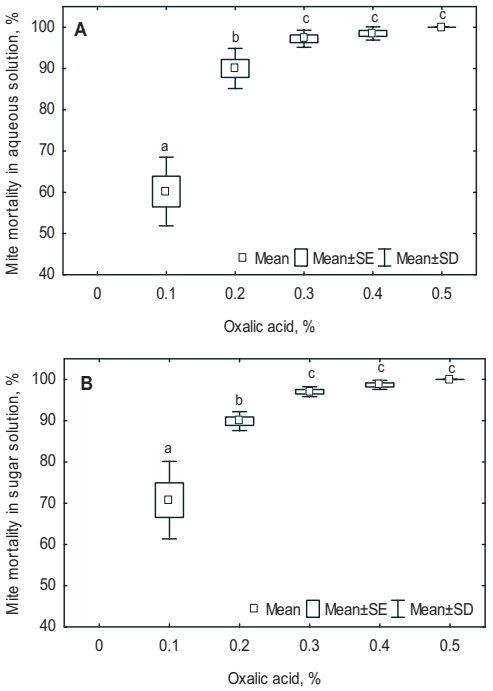

Figure 2. Effect of 0.1–0.5% oxalic acid dihydrate (OA) in aqueous (A) and in 50% sucrose solution (B) on mite mortality in submersion tests (n = 5 experimental series) at the end of July 2007. Different letters indicate statistically significant differences (ANOVA one-way, LSD test, aqueous solution $F_{4;20}$ = 69.5849, P = 0.000; sucrose solution $F_{4;20}$ = 38.1801, P = 0.000). There was little difference between the effectiveness of the OA in aqueous and sucrose solution at 0.1–0.5% concentrations of OA. Higher acaricidal activity was found only in the concentration of 0.1% in aqueous solution compared to the same concentration in sugar solution.

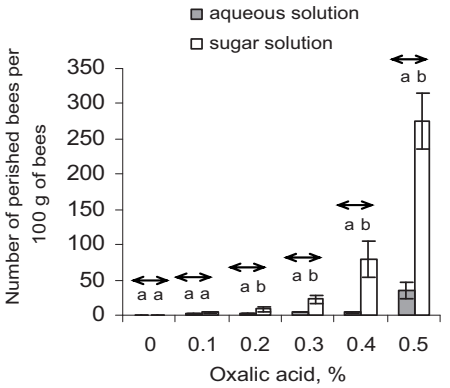

Figure 3. Effect of 0.1–0.5% oxalic acid dihydrate in aqueous and in 50% sucrose solution on bees in submersion tests (n = 5 experimental series) at the end of July 2007. Different letters indicate statistically significant differences. As the concentration of OA increased above 0.2%, the sucrose solution became increasingly more toxic to bees than the aqueous solution (t-test, $P < 0.05$).

3.2. Spraying experiments

Experiment 3, in which bees in brood-free colonies were sprayed four times in early spring (April) with 0.5 to 1.5% OA in aqueous solution (Fig. 4) confirmed the results of the submersion test (Experiment 1) (Fig. 1). Solutions of 1.0 and 1.5% OA gave similar mite control (98.39 ± 0.01% and 98.28 ± 0.01%); the 0.5% aqueous solution gave a weaker, but significant effect (94.92 ± 0.00%) (ANOVA, LSD test, $F_{3;8}$ = 4466.64, P = 0.00) (Fig. 4). However, the 1.0 and 1.5% concentrations were highly toxic to bees; twice the number died (Fig. 5, t-test, $P < 0.05$). In both groups, two of the three colonies were severely affected. The weakening was noticeable after the third and fourth spraying. In cool weather, dead bees were found in the hive behind the partition board and a few bees were seen to leave the hive, stagger and die in front of the entrance.

Experiment 4, in which bees in brood-free colonies were sprayed five times in May with a 0.5% OA in aqueous solution at a dose of 25 mL per comb proved highly effective in mite control (99.01 ± 0.24%) and non-toxic to bees. The colonies did not weaken after spraying (t-test, t = 0.4246, P = 0.6823).

Experiment 5, in which spray was applied five times in April with a 0.5% OA was similarly effective, 99.42 ± 0.10%. Most of the mites (647.1 ± 154.3 or 78.3%) died within two days of the first spraying, fewer within two days of the second spraying (139.6 ± 23.7 or 16.9%), and very few after later sprayings (Fig. 6). However, 4 of the 11 test colonies weakened considerably, indicating toxicity of this treatment to bees. Noticeable weakening occurred after the fourth and fifth spraying, and was most noticeable 12 days later (Fig. 7, ANOVA, LSD test, $F_{3;40}$ = 10.2377, P =

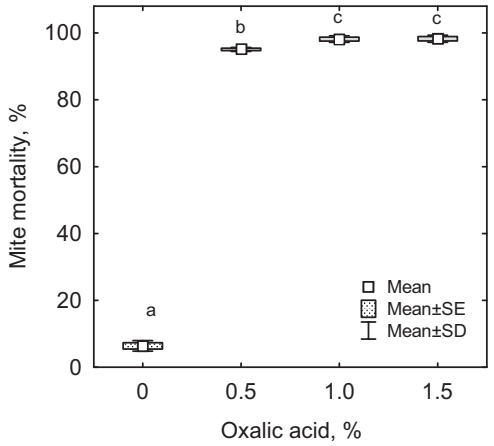

Figure 4. Effect of spraying bees in brood-free colonies in April 2003 with 0.5, 1.0 and 1.5% oxalic acid dihydrate in aqueous solution four times on the mortality of *Varroa* mites (dose 25 mL per comb, n = 3 experimental series). Mean values marked with same letters do not differ significantly. The 0.5% concentration was significantly less effective than the 1.0 and 1.5% concentrations (ANOVA one-way, LSD test, $F_{3;8} = 4466.64$, $P = 0.000$).

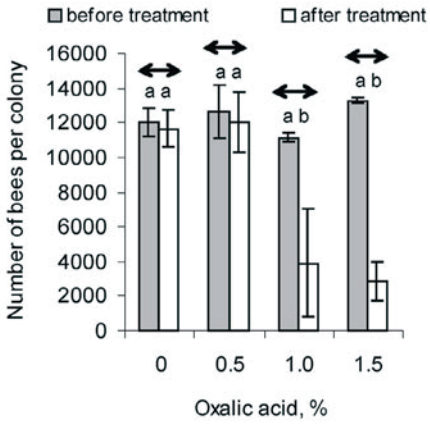

Figure 5. Effect on colony size of spraying bees four times in brood-free colonies in Spring 2003 with 0.5, 1.0 and 1.5% oxalic acid dihydrate in aqueous solution (dose 25 mL per comb, n = 3 experimental series). Mean values (±SE) marked with different letters are significantly different.
The 1.0 and 1.5% concentrations weakened the colonies considerably ($t = 2.3206$, $P = 0.05$ and $t = 9.3228$, $P = 0.0007$, t-test) whereas the 0.5% solution had no noticeable toxic effect ($t = 0.2579$, $P = 0.8092$, t-test).

0.00). In the control group colonies, no similar weakening occurred. The morning after the third spraying, a noticeable number (20–50) of dead bees were seen in front of the entrances of some test colonies. Following the fourth and fifth spraying, most of the test colonies had numerous dead bees in front of the entrance. In some colonies, bees showing toxic symptoms were seen to fly out of the hive, then fall and crawl in front of the hive.

Experiment 6 showed that spraying colonies with 0.5% OA in aqueous solution, in September when they had little sealed brood, was highly effective in killing mites. Average efficacy in colonies sprayed once was 92.94 ± 0.01% and in those sprayed twice, 91.84 ± 0.02%; in control groups only 5.41 ± 0.01% (ANOVA, $F_{3;36} = 683.96$, $P = 0.00$) of mites died. Treatment effects were smaller in colonies with more capped brood during spraying (r = −0.8276, n = 30). Thus there were 5 colonies in the test with 14.6–85.1 cm^2 of capped brood and a treatment efficacy of 98.64–99.9%. The same test included 11 colonies with 259.6–529.2 cm^2 of capped brood and a treatment efficacy of 86.16–89.67%. Spraying once or twice with a dose of 25 ml per comb proved non-toxic to bees; test colonies were no weaker than control colonies after the autumn spraying or the following spring (Fig. 8, ANOVA, LSD test, $P > 0.05$). The number of dead mites ranged from 436 to 9164 per colony, and on average 3329.74 ± 500.94 mites.

Experiment 7 showed that, although 0.3% OA in aqueous solution had relatively high efficacy in submersion tests (Experiment 2), this was not the case when bees were sprayed once with the same solution in the hive. The average effectiveness of spraying with 0.3% aqueous solution was 76.44 ± 4.02%, significantly lower than the 92 to 93% effectiveness obtained the previous autumn (Experiment 6) when spraying with the same dose of 0.5% solution (t-test, $t = -5.6637$, $P = 0.000$; LSD Test, $P = 0.000$).

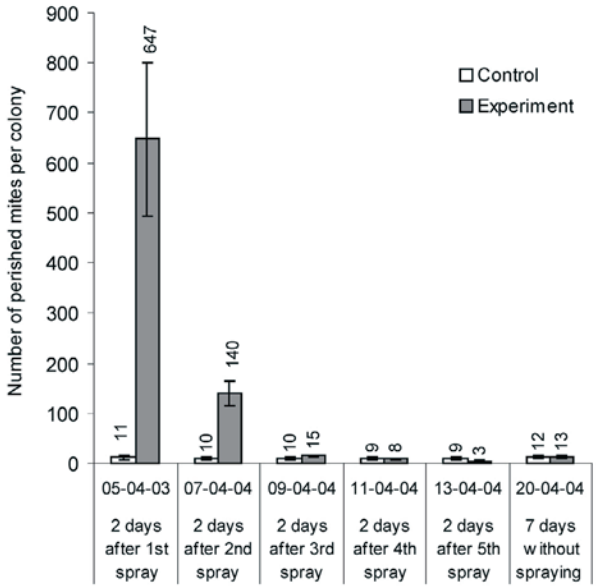

Figure 6. Dynamics of mite mortality in brood-free colonies after spraying the bees five times with 0.5% oxalic acid dihydrate in aqueous solution (dose 25 mL per comb, n = 11) in 2004. Data are presented as mean ± standard error.

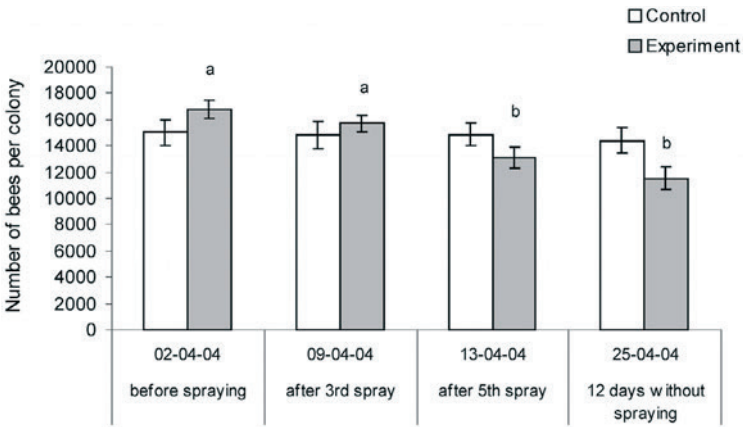

Figure 7. Effect on colony size of spraying bees five times in brood-free bee colonies (dose 25 mL per comb, n = 11) with 0.5% oxalic acid dihydrate in aqueous solution in Spring 2004. Different letters indicate statistically significant differences. Test colonies were weakened slightly more than the control colonies after the third spraying and considerably more after the fifth spraying. They continued to be weaker 12 days after the last spraying (ANOVA one-way, LSD test, $F_{3;40} = 10.2377$, $P = 0.000$).

4. DISCUSSION

4.1. Submersion experiments

The 0.5% OA in aqueous solution was as effective against mites as the 1, 1.5 and 2.0% solutions but was less toxic to bees (Fig. 1). When caged bees were submerged into 0.1–0.5% aqueous OA solutions, even the 0.1% solution had a strong effect against the mite. Increasing concentrations to 0.2–0.3% substantially increased the effectiveness of mite control (Fig. 2A), but further increase from 0.3 to 0.5% did not raise mite mortality

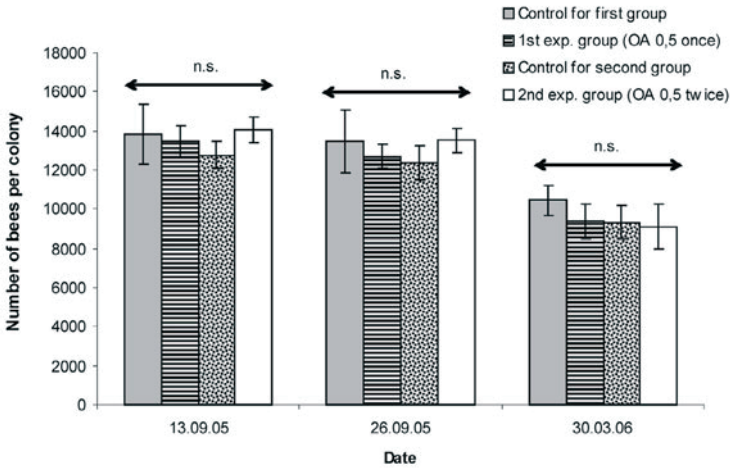

Figure 8. Effect on colony size of spraying bees in colonies containing a small amount of capped brood either once or twice with 0.5% oxalic acid dihydrate in aqueous solution (dose: 25 mL per comb, n = 15) in September 2005. Mean values marked with n.s. do not differ significantly. Test colonies were not significantly weaker than control colonies after spraying in autumn or in the following spring (ANOVA, LSD test, $P > 0.05$).

(Fig. 2A). The same concentrations of OA in sucrose solution showed a similar rise in mite mortality (Fig. 2B). A slightly higher acaricidal activity was found only in the concentration of 0.1% in sucrose solution compared to the same concentration in aqueous solution (Fig. 2A, B). At the same time, the oxalic acid in sugar solution increased the toxic effect to bees at the lower concentrations of 0.2–0.3%. Oxalic acid concentrations of 0.4–0.5% increased the rapid death of bees in comparison with solutions without sugar in the treatment solution (Fig. 3). It is possible the sugar solution containing oxalic acid crystals stuck to the cuticle and spiracles of bees prolonging the toxic effect, whereas the water from an aqueous solution evaporated and the remaining oxalic acid crystals fell from the bees. In our submersion tests bees were equally wet in both aqueous and sucrose solutions.

A few studies have been conducted to compare the effects of water and sugar solutions. For example, adding sugar to a trickling solution of OA increased the effectiveness of controlling the mite from 92.5% to 97.5%, thus only by 5% (Charriere, 2001). Some researchers have established that lower sugar content in oxalic acid solution increased bee tolerance but decreased its effectiveness in mite control (Milani, 2001; Gregorc and Planinc, 2001; Charriere and Imdorf, 2002; Nanetti et al., 2003). In most cases solutions without sugar were not very effective, and 30% sugar solutions tended to have slightly lower acaricidal efficacy compared to 60% sugar solutions (Nanetti et al., 2003). The importance of sugar in increasing the efficacy is still an open question and more tests are required using lower concentrations of sugar.

4.2. Spraying experiments

In spraying experiments, 0.5% OA in aqueous solution was as effective as 1 and 1.5% solutions for mite control (Fig. 4) but was less toxic to bees (Fig. 5). Spraying five times with 0.5% OA in aqueous solution in May 2003 was non-toxic to bees; colonies were as strong post-treatment as pre-treatment. Spraying five times in April 2004, however, was toxic to bees. The toxic effect was not significant after

the first three sprayings and test colonies did not differ significantly from the control. A noticeable toxic effect was seen after the third, and particularly after further sprays (Fig. 7). One possible explanation is that in April 2004, compared to the experiment conducted in May 2003, the bees were less able to fly from the hive on cleansing flights due to cold weather; the cleansing flights might have helped rid the bees of oxalic acid residues. Furthermore, the bees in the experimental hives in April 2004 were comprised mainly of old overwintered individuals. The brood area was small, whereas by May, a new generation of bees had emerged before treatment. The test colonies were weakened by the death of old bees and shortage of young bees in 2004. Due to high efficacy of 0.5% OA for mite control, there was no need to treat more than twice as most of the mites died after the first and second spraying (Fig. 6).

Most mites died after the first spraying and a large number died after the second spraying as well (Fig. 6). As first and second sprayings were only two days apart, some of the mites presumably died as a result of the first spraying in our experiment. In submersion tests, about 90% of the mites fell within twenty-four hours presumably because they became completely wet. Higher doses of treatment solution may have led to better distribution of OA due to water improving the transfer and contact with OA, resulting in the more rapid death of mites. It has been recommended that experimental data should not be gathered for at least one week after spraying (Radetzki et al., 1994; Brødsgaard et al., 1999) as most mites fall during the week following a treatment (Brødsgaard et al., 1999). The experiment with 0.5% OA in aqueous solution in autumn of 2005 showed that there was no difference in the effectiveness of control after spraying once or twice (ANOVA one-way, $F_{3;36} = 683.96$, $P = 0.00$). The effectiveness of mite control increased as the area of capped brood in hives decreased ($r = -0.8276$, $n = 30$).

During experiment 6 (spraying once or twice with 0.5% OA in aqueous solution) we observed large differences in the number of mites among different colonies within one apiary. The differences were greater than 10 times and, in some cases, even up to 20 times. This was also noted by Fries et al. (1991) who suggested the variation may be affected by several factors, such as differences in the actual initial dose of fertile mites, differences in brood-rearing pattern between colonies, variation in mite fertility between colonies, differences in grooming behaviour between colonies, and possible uneven transfer of mites between colonies, in spite of precautions to avoid drifting.

Spraying once or twice with 0.5% OA in aqueous solution in September 2005 showed equally high efficacy of mite control and little toxicity to bees (Fig. 8). We conclude that a single spray of this concentration is sufficient to achieve adequate *Varroa* control. In our spraying experiments during the treatments in April, colonies had mainly capped honey and only a little fresh nectar from willows (*Salix* sp.) and coltsfoot (*Tussilago farfara*). In autumn, honey combs were capped and contact with OA-residues was also restricted. For this reason only a little amount of OA would contaminate the honey. Due to lower amount of OA in our treatments 0.125 g per comb (in 0.5% OA solution) compared with the experiments (in 3.0% OA solution 0.195 g per comb) of Radetzki et al. (1994) and Charrière and Imdorf (2002), the residues in honey would be even lower in our experiments. In Central European conditions it may prove possible to treat in November or even in December. As a sprayed concentration of 0.3% was less effective in practice, a concentration of 0.4–0.5% in aqueous solution at a dose of ca 25 mL per comb should be used. With the 0.5% solution, we must accept some death of bees, although this is small and may be acceptable. Spraying later, in autumn, when there is less brood in the hive, can increase its effectiveness. The treatment effect can also be increased by spraying bees on combs from different directions, i.e. wetting them as much as possible with an OA solution.

Some experiments have shown that a single spray with OA in aqueous or sucrose solution is considerably more effective than trickling (Brødsgaard et al., 1999; Bahreini, 2003), indicating that greater wetting of bees increases the effectiveness of the oxalic acid in mite

control. Our submersion tests also showed the importance of thorough wetting for good mite control. A lower concentration may be considerably less effective in trickling due to less contact of the solution with the bees and mites. Fries (2001) found that trickling a 1.6% oxalic acid solution at a higher dose (60 mL per colony) was 68.3% effective whereas a 3.25% solution, 30 mL per colony, was 92.2% effective. Although concentrations of <4.6% have been tolerated well by bees in experiments by many researchers, in some experiments colonies have been weakened considerably following a single trickling treatment. Multiple autumn or summer treatments (two to three times) by trickling were poorly tolerated by the bees in Central Europe (Rademacher and Harz, 2006). These facts refer to the toxicity of the OA concentrations to bees used with the trickling method, which is not always revealed and depends, in all tests, on the individual tolerance of bee colonies. Concentrations, doses and efficacy of oxalic acid sugar concentrations less toxic to bees as well as the toxicity of the concentrations in their repeated use require further research.

The main advantage of treatment in the spring rather than in the autumn is the reduced danger of disturbance of colony homeostasis, especially of harm to the queen. Autumn treatment may disturb preparations for the wintering period. On the other hand, if the population of mites is high and autumn treatment for mite control considered essential it must be done carefully. Treatment by spraying is very laborious whereas the trickling method is more convenient in practice.

The spraying method is the most suitable in hobby beekeeping apiaries. Use of low spray concentrations of OA harmless to bees probably could be successfully integrated in normal beekeeping practice when there is no or only small areas of capped brood (eg. after hiving of swarms, broodless periods in swarmed colonies and nucleus colonies, after heavy honeydew flow and after honey harvesting with only a small amount of brood in the hive, in broodless periods in late autumn and early spring, or after use of the shaking against foulbrood diseases). Spraying with a weak solution of OA is less harmful to the honey bees and to the people applying the treatment.

ACKNOWLEDGEMENTS

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Effet de l'acide oxalique dissout à différentes concentrations dans une solution d'eau distillée ou de sucrose sur le varroa et sur les abeilles.

***Apis mellifera* / *Varroa destructor* / acide oxalique / toxicité**

Zusammenfassung – Der Effekt unterschiedlicher Oxalsäurekonzentrationen in Wasser- und Zuckerlösungen auf Varroamilben und Honigbienen. In dieser Untersuchung sollte diejenige Konzentration von Oxalsäure-Dihydrat (OS) ermittelt werden, die eine wirksame Varroakontrolle bei gleichzeitiger geringer Bienentoxizität sicher stellt. Von 2002 bis 2007 verglichen wir unterschiedliche OS-Konzentrationen in wässrigen Lösungen und Zuckerwasserlösungen, indem wir zunächst gekäfigte und mit Varroamilben befallene Bienen in die entsprechenden OS-Lösungen eintauchten. Daneben wurden Bienen in befallenen Bienenvölkern mit und ohne Brut mit OS- Lösungen besprüht. Die Anzahl der überlebenden Milben nach den Behandlungen wurde mit Apistan-Streifen ermittelt.
In den „Tauchtests" waren wässrige OS-Konzentrationen zwischen 0,5–2,0 % toxisch für Milben, aber bereits Lösungen von 1 % – und mehr noch die 1,5 – und 2,0-prozentigen Lösungen – waren auch für Bienen hochtoxisch (Abb. 1). In 50 %-iger Zuckerlösung war OS (0,1–0,5 %) bei 0,1 % wirksamer gegenüber Milben (Abb. 2A, B) aber bereits bei Konzentrationen von 0,2–0,3 % auch toxischer gegenüber Bienen (Abb. 3).
In Sprühversuchen waren wässrige 1,0 und 1,5 % OS-Lösungen gleichermaßen toxisch für Milben (98,4 ± 0,01 % und 98,3 ± 0,01 %), während eine 0,5 %-ige Lösung einen schwächeren Wirkungsgrad hatte (94,9 ± 0,00 %) (Abb. 4). Allerdings waren die 1,0- und 1,5 %-igen Lösungen sehr toxisch für Bienen (Abb. 5). Frühjahrsbehandlungen, bei denen brutfreie Bienenvölker fünfmal mit einer 0,5 %-igen wässrigen OS-Lösung mit 25 ml pro Wabe besprüht wurden, ergaben einen durchschnittlichen Wirkungsgrad von 99,0 ± 0,24 %.

Die meisten Milben starben dabei nach der ersten Behandlung (Abb. 6). Jedoch war die fünfmalige Sprühbehandlung im Frühjahr toxisch für Bienen (Abb. 7). Ein- bis zweimalige Herbstbehandlungen von Bienen in Völkern mit wenig verdeckelter Brut mit 0,5 %-iger OS-Lösung zeigten mit 92,9 ± 0.01 % bzw. 91.8 ± 0.02 % fast den gleichen Effekt (Abb. 8) und blieben ohne erkennbare Toxizität gegenüber den Bienen (Abb. 8). In Bienenvölkern mit einem größeren Umfang an verdeckelter Brut waren die Behandlungen weniger effektiv.

Wir schließen aus unseren Versuchen, dass mit einer 0,5 %-igen wässrigen OS-Lösung, die einmal auf Bienen in Völkern ohne oder mit wenig Brut gesprüht wird, eine gute Wirksamkeit gegenüber den Varroamilben bei gleichzeitigen geringen Bienenschäden erreicht wird.

Apis mellifera **/ Honigbiene /** ***Varroa destructor*** **/ Oxalsäure**

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II

Toomemaa, K., Martin, A-J., Mänd, M., Williams, I. H. 2010

USING OXALIC ACID IN WATER SOLUTION IN CONTROL OF VARROA MITES AND ITS INFLUENCE ON HONEY BEES

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Using oxalic acid in water solution in control of Varroa mites and its influence on honey bees

K. Toomemaa, A.-J. Martin, M. Mänd, I. H. Williams

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 1A Kreutzwaldi St, 51014 Tartu, Estonia; e-mail: ktoomemaa@gmail.com; ants.martin@emu.ee

Abstract. We studied the toxicity of water solutions containing various concentrations of oxalic acid dihydrate to bees and Varroa mites (*Varroa destructor*) using by spraying honey bee (*Apis mellifera*) colonies with no brood or little brood in beehive conditions in West-Viru County, Estonia. A water solution of 0.5% OA gave effective control of the mite and was not toxic to bees whereas higher concentrations of OA (1.0 and 1.5%) were highly toxic to bees. In autumn, spraying test colonies that had little capped brood once or twice with a 0.5% OA solution gave effective mite control (92.94 ± 0.01% and 91.84 ± 0.02%, respectively) with no noticeable toxicity to bees.

Keywords: *Apis mellifera*, honey bee, *Varroa destructor*, oxalic acid.

INTRODUCTION

Some synthetic drugs such as **Apistan®** and **Bayvarol®**. are successfully used against honey bee parasitic mites *Varroa destructor,* but they leave the residues in honey and wax and may be dangerous for human health. Spraying honey bee colonies with 2 or 3% oxalic acid water solution has been used to control the Varroa mite since the 1980's. At first, 2% oxalic acid, at a dose of 100–150 ml per colony, was found to be very effective in mite control and, at the same time, harmless to bees (Takeuchi and Harada, 1983). More recently, spraying 5–8 ml of 3% OA solution per comb has been recommended (Radetzki et al., 1994). A single spray applied during a brood-free period, has given, on average, 94.5–98.8% (Imdorf et al., 1997) and 97.29% (Radetzki et al., 1994) effectiveness against the mite, whereas spraying twice has given up to 99.5% (Nanetti et al., 1995). Usually, no negative toxic effects have been observed in bees. Lately however, some negative after effects have been reported, namely a decrease in brood area after spraying four-times (Higes et al., 1999); others found that this concentration weakened colonies in the winter following treatment, particularly after two or three treatments during the summer or autumn (Rademacher & Harz, 2006).

The effect of solutions containing less than 2% OA has been little studied so far. Aqueous oxalic acid solutions in concentrations 1.7 and 1.4% have been reported to kill Varroa mites whereas a 0.7% aqueous solution had no effect (Takeuchi & Sakai,

1983). We found that even 0.5% OA solution guarantees the highly effective mite control in our pilot tests. Despite extensive recent research, optimum OA concentrations as well as OA amount per comb have not been established so far. The aim of our present study was to determine the concentration of oxalic acid solution that would guarantee sufficient control of the Varroa mite disease with low bee mortality.

MATERIALS AND METHODS

Two experiments were conducted from 2003–06 to test a range of different concentrations of oxalic acid for their toxicity to Varroa mites and honeybees. The studies were carried out at an apiary in West-Viru County, Estonia. The bees were housed in Estonian standard one-box long hives with a frame size of 414×277 mm, with a side area of 1000 cm^2. The ambient temperature during the experiments was 4–12°C. Hives were provided with Varroa screens. The solutions with different concentrations were sprayed using a hand-operated atomizer directly onto bees on combs removed from the hives and replaced after spraying. The strength of each colony was estimated according to the Liebefeld method (Gerig, 1983, Imdorf et al., 1987) before and after the spraying experiments. Seven days after the last spraying, dead mites were counted and colony strength estimated again. Apistan strips were then placed in each test and control colony to determine the number of mites that had survived the test. A strip was placed into each comb space, except for both side comb spaces and kept there for 6 days, after which mites had fallen onto the sheet of paper placed under the varroa screen on the bottom of the hive were counted. In determining the effectiveness of the control, the cumulative mite-fall after oxalic acid and subsequent control treatment was assumed to be 100%.

Experiment 1. Water solutions with 0.5, 1.0, 1.5% OA (April 2003).Twelve colonies were used with three test hives and a control colony in each experimental group. The brood was cut out of the brood comb and the queens were caged to prevent egg-laying prior to treatment. Test colonies were sprayed with different concentrations of OA aqueous solutions four times at 3–6 day intervals depending on the weather, control colonies were sprayed with same amount of water. The bees were sprayed until wet at dosage 25 ml per comb. The bees outside the combs were sprayed additionally with 25 ml OA solution. Thus for an 8-frame colony $8 \times 25 + 25 = 225$ ml solution of OA was applied.

Experiment 2. A water solution with 0.5% OA was applied once or twice (September 2005). Forty colonies were split into 2 groups, each having 15 test colonies and 5 control colonies. The area of capped brood was assessed in each colony. Group 1 test colonies were sprayed once and group 2, twice with a 6-day interval. Dosage per comb was the same. Group 1 control colonies were sprayed once and group 2 control colonies twice with the same amount of water. Dead mites were counted and colony strength assessed 7 days after the final spraying. The following spring (30 March 2006), colony strength was reassessed and mite survival assessed.

For statistical analyses the program Statistica 7.0 and one-way ANOVA was used. The means were compared by the LSD Test, and *t*-test was applied at a significance level of $p < 0.05$.

RESULTS

Experiment 1, in which bees in brood-free colonies were sprayed four times in early spring (April) with 0.5, 1.0 and 1.5% OA in aqueous solution confirmed the results of the earlier pilot research (Fig. 1).

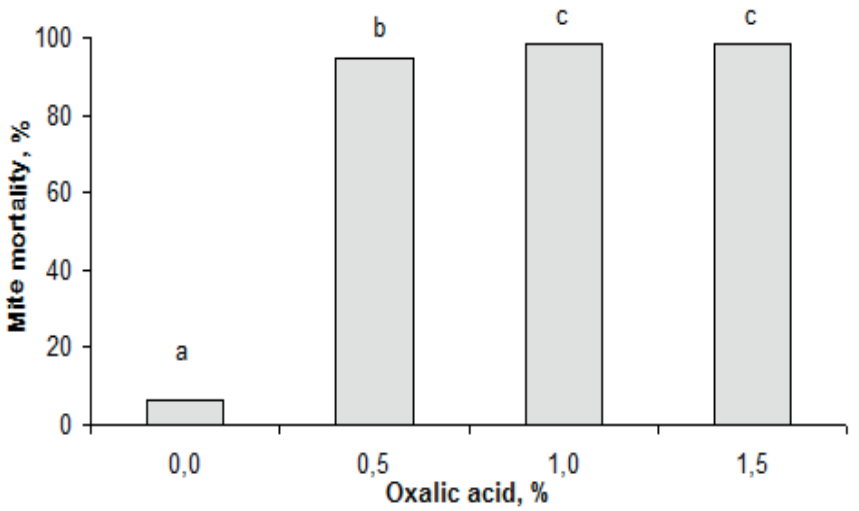

Figure 1. Effect of spraying bees in brood-free colonies in April 2003 with 0.5; 1.0 and 1.5% oxalic acid dihydrate in aqueous solution four times on the mortality of Varroa mites (dose 25 ml per comb, n = 3). Mean values marked with same letters do not differ significantly. The 0.5% concentration was significantly less effective than the 1.0 and 1.5% concentrations (ANOVA one-way, LSD test, $F_{3;8} = 4466.64$, $p = 0.000$).

Solutions of 1.0 and 1.5% OA gave similar mite control (98,39 ± 0,01% and 98,28 ± 0,01%); the 0.5% aqueous solution gave a weaker, but significant effect (94,92 ± 0,00%) (ANOVA, LSD test, $F_{3;8} = 4466.64$, $p = 0.00$) (Fig. 1). However, the 1.0 and 1.5% concentrations were highly toxic to bees: about twice the number of bees in the colony died according as estimated in the Liebefeld method (Gerig; 1983, Imdorf et al., 1987) before and after the spraying experiments (*t*-test, $p < 0.05$). The 1.0 and 1.5% concentrations weakened the colonies considerably, whereas the 0.5% solution had no noticeable toxic effect (Table 1). In both groups, two of the three colonies were severely affected; the weakening was noticeable after the third and fourth spraying. In cool weather, dead bees were found in the hive behind the partition board and a few bees were seen to leave the hive, stagger and die in front of the entrance.

Experiment 2 showed that spraying colonies with 0.5% OA in aqueous solution, in September when they had little sealed brood, was highly effective in killing mites

(Fig. 2). The average efficacy in colonies sprayed once was 92.94 ± 0.01% and in those sprayed twice, 91.84 ± 0.02%; in control groups only 5.41 ± 0.01% (ANOVA, $F_{3;36}$ = 683.96, p = 0.00) of mites died. Treatment effects were significantly lower in colonies with more capped brood during spraying (r = -0.806, n = 30). Spraying once or twice with a dose of 25 ml per comb proved non-toxic to bees: test colonies were no weaker than control colonies after the autumn spraying or the following spring (ANOVA, LSD test, $p > 0.05$). The number of dead mites ranged from 436 to 9164 per colony, on average 3329.74 ± 500.94 mites.

Table 1. Effect of spraying bees in brood-free colonies in April 2003 with 0.5; 1.0 and 1.5% oxalic acid dihydrate in aqueous solution (dose 25 ml per comb, n = 3) four times on colony size. The 1.0 and 1.5% concentrations weakened the colonies considerably (t = 2.3206, p = 0.05 and t = 9.3228, p = 0.0007, t-test) whereas the 0.5% solution had no noticeable toxic effect (t = 0.2579, p = 0.8092, t-test).

Concentration of OA in solution, %	Number of experiments (N)	Number of bees in colony		Decreasing no of bees, %
		Before treatment	After treatment	
0	1–3	12,026 ± 817	11,660 ± 1040	3.1 ± 2.4
0.5	4–6	12,613 ± 1505	12,027 ± 1706	4.7 ± 2.6
1.0	7–9	11,147 ± 264	3887 ± 3117	65.1 ± 26.6
1.5	10–12	13,273 ± 147	2860 ± 1107	78.5 ± 8.7

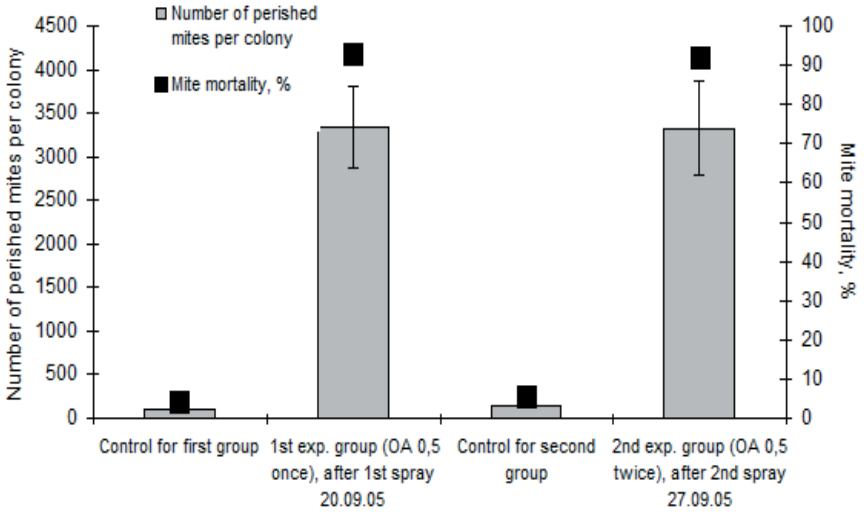

Figure 2. Effect of spraying bees in colonies with some capped brood with 0.5% oxalic acid dihydrate in aqueous solution (dose: 25 ml per comb, n = 15), either once (13.09.05) or twice (13.09.05 and 19.09.05) in autumn on Varroa mite mortality

Different letters indicate statistically significant differences (ANOVA one-way, $F_{3;36} = 683.96$, $p = 0.00$).

DISCUSSION

In the spraying experiments, 0.5% OA in aqueous solution was as effective as 1 and 1.5% solutions for mite control (Fig. 1) but less toxic to bees (Table 1). Spraying once or twice with 0.5% OA in aqueous solution, in September 2005, showed equally high efficacy on mite control (Fig. 2) and little toxicity to bees. We conclude that a single spray of this concentration and at a dose of ca 25 ml per comb should be used for effective Varroa control. Spraying later, mainly at the end of September for Estonian conditions, when there is less brood in the hive, can increase its effectiveness. In Central European conditions presumably it would be suitable to treat in November or even in December depending on the presence of the brood. The treatment effect can also be increased by spraying bees on combs from different directions, i.e. wetting them as much as possible with an OA solution. The spraying method is the more suitable in amateur apiaries. Use of low spray concentrations of OA harmless to bees could probably be successfully integrated in normal beekeeping practice with or without only small areas of capped brood. If the number of mites is high the colonies should be treated in early autumn, after the honey harvest, using the spraying with 0.5% OA or other natural substances like formic- and lactic-acid which are less harmful to the honey bees and to the person applying the treatment.

CONCLUSIONS

We conclude that 0.5% oxalic acid in a water solution at a dose of 25 ml per comb applied as a single spray in honeybee colonies with little or no brood achieves effective control of the Varroa mite. Spraying with a weak solution of OA is less harmful to the honey bees and to the person applying the treatment.

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III

Toomemaa, K., Martin, A-J., Mänd, M., Williams, I. H. 2013

DETERMINING THE AMOUNT OF WATER CONDENSED ABOVE AND BELOW THE WINTER CLUSTER OF HONEY BEES IN A NORTH-EUROPEAN CLIMATE

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ORIGINAL RESEARCH ARTICLE

Determining the amount of water condensed above and below the winter cluster of honey bees in a North - European Climate

Kalle Toomemaa[1*], Ants-Johannes Martin[1], Marika Mänd[1] and Ingrid H Williams[1]

[1]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, 1A Kreutzwaldi St, 51014 Tartu, Estonia.

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*Corresponding author: Email: ktoomemaa@gmail.com

Summary

The amount of water condensed in the hives of overwintering honey bee colonies was investigated. In autumn, condensers of thin sheet metal were placed in hives above and below the frames of the winter nests of the experimental colonies. The condensed water could flow into plastic bottles for collection. Most of the water collected during the winter condensed on the lower devices, only 2.5 ± 1.31% on the upper ones. At an average food consumption of 7.17 ± 0.35 kg, the amount of water condensed was 445.27 ± 45.8 g. The total amount of water released by such food consumption would be 4.88 ± 0.24 kg. The upper condensers increased moisture and wet surfaces in the hive, whereas the lower ones did not. Test and control colonies did not differ significantly in food consumption and bee mortality. The top condenser created additional airspace above the cluster and probably metabolic water vapour released from the cluster, having risen up first started descending, but due to the longer distance to the lower condenser it cooled more and condensed on the hive walls. The results show that in hives without top ventilation, it would be appropriate to remove descended water vapour from below of the nest or to retain it in the hive for spring consumption of the bees. It enables to reduce humidity in the nest of bees without strengthened ventilation to withdraw increased heat loss and enforces the bees to rise the metabolic rate and heat production, which in turn would increase food consumption and water production.

Determinación de la cantidad de agua condensada por encima y por debajo del grupo de invierno de abejas melíferas en un clima del norte de Europa

Resumen

Se ha investigado la cantidad de agua condensada en las colmenas de colonias de abejas de invierno. En otoño, se colocaron condensadores de hojas delgadas de metal en colmenas por encima y por debajo de los cuadros de los nidos de invierno en colonias experimentales. El agua condensada pudo fluir a botellas de plástico para ser recogida. La mayor parte del agua condensada durante el invierno fue recogida en los dispositivos inferiores y sólo un 2,5 ± 1,31% sobre los superiores. Con un consumo medio de alimentos del 7,17 ± 0,35 kg, la cantidad de agua condensada fue 445,27 ± 45,8 g. La cantidad total de agua liberada por tal consumo de alimentos sería de 4,88 ± 0,24 kg. Los condensadores superiores aumentaron la humedad y las superficies húmedas de la colmena, mientras que los inferiores no lo hicieron. Las colonias de prueba y de control no difirieron significativamente en el consumo de alimento y la mortalidad de las abejas. El condensador superior creó un espacio aéreo adicional por encima del grupo de abejas y el vapor de agua liberado por el grupo de abejas probablemente, después de haber aumentado comenzó a descender, pero debido a la mayor distancia respecto al condensador inferior se enfrió y se condensó en las paredes de la colmena. Los resultados muestran que en las colmenas sin ventilación superior, sería adecuado eliminar el vapor de agua descendente desde abajo del nido o retenerlo en la colmena para el consumo de las abejas en primavera. Esto permite reducir la humedad en el nido de abejas sin forzar la ventilación para evitar una pérdida de calor mayor y forzar a las abejas a aumentar el metabolismo y la producción de calor, lo que a su vez aumentaría el consumo de alimentos y la producción de agua.

Keywords: honey bee, wintering, water vapour, moisture, condensation

Introduction

In practical apiculture, especially in the northern countries, bad overwintering and even dying of bee colonies is a frequent problem. Very little is known about the physiology of releasing the metabolic water from the bees in a winter cluster. Honey bee clusters' water and heat balance and water economy during winter period has been little studied. In case of bad overwintering the metabolic rate of bees is increased, resulting in a higher temperature of the winter cluster, increased food consumption, exhaustion of the bees and overfulfilment of their hindgut. The water balance of a colony would be destroyed (disturbed) and it will lead to excess moisture (accompanied often with mould and wet surfaces within the hive and diarrhoea of bees), or vice versa, to lack of water in a hive. It occurs usually in the presence of crystallised honey in stores.

Good hive ventilation is recommended to avoid excessive moisture within the hive. In winter, both upper and lower entrances are opened and extra openings are made in the top part or the top of the hive, thereby creating a constant airflow through the hive. However, provision of too much ventilation also removes heat vital for bees and forces them to increase their metabolic rate to compensate for the heat loss. Increased food consumption results in increased production of metabolic water and increased moisture in the hive.

Some researchers have successfully overwintered bees in conditions of limited ventilation. In experiments with colonies which had closed hive entrances food consumption and bees' mortality decreased, and the amount of faeces in the hindgut of bees decreased 15.0% on average and up to 37.6% maximum as there was no free flow of cool air into the hive and the bees were calm (Perepelova, 1947). Similar results were obtained by Michailov (1964). In his experiments, the wintering of honey bee colonies was most successful inside a building. In this case, both entrances were closed and the colonies were covered on top with a cover cloth only. The upper entrances were opened on 20 February when brood emerged in the colonies. Colonies with weak ventilation consumed 15.6% less food and reared 54.1% more brood in spring. Later experiments with 227 colonies in two winters confirmed the results: poorly ventilated colonies consumed 0.8 kg (7.0 and 6.6%) less food on average, had fewer dead bees in winter and reared more brood in spring (Michailov, 1964). It shows that weak ventilation reduced heat loss and created more favourable conditions for the bees, reducing the metabolism level, food consumption and mortality of bees and helped to preserve their energy for spring development.

The absolute air humidity in a honey bee winter cluster has been reported to be 11.5–17.0 g/m3 in the first half of the winter. In the second half of the winter, when brood is emerging, it reaches 13.0-18.5 g/m3 (Simpson, 1950). It has been found that decreasing of outdoor temperature reduces relative humidity inside the hive. The relative air humidity in the unoccupied by bees area above the cluster was measured 79% at -10°C and 67% at -22°C (Eskov, 1995). Earlier it was thought that air humidity in the hive tends to approximate to that of the atmosphere outside and that bees do not actively regulate it (Oertel, 1949; Lindauer, 1955). But newer data show, that humidity is regulated by the workers, although not precisely, during summer time. Absolute humidity was always higher in the brood area and lower in the nectar stores (Human et al, 2006). Probably bees regulate humidity in the centre and periphery of the winter cluster as well. Very little is known about this process, and of water vapour releasing from the individual bees. As water vapour cannot penetrate the chitinous cuticle of the honey bee, an important role is played by the respiratory organs. But the relatively high density of honey bees and the high air humidity in the peripheral part of the cluster create conditions unfavourable for water evaporation from the tracheae. Thus, evaporation decreases from the centre to the periphery of a winter cluster, which may result in bees in the centre becoming dehydrated and those on the periphery hydrated as found in a theoretical study (Omholt, 1987). It has been suggested that with food transfer from the periphery, where honey is usually stored, to the centre, simultaneous transport of water contained in honey from hydrated bees to dehydrated bees may occur (Omholt, 1987). The exchange of food between colony members (trophallaxis) is a well-known phenomenon. It has been found that bees 12-21 days of age are most active in that (Moritz and Hallmen, 1986). The possibility of trophallaxis occurrence in the winter cluster has not been studied.

The amount of water vapour produced by the winter cluster within a hive has not been established experimentally yet, as it is impossible to enclose bees in an airtight hive for winter. Its natural circulation in the hive and its way out of the hive is also poorly understood. Metabolic water is produced when sugars in honey are oxidized in tissues of bees, releasing energy in the form of ATP (adenosine triphosphate) and heat. Theoretically consumption of 1 g of honey produces 0.68 g water (Cheshire, 1879; Frisch, 1921; Wedmore, 1947, reviewed by Johansson and Johansson, 1979). An undisturbed overwintering colony may thus produce 3-5 g/h of water vapour (McCutcheon, 1984) while an indoor wintering colony may produce an average 1.92-3.33 g/h (Lebedev and Toroptsev, 1996). Water production is directly dependent on food consumption and if the latter is too high for whatever reason, problems occur with excess moisture in hive. The borderline between normal and excessive food consumption has not been set yet, i.e. the optimum value is presently unknown. It has been found that winter severity has no significant variance in consumption between very warm or very cold winters (Severson and Erickson, 1985). Tests with caged bees have shown, that food consumption of bees from different colonies differs, and it may be partly genetically determined (Free, 1980; Kulinčević and Rothenbuhler, 1989).

The aim of this research was to determine experimentally water vapour movement and condensing place, as well as the actual amount of condensed water in hives without top ventilation. In other

words, there has been an on-going search for ways of ensuring the export of metabolic water from the nest of the bees without simultaneously increasing the loss of heat, and hence increasing demands of the bees for extra food consumption.

Materials and methods

The study was carried out in West-Viru County in Estonia (26°29' E, 59°10' N) during the winters of 2000/1 to 2004/5. The ambient temperatures were received from the local meteorological station, located 15 km from the experimental apiary and have been presented in the following table (Table 1).

Table 1. Ambient temperatures (°C) in winter months of the experimental years 2000/01–2004/05.

Temp	Year	Month				
		November	December	January	February	March
Mean	2000/01	3.0	-0.1	-2.3	-6.3	-4.3
	2001/02	-1.0	-8.7	-3.4	-1.1	0.5
	2002/03	-2.2	-9.5	-8.8	-6.8	-1.9
	2003/04	2.0	-0.9	-8.3	-5.0	-1.0
	2004/05	-0.7	-0.8	-2.1	-7.6	-7.1
Min	2000/01	-6.3	-18.4	-12.3	-23.4	-17.1
	2001/02	-11.6	-26.8	-24.1	-18.2	-9.4
	2002/03	-15.7	-27.3	-33.3	-24.8	-20.3
	2003/04	-4.5	-15.4	-20.9	-19.4	-14.4
	2004/05	-11.7	-13.9	-18.9	-19.5	-25.3
Max	2000/01	9.9	7.5	2.7	3.7	8.5
	2001/02	9.9	1.4	3.5	5.3	14.1
	2002/03	5.5	1.6	3.1	1.3	10.4
	2003/04	8.5	4.8	-1.0	3.5	11.7
	2004/05	8.5	5.7	7.0	0.9	8.1

Colonies

Estonian standard one-box long hives were used with a lower hive entrance and a frame size of 414 x 277 mm. Each hive was evaluated for colony strength, lifting out each comb to estimate the number of bees on it according to the Liebefeld method (Gerig, 1983). Colonies selected for the study were those with bees covering six comb frames, i.e., with about 13,200 bees; selected colonies were allocated at random to test and control groups with three colonies in each group in each of the five years.

Condensers

Condensers were constructed of thin sheet metal, onto which water vapour from the winter clusters could condense and flow into a plastic collecting bottle for measurement. They were of three designs (Fig. 1). Upper condensers were roof-shaped (Fig. 1a) and placed above the frames, fully covering the entire 6-frame winter nest. Water condensing on the inner surface of the inclined sidewalls and vertical front and back walls could flow into the gutter and from there into the collection bottle, as the outflow side of the condenser was placed 1 cm lower than the opposite side. The upper condenser created an empty space - with the volume of 7200 cm^3 - above the nest. That is approximately equal to the unoccupied by bees and honey combs airspace within the nest cavity (the area of the hive where the nest located).

The lower condensers were of two designs. The lower 'wall' condenser was installed into the base of the hive to collect condensed water from the sides and back wall of the condenser (Fig. 1b), near the winter cluster in the lower half of the height of the nest (Fig. 1b). The walls of the condenser passed through the hive floor into the gutter, so the lower 'wall' condenser did not create an empty space under the frames. The lower 'gutter' condenser was installed inside the hive floor (Fig. 1c). It was similar to the upper roof-shaped condenser but inverted and covered with wire mesh to avoid bees from falling into it (Fig. 1c). It was meant to collect moisture gathered under the frames. It increased the space under the frames to the same extent as the upper condenser increased space above the frames. The condensers were installed into the hives and removed from them before the weighing of colonies in October and in March.

Experimental design

Two variants of the experiments were conducted. The first variant (2000/01 and 2001/02) included two test groups and one control group; hives of one test group were each fitted with an upper condenser (Fig. 1a), while those of the second test group were each fitted with a lower 'wall' condenser (Fig. 1b). The second variant (2002/03, 2003/04, and 2004/05) had one test group and one control group. Each test hive was fitted with two condensers, an upper one and a lower 'gutter' condenser (Fig. 1c). All openings above and between the side walls and partition boards and the floor were sealed with silicone. In spring, the amounts of condensate collected by the upper and lower condensers were measured separately.

The control hives were not hermetically sealed and there was a 0.5 cm wide space between the partition boards and the floor. The hive entrances (area 15×30 mm) of all colonies were open and all colonies were equally well insulated above and on the sides. Food consumption by colonies was determined by weighing colonies. They were lifted out of their hives together with the frames, in a special weighing box in October and again in March. Wet and dry weight of dead bees (mortality) on the hive floor and the numbers of bees that flew off the hives during the winter and perished outdoors were determined. To find the latter indicator, wire mesh cages for catching bees were placed in front of the entrances of both test and control colonies. The dead bees removed from the hive and the cages were weighed when wet and after drying them in a thermostat for 24 hours. In the spring, any area of wet surfaces of hive walls, bottom and frames was measured in cm^2.

Analyses

The data obtained were statistically analysed using the t-test at the significance level of $p < 0.05$.

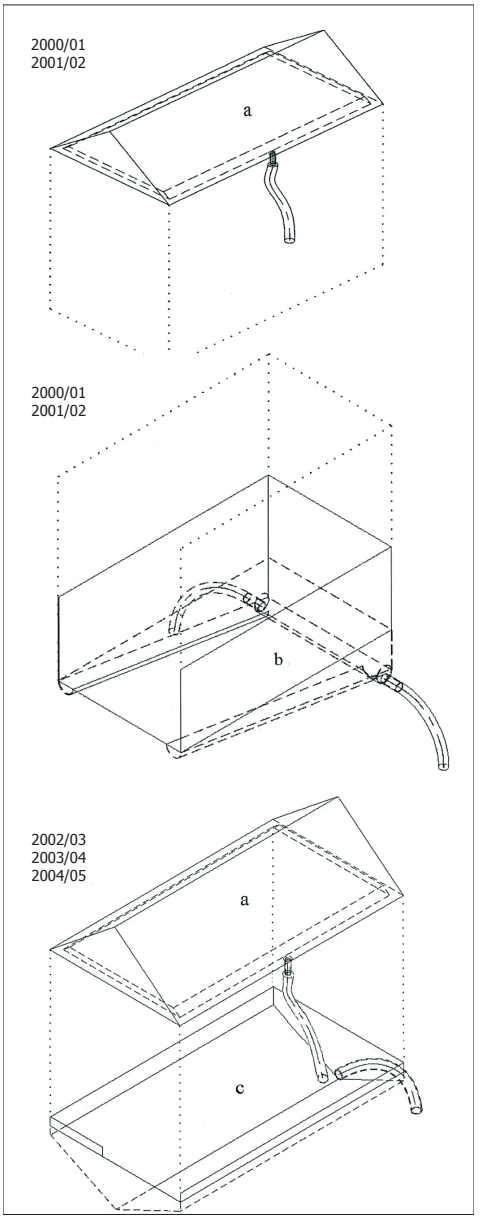

Fig. 1. Design of the upper and lower condensers used in experiments in 2000/01 to 2004/05 (a - upper condenser; b - lower ´wall´ condenser; c - lower ´gutter´ condenser). Dotted line marks the contours of the area where the nest located.

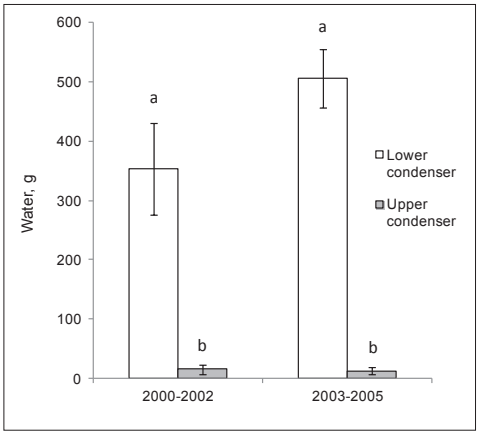

Fig. 2. The mean (± SE) amounts (g) of condensed water collected into upper and lower condensers during experiments 2000-2 and 2003 -5. Most of the water condensed on the lower condenser. Different letters indicate statistically significant differences.

Results

There was a considerable difference in condensation of metabolic water on the upper and lower condensers (Fig. 2); most of the water condensed on the lower condensers. Both designs of lower condensers removed significantly more condensed water than the upper ones (t-test: for first variant t = 4.340, P = 0.0014, df = 10; for second variant t = 9.931, P = 3.02309E-08, df = 16). In 10 of the 15 colonies fitted with upper condensers during the five winters the amount of water gathered in the upper condensers was small: < 56.7 g. In hives with both upper and lower condensers fitted, the upper condenser helped remove 0.6 ± 0.34% in 2002/03, 0.8 ± 0.39% in 2003/04 and 6.1 ± 3.25% in 2004/05 of metabolic water collected in winter, i.e. the average for all three winters was only 2.5 ± 1.31%.

The areas of wet surfaces inside the hives in spring showed that the upper condenser hindered removal of moisture. Test colonies fitted with upper condensers had considerably larger areas of moist or wet surfaces than control colonies or those with lower condensers (Fig. 3) (t-test: for first variant with upper condenser t = -2.948, P = 0.0145, df = 10). The lower condensers did not cause an increase in humidity in the hive compared with the control (Fig. 3) (t-test: for the first variant with lower 'wall' condenser t = 1.478, P = 0.1699, df = 10). The presence of upper condenser simultaneously with the lower one increased humidity in the nest (Fig 3) (t-test for second variant with upper and lower 'gutter' condenser t = -5.032, P = 0.0001, df = 16).

Test and control colonies did not differ significantly in food consumption nor in total bee mortality (Fig. 4, Table 2, t-test: p>0.05). Food consumption of all 15 test colonies fitted with lower condensers

Table 2. Mean food consumption and total bees mortality per colony ± standard error of the experiments in 2000/01-2004/05. Differences are not statistically significant (t-test, p > 0.05).

Year of experiment	Variant of experiment	Food consumption, kg ± SE		Probability	Total bees mortality, g ± SE		Probability
		control	experiment	p	control	experiment	p
2000/01	Upper cond.	5.430 ± 0.140	5.203 ± 0.693	0.765	43.002 ± 6.734	50.867 ± 7.340	0.474
	Lower cond.	5.430 ± 0.140	5.037 ± 0.153	0.133	43.002 ± 6.734	61.297 ± 17.644	0.387
2001/02	Upper cond.	7.753 ± 0.160	7.651 ± 0.265	0.755	91.833 ± 9.552	104.171 ± 7.636	0.370
	Lower cond.	7.753 ± 0.160	7.492 ± 0.210	0.376	91.833 ± 9.552	81.530 ± 9.552	0.604
2002/03	upper+lower	6,950 ± 0.399	7.247 ± 0.940	0.786	59.033 ± 7.134	96.671 ± 16.624	0.106
2003/04	Upper+lower	7.963 ± 0.069	7.507 ± 0.369	0.291	98.569 ± 12.753	103.603 ± 9.546	0.768
2004/05	Upper+lower	7.840 ± 0.523	8.587 ± 0.122	0.235	99.233 ± 22.641	72.433 ± 7.604	0.325

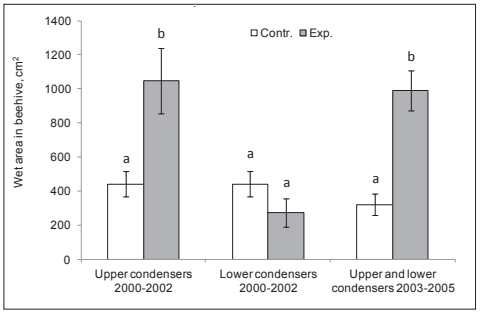

Fig. 3. The mean (± SE) areas of moist surfaces (cm^2) in the hives of control (without condensers) and experimental colonies (with either upper or lower condensers or with both simultaneously) after wintering. Presence of upper condensers increased moisture in hive. Different letters indicate statistically significant differences.

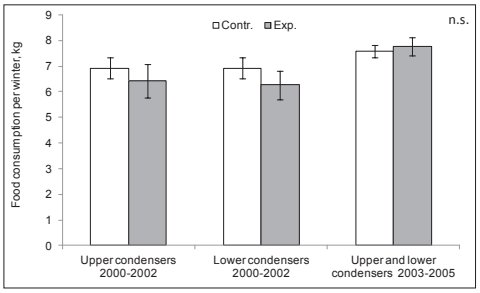

Fig. 4. The mean (± SE) food consumption (kg) per winter in the hives of control and experimental colonies supplied with upper and/or lower condensers. Differences are not statistically significant (p > 0.05).

or with both during the five winters was 7.17 ± 0.36 kg on average and the amount of water collected was 445.27 ± 45.80g on average. Listening regularly to test and control colonies in winter, no intensive ventilation buzz was detected and observation of entrances showed that ice crystals rarely formed indicating no intensive passage of water vapour through the entrance.

Discussion

The upper condenser increased the extent of moist or wet surfaces in the hive (Fig. 3) even when the hive was also fitted with a lower condenser (Fig. 1c). The top condenser created a 7200 cm^3 airspace above the nest. Heat rising from the winter cluster did not enable water vapour to condense on the condenser located above the cluster. It was only possible to a small extent on its vertical front and back walls and on the inclined surfaces at a distance of 6–10 cm from it, i.e. at maximum distance from the bee cluster. The risen water vapour cooled and started descending. As its path to the bottom condenser or to the bottom entrance was longer than in hives without the top condenser, the vapour cooled more and condensed also on the hive walls and lower parts of the frames, creating more humidity. The same may happen in hives with higher nest cavity (two box hives). The distance between the bottom and ceiling of the nest was 28 cm in our experiments.

Condensers, neither top nor bottom ones, had no impact on food consumption and bee mortality (Fig. 4, Table 2). That is because in all variants (with top, bottom or both condensers simultaneously and also in control) water vapour exited from the underneath of the cluster. Had the water vapour in control exited from the top, food consumption and possibly bee mortality might have been higher, as bees would have had to increase metabolism and food consumption to compensate for the increased heat loss.

A condenser above the cluster collected far less water than the one below the cluster (Fig. 2). The upper condenser was probably heated by the warm air raising from the cluster, which decreased its ability to condense humidity. The bottom condenser had always been cooler due to being placed under the nest of bees and could thus condensate the water very efficiently.

The lower 'gutter' condenser collected water more effectively than the lower 'wall' condenser (Fig. 2) It is probably thanks to the empty space created below the frames, where water vapour could descend unhindered by the hive floor, which was an obstacle in the case of the 'wall' condenser.

The movement of air inside the hive and condensation in various places could hypothetically have taken place in the following way. In a

way consistent with convection laws, most of the metabolic water produced by the winter cluster rose together with the air warmed by the cluster. As it reached the upper condenser some of the water vapour condensed onto its cooler front and back walls and flowed into a collecting bottle. As it rose, this warm air would have given up part of its warmth to higher-located honey combs and the nest ceiling. If a top opening, or other openings e.g., between hive walls and partition board, had been present, this upward-moving warm moist air would have exited the hive. At the same time it would have meant a considerable loss of heat.

Probably new flows of warm moist air from the cluster then helped force the moist air, cooled under the nest ceiling peripherally downwards between the hive walls and frames. As it descended, the moist air cooled further, condensing onto the sides and back wall of the hive and the lower condenser. It gave part of its heat to the walls of the hive and empty frames, and finally left the hive through the lower entrance. This, at the same time, let in the same amount of fresh cool air. This mixed with the descending hive air near the lower entrance and under the frames, cooling the latter and increasing its relative humidity. With good insulation and a low nest ceiling, no condensation would occur on the ceiling, but the moisture would descend as vapour and condense on the floor or leave the nest through the lower entrance.

It has been found that ventilation activity of bees shows long-term cycles. Periodic active fanning moves an air current out, followed by a passive influx of air. The frequency of such "breathing" of the hive was 2.9 ± 0.84 "breaths"/min during daytime and seven times lower during the night (0.4 ± 0.20 "breaths"/min), the air volume flow rates were an average of 0.42 ± 0.01 l/min (Southwick and Moritz, 1987). It has also been found, that honey bees synchronize their individual circadian rhythms of oxygen consumption and respiratory frequency to an overall group rhythm – this is theoretically explained as a self-organization phenomenon (Moritz and Kryger, 1994). The above-mentioned respiratory frequency was carried out during summertime, the winter period "hive breathing" is probably very low.

The amount of metabolic water collected into the condensers was substantially less than the production expected from the amount of food consumed. Although bees consumed, on average, 7.17 ± 0.36 kg (n = 15) of food per colony during the five winters, the total amount of water collected by the condensers below the nest was, on average, only 445.27 ± 45.80 g. Previous research suggests that the expected total amount of water released from such food consumption would be 4.88 ± 0.24 kg (Cheshire, 1879; Frisch, 1921; Wedmore, 1947, reviewed by Johansson and Johansson, 1979); thus 4.43 kg of water remained uncollected. It is unlikely that it was all absorbed into the hive walls or left the hive through the entrance.

The actual amount of released metabolic water has not yet been experimentally determined and it may be significantly different from the theoretical value. Apicultural literature talks about more than 5l of water that has to be removed from the hive by the bees. That is an amount calculated directly from food consumption. It is not clear how much of honey bees' metabolic water returns to the bees' life cycle. Neither is it known how much water vapour from the hive atmosphere is absorbed by honey exposed in uncapped cells, and is being consumed with the honey by bees themselves. Crystallised honey needs more moisture, and the humidity within the cluster is not enough to liquefy it, causing bees to become restless. They increase the temperature of the nest, enforce the ventilation at the entrance, fly out in search of water and even cluster outside the nest in the empty hive space. Bees calm down quickly and decrease the nest temperature when water is given into the nest (the author´s observations, unpublished). A brood rearing if it occurs decreases considerably the humidity within the winter cluster. It has been suggested that the phenomenon of brood rearing in the winter cluster is likely to be a strategy to reduce their own water content when this has reached a specific level (Omholt, 1987). Thus the actual amount of water produced in winter by the honey bee colony probably varies. It depends on several factors such as the crystallisation level of the honey, the brood rearing in winter, to some extent the water content of winter reserves (honey) and probably, also the humidity of the external air. For example, in a humid climate, moisture entering the nest together with atmospheric air probably considerably increases the humidity of the air inside the hive.

Our experiments showed that some water vapour produced by the cluster gathered in the upper condenser. The rest of it accumulated into the empty space under the frames and flowed as water into the lower condenser. Excess metabolic water should either be removed from the hive or directed into a drinking dish inside the hive. Water reserves in the hive may be very important in early spring when it is difficult for bees to fly to collect water. In a natural tree cavity, there is probably no lack of water as there is always enough room under the nest (unless the cavity is too low) where condensed metabolic water can accumulate. In Russia, Bashkortostan natural reserve, where winter lasts at least half a year and the temperature often drops to -40-45°C, observations have shown, that water vapour condenses at the lower, colder part of the tree cavity, turning to ice. The ice gradually rises from the bottom of the cavity along the walls toward the cluster (Petrov, 1970). Generally, a lack of water is also quite rare in a hive as moisture usually gathers in hive corners further from the winter cluster and in the bottom part of the back wall.

Many beekeepers think that honey bees winter ideally in tree cavities where there is no excessive humidity as metabolic water from the bees is absorbed onto the cavity walls and into the wood due to surface tension forces. It can even freeze in very cold periods. Bees tend to cover the walls and ceiling of their nest cavity with a propolis layer whether the nest is in a tree cavity or a man-made hive. In natural nests, the propolis layer is thick and can completely cover the nest cavity floor, walls and ceiling forming a propolis envelope. The

thickness of this layer varies between 0.1 and 2.3 mm, but is generally in the 0.3 to 0.5 mm range (Seeley and Morse, 1986); these authors noted that since propolis repels water, the propolis envelope may waterproof the nest from free tree sap and other external moisture. On the other hand the propolis envelope makes it impossible to absorb water vapour by cavity walls and ceiling, therefore requiring another mechanism to avoid excessive moisture in the nest cavity.

This research has shown that cooled water vapour from the honey bee winter cluster probably descended gradually, after its partial condensation in the upper condenser, and condensed mostly below the nest. It would be desirable to remove this moisture or to maintain it in the hive as water in place which it has reached naturally. Before removing the condensed water from the hive it would be necessary to determine experimentally the need of water by bees in spring and sometimes also in winter, as well as the impact of water stored inside the hive on the spring development. This would help resolve an important problem in apiculture: removal of excessive moisture from the winter nest or its maintenance in the hive without increased ventilation and excessive heat loss.

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IV

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WINTERING OF HONEY BEE COLONIES IN CYLINDRICAL NEST CAVITIES VERSUS OBLONG BOX-HIVES IN A NORTH EUROPEAN CLIMATE

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ORIGINAL RESEARCH ARTICLE

Wintering of honey bee colonies in cylindrical nest cavities versus oblong box-hives in a North European climate

Kalle Toomemaa*, Marika Mänd and Ingrid H Williams

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

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We compared the efficacy of wintering honey bee colonies in wooden cylinders and wooden oblong box hives. Five colonies in "cylinders" and three colonies in "box-hives" were compared over two winters. The cylinders were 450 mm high with an internal diameter of 280 mm. The oblong box hives were Estonian standard one-box long hives with a frame size of 414 × 277 mm. Eight vertical rows of thermistors were fixed along the inner walls of both cylinders and hives and temperatures were measured at about weekly intervals. Food consumption and bee mortality of colonies were also assessed. In the cylinders, the winter clusters remained close to their front walls and away from the back wall, especially in cold weather. It was considerably warmer by the inner walls of the cylinders than in the hives, especially in the lower part. The surface of warm area decreased with cold weather and increased at around 0 °C indicating contraction and expansion of the cluster, respectively. In the second half of winter, there was a larger warm area in the cylinders than in the hives indicating that the clusters touched the walls of the cylinder more, thereby reducing heat losses further. Considerably, less food (13.5%) was consumed in the cylinders than in the hives. The numbers of dead bees on the floor was significantly lower in the cylinders than in the hives, but total bee mortality (dead bees on the floor + bees flown out in winter) differed little between cylinders and hives.

La invernada de las colonias de abeja de la miel en nidos cilíndricos frente a las cajas-nido oblongas en el clima noreuropeo

Se comparó la eficacia de la invernada de las colonias de abeja de la miel en cilindros de madera y en cajas-nido oblongas. Cinco colonias en "cilindro" y tres colonias en "cajas-nido" se compararon en dos inviernos. Los cilindros presentaban 450 mm de alto con un diámetro interno de 280 mm. Las cajas-nido oblongas eran cajas estonias estándar para una única colonia con un tamaño de 414 × 277 mm. Ocho filas verticales con termistores fueron implementados a lo largo de las paredes internas de los dos tipos de caja y la temperatura fue medida en intervalos semanales. El consumo de comida y la mortalidad de las abejas también se medieron. En los cilindros, el clúster invernal permaneció más cercano a la pared frontal que a la pared trasera, especialmente durante con tiempo más frío. Las paredes interiores estuvieron considerablemente más cálidas en los cilindros que en las cajas-nido, especialmente en la parte baja. La superficie de área cálida decreció con tiempo frío y se incrementó sobre los 0 °C indicando contracción y expansión del clúster, respectivamente. En la segunda parte del invierno, el área cálida de los cilindros fue mayor que en las cajas-nido indicando que los clústers tocaron más las paredes del cilindro, reduciendo más las pérdidas de calor. Se consumió considerablemente menos comida (13.5%) en los cilindros que en las cajas-nido. El número de abejas muertas en el suelo fue significantemente menor en los cilindros que en las cajas-nido, pero la mortalidad total (abejas muertas en el suelo + abejas perdidas en el invierno) no se diferenció mucho entre los cilindros y las cajas-nido.

Keywords: honey bee; winter cluster; wintering; tree cavity; hive; temperatures; food consumption

Introduction

Poor overwintering leading to weakening and collapse of colonies is a well-known problem in beekeeping, especially in Nordic countries. Formerly, 5–10% winter losses were common, but since 2006, much higher colony losses (up to 20% and more) have been reported in Europe and North America (Ellis, Evans, & Pettis, 2010; Le Conte, Ellis, & Ritter, 2010; Van Engelsdorp, Hayes, Underwood, & Pettis, 2010).

Winter colony mortality can have various causes: queen loss, inadequate food reserves, low autumn population, poor foraging conditions, and diseases or parasites including failure of control varroa mites adequately (Le Conte et al., 2010). Also, little is known about the biology of the winter cluster of honey bees; the temperature and humidity regimes of the cluster and therefore the most suitable conditions for overwintering of bees have not been established. Poor overwintering of colonies is often associated with a heightened metabolism level of bees, which, in turn, may result in a higher temperature in the winter cluster, greater food consumption, shortening of lifespan of bees and their excessive exhaustion, overfilling of their hind gut, diarrhea, nosemosis, and excessive humidity in the hive as

*Corresponding author. Email: ktoomemaa@gmail.com

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observed by many beekeepers. The factors causing the increased activity and metabolism level of bees in winter cluster have been little studied or established.

Sokolov (1900) suggested that, in nature, a honey bee colony will seek a nest site in a tree cavity where it can fill up the entire cross-section of the cavity. It coats the walls and ceiling of the nest cavity above the winter cluster with propolis and at the first frosts seals any space with a large number of bees forming a living cork, thus blocking access by cold air to the stored honey. This enables the colony to keep the honey warm and liquid, as in the summer, and in a proper state for consumption during winter. The tree cavity may therefore provide the ideal habitat for honey bees in winter. In hives the whole surface of the winter cluster is exposed to cold air, whereas in the tree cavity the bees press themselves against the cavity walls protecting themselves from the cold air coming up from below. Consequently, they eat less, secrete less water vapor and carbon dioxide, and need less oxygen (Pobоženski, 1994). Their life processes slow down and they retain their vitality. Bee colonies wintering in tree cavities reportedly consume as much as three times less honey than those in a hive (Nesterov, 2003), but no comprehensive experimental data have been reported. Colonies overwintered in tree cavities are noted to usually be clean, with no fecal stains in spring and few dead bees (Petrov, 1970). It has been reported, that in Bashkortostan (Russia), colonies with food reserves of under 5 kg perish in winter while those with reserves of over 5 kg survive the winter (Petrov, 1970).

With decrease or increase in external temperature, the cluster contracts and expands, respectively, thereby reducing or increasing the surface/mass ratio, and causing a change in the rate of flow of heat from the cluster (Johansson & Johansson, 1979). However, it has been also suggested that no precise relationship exists between cluster expansion/contraction and ambient temperature and that the limits of cluster contraction are reached at about 0–5 °C (Seeley & Heinrich, 1981; Southwick, 1988). But Severson and Erickson (1990) have given the first quantitative evidence for a relationship between cluster contraction and ambient temperature: they observed decreases of about 55% in cluster volume and 40% in cluster surface area as ambient temperature decreased from 4 °C to −23 °C. As the number of individual bees within the cluster increases, proportionally fewer bees are exposed to low ambient temperature at the surface.

Heinrich (1981) found that the active thermoregulatory metabolism of the mantle (outer) bees of the cluster indicates their primary role in controlling heat production and loss by the cluster. But later research has shown that the endothermic heat production by "shivering" thermogenesis of the core bees plays an active role in thermal control (Stabentheiner, Pressl, Papst, Hrassnigg, & Crailsheim, 2003). The "superorganism model" presents the hypothesis of self-organized cluster thermoregulation with insulating mantle bees and heat-producing core bees (Moritz & Southwick, 1992).

In natural conditions, bees overwinter in tree cavities, caves, attics, or any suitable shelter. Natural wintering has been little studied. The winter cluster is round or oval. A round (cylindrical) cavity thus corresponds more to the shape of the cluster than the quadrangular nest space of a man-made hive where the cluster is unable to occupy the corners. The aim of our research was to establish whether wintering in a round (cylindrical) cavity was more beneficial than wintering in an oblong cavity (hive). We studied temperature changes next to the walls of the cylinders and hives to establish the position and movement of winter clusters. In addition, we compared the amount of food consumed and bee mortality during winter in both hives and cylinders.

Materials and methods

The experiments were carried out in West-Viru County, Estonia (26°29′ E, 59°10′ N), in 2001–02 and 2003–04. The five experimental colonies in wooden cylinders and the three control colonies in Estonian standard wooden one-box long hives (frame size of 414 × 277 mm) were located outdoors in both winters.

The cylinders were made of wood, opening vertically in the middle; their inner height was 450 mm, the inner diameter 280 mm, and the wall thickness 60 mm. The walls were painted twice with vegetable oil to avoid invasion of water vapor in winter and distortion of weighing data. Seven parallel comb foundations were fixed at 35 mm intervals inside the cylinders. Swarms of equal weight (2.5 kg) were driven into the cylinders in June. The strength of both control and experimental colonies was assessed in October. The cylinders were opened up from below and vertically from the middle and the density of honey bees on the central combs, below them and on the walls of the cylinders was assessed. The bees covering the lower parts of the combs were then forced by smoke to go deeper inside the cavity; when they ceased to go farther or started to run out, the comb spaces were considered filled with bees.

The colonies in the box hives were about 15,400 bees strong according to the Liebefeld assessment method (Gerig, 1983) and densely covered seven frames with a total comb area of 14,000 cm^2. Colony strength in the cylinders was similar with seven densely covered combs and a total area of 13,800 cm^2.

The colonies in both cylinders and box hives were insulated at the top and on the sides with 10-cm-thick rock wool and with an additional 5-cm-thick cotton wool mat. The cylinders were located in box hives in order to protect them from the wind and external moisture and had open lower entrances measuring 100 × 15 mm in front and 70 × 15 mm lower entrances on their sides, opening on the floor of the surrounding hive.

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Temperatures were measured by thermistors (NTC 4.7 kΩ), of which there were a total of 56 in the cylinders and 32 in the hives. Prior to their installation, these were tested at temperatures of 5, 10, 20, 30, 35 °C, and any with a deviation exceeding 0.5 °C were replaced (Szabo, 1985). The cylinders each had eight rows of thermistors fixed vertically to their inner walls with a space of 11 cm between the rows. Seven thermistors were positioned vertically, with a distance of 7 cm between them, the lowest of them 1 cm above the floor and the upper one 2 cm below the ceiling (Figure 1(B)). The box hives had eight rows of thermistors installed vertically, four in each row, spaced 8 cm apart. The lower thermistor was 1 cm above the floor and the upper one 2 cm below the ceiling. The thermistor rows were located in the middle of the back wall and front wall (i.e., by the entrance), in the middle of both partition boards and in all four corners of the nest cavity (Figure 1(A)). Temperatures were measured in winter (from November to March) at about one-week intervals

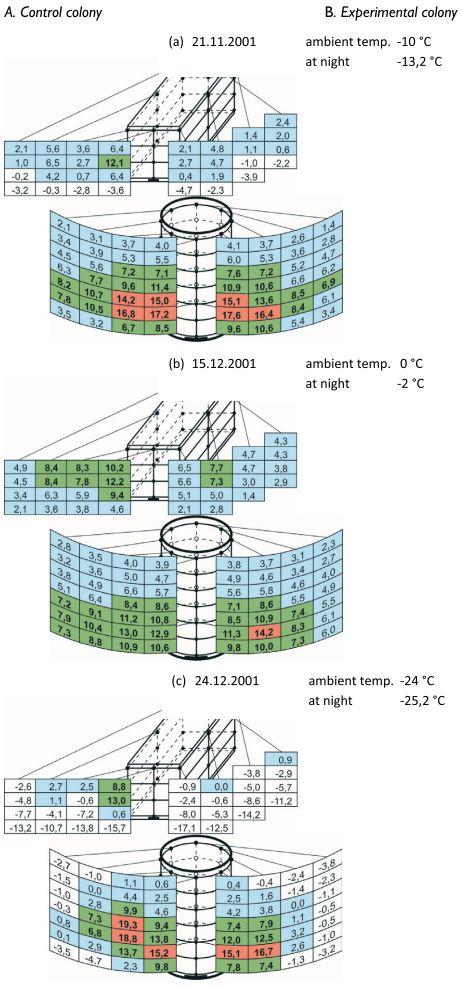
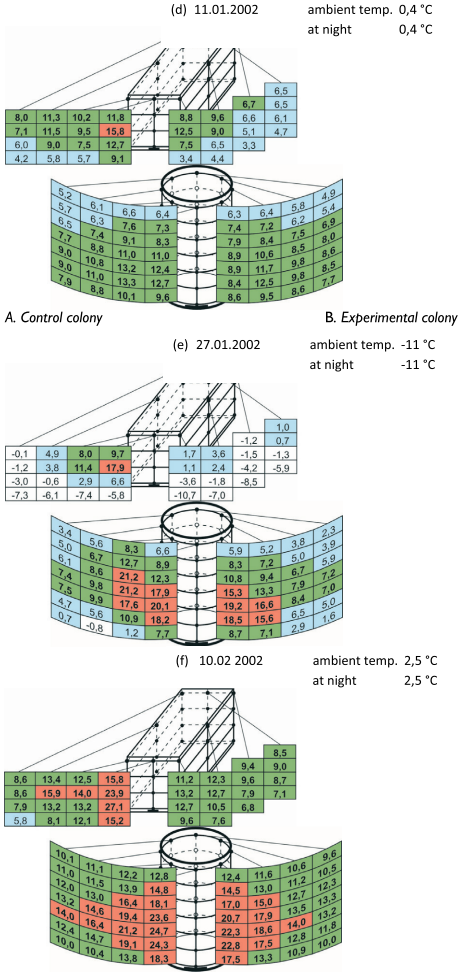


Figure 1. Temperatures by the inner walls of the nest cavity (the area of the hive where the nest located) of the control (in box hive) colony (A) and the experimental (in cylinder) colony (B); and the ambient temperatures at the time of the measurement and in previous night. Red areas indicate the center of the cluster (14.0–24.7 °C), green – the periphery of the cluster (6.7–13.9 °C), blue – unoccupied by bees areas (above 0 °C), and white – unoccupied by bees areas (below 0 °C).



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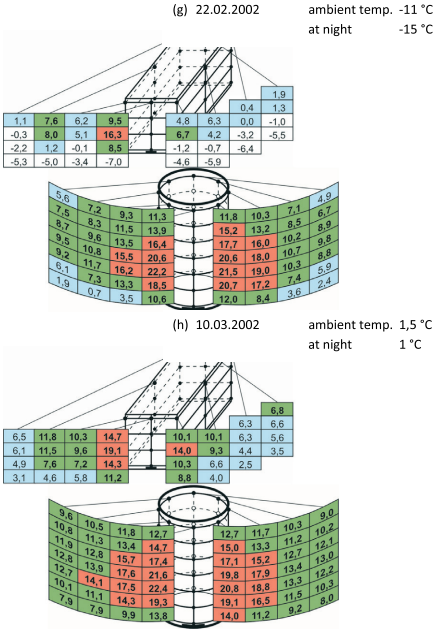

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and it was tried to establish extreme ambient temperatures. Any temperatures of 6.7 °C or above were considered to be from inside or in the outer surface of the winter cluster, temperatures lower than this were considered to be outside the cluster (Owens, 1971).

Food consumption of colonies was determined by weighing them in autumn (October) and spring (March). The colonies in the cylinders were weighed together with the cylinders but the colonies in the box hives were weighed separately from the hives.

Overwintering mortality of bees was assessed in spring. The number of dead bees on the floor and bees flown out of the hive were determined. To establish the latter indicator, traps covered with wire mesh were placed in front of the hive entrances in autumn. The dead bees removed from the hive or the trap were weighed in the wet state and after drying them in a thermostat for 24 h.

The data were analyzed statistically using the *t*-test at the significance level of $p < 0.05$.

Results

Each cylinder had a round cavity with seven parallel combs; the ones situated at the edges were usually slightly curled. The combs were attached to the ceiling and the walls and the bees had made passages, a few centimeters apart, to where the combs were fixed to the side walls. All the walls and the ceilings of the cylindrical cavities were lined with propolis and a layer of wax.

Temperatures by the inner walls of the hives and cylinders

Temperature changes by the inner walls of both the cylinders and box hives were alike. Data of a typical colony from each group are shown in Figure 1(A) and (B). The area of warm surface was greater in the cylinders than in the box hives but the lower part of the latter was considerably cooler than the corresponding area in the cylinders (Figure 1(A) and (B)). In the hives it was always warmer under the ceiling of the nest than down on the floor (Figure 1(A)). Temperatures in areas unoccupied by bees under the ceiling of the cylinders fell rather low, nearly as low or even lower as under the cluster in the first half of the winter (Figure 1(B), (a)–(c)) but not anymore in the second half of the winter (Figure 1(B), (e), (g)). Both in the hives and the cylinders, the winter cluster touched the front wall but stayed away from the back wall, especially with cold weather (Figure 1(A), (a), (c); Figure 1(B), (a), (c)). The area of warm surface increased as the weather got warmer (Figure 1(A), (b), (d), (f)); Figure 1(B), (d), (f)) while it decreased in both hives and cylinders, particularly at the back side, with cooler weather (Figure 1(A), (c), (e), (g)); Figure 1(B), (d), (f). In the second part of the winter, the area of warm surface was impressively larger in the cylinders than in the hives at low temperatures (Figure 1(B), (e), (g)); Figure 1(A), (e), (g) as well as at high temperatures (Figure 1(B), (f), (h); Figure 1(A), (f), (h)). Comparing the surface of warm area at the same ambient temperature both in the first and the second half of the winter, increase in the size, especially in the cylinders, can be detected in the second half of the season (Figure 1(B), (a), (e), (g)). Also, the surface of warm area shifted slightly upward during the second part of winter (Figure 1(B), (a), (e)).

Temperatures by the inner walls of the hives were lower than those in the cylinders, especially in the lower parts. The coolest places in the hives were the corners on the bottom and by the lower entrance (Figure 1(A), (c)), in the cylinders, by the back wall on the bottom and by the entrances on the sides (Figure 1(B), (c)). With the outside temperature of −24 °C minus degrees dominated by the inner walls of the hives, the lowest was the temperature in the corners and by the lower entrance, falling down to −17.1 °C (Figure 1(A), (c)). The lowest registered temperatures were −18.2 °C in a hind corner of the hive and −6.8 °C by the lateral entrance on the floor of the cylinder. Those low inner temperatures were, respectively, 8 °C and 17.2 °C higher than the temperature outside. The mean temperature by the back side on the floor was −10.5 ± 0.9 °C ($n = 3$) in the hives and −2.9 ± 0.3 °C ($n = 5$) in the cylinders at an ambient temperature of −24 °C.

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The coolest part of both inside the hives and the cylinders was the back wall. It was recorded by three rows of thermistors (except for lower ones) fixed vertically on the back wall measuring temperatures above the floor, next to the cluster of bees. The average temperature of the back wall was −4.0 ± 0.4 °C in the hives and −0.6 ± 0.2 °C in the cylinders at an ambient temperature of −24 °C. Also in the case of higher outside temperatures, it was warmer by the back wall in the cylinders while divergence was greater in the second half of the winter. With the similar ambient temperature (−10–11 °C), by the back wall there was measured the temperature of 1.4 ± 0.2 °C in the hive (Figure 1(A), (a)) and 3.9 ± 0.3 °C in the cylinder (Figure 1(B), (a)) on 21 November 2001, and −0.4 ± 0.4 °C (Figure 1(A), (e)) and 4.3 ± 0.2 °C (Figure 1(B), (e)), respectively, on 27 January 2002 and 1.7 ± 0.7 °C (Figure 1(A), (g)) and 6.8 ± 0.2 °C (Figure 1(B), (g)) on 22 February 2002. With the air temperature close to 0 °C outside, differences were smaller: 4.0 ± 0.2 °C in the hives (Figure 1(A), (b)) and 4.3 ± 0.1 °C (Figure 1(B), (b)) in the cylinders on 15 December 2001, 5.7 ± 0.2 °C (Figure 1(A), (d)) and 7.2 ± 0.4 °C (Figure 1(B), (d)), respectively, on 11 January 2002 and 9.4 ± 0.7 °C (Figure 1(A), (e)) and 10.8 ± 0.2 °C (Figure 1(B), (e)) on 10 February 2002. In March, the difference was again greater: 6.3 ± 0.3 °C in the hives (Figure 1(A), (h)) and 10.6 ± 0.2 °C in the cylinders (Figure 1(B), (h)). Both in hives and in cylinders, sidewalls and the front wall were always warmer than the back wall, but no cold corners detected with the hives (Figure 1(A), (a), (c), (e), (g)), singularly with a chilly weather, were noted in the cylinders (Figure 1(B), (a), (c), (e), (g)).

Food consumption and mortality of bees

The colonies in cylinders consumed significantly less honey than those in the hives (Figure 2; cylinders 7.18 ± 0.32 kg, box hives 8.30 ± 0.24 kg; $t = 2.474$, df = 14, $p = 0.027$). There were significantly fewer dead bees on the floors of the cylinders than on those of the hives ($t = 2.960$, df = 14, $p = 0.010$) (Figure 3), although the numbers of bees flown out from the cylinders in winter did not differ significantly from those from hives ($t = 1.141$, df = 14, $p = 0.273$) (Figure 3). The total number of dead bees (dead bees on bottom + bees flown out) did not differ significantly between cylinders and hives ($t = 1.202$, df = 14, $p = 0.249$) (Figure 3).

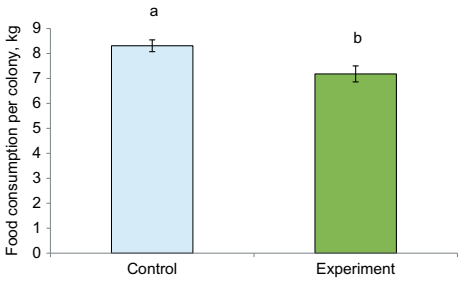

Figure 2. The mean (±SE) food consumption (kg) of control (in box hive) and experimental (in cylinder) colonies. The colonies of experimental group had less food consumption. Different letters indicate statistically significant differences (*t*-test, $p < 0.05$).

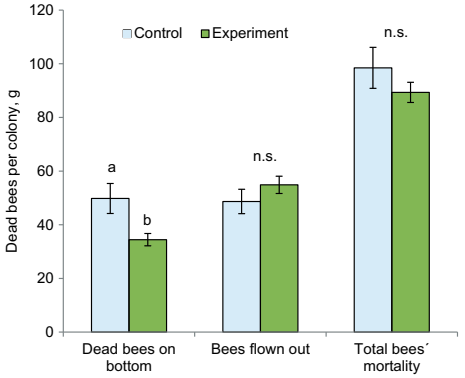

Figure 3. The mean (±SE) amount (g) of dead bees on bottom, bees flown out in winter, and the total bee mortality in control (in box hive) and experimental (in cylinder) colonies. The colonies of experimental group had less dead bees on bottom. Different letters indicate statistically significant differences (*t*-test, $p < 0.05$), n. s. – not significant.

Discussion

Our experiments showed that, in cold weather, the winter clusters in the cylinders did not occupy the entire cross section of the cavities but stayed away from the back walls. Thus, they did not close as a cap all the cross-section of the round cavities, as Sokolov (1900) suggested. Cold air moved easily above the clusters, causing the temperatures under the cylinder ceilings to be nearly as low as those under the clusters. However, this was not the case in the second part of winter as, by then, the clusters had moved upward. All clusters both in hives and cylinders displayed contraction in cold weather and expansion in warmer weather or during a thaw. Southwick (1983) showed that tight clustering can reduce the effective area of heat exchange by as much as 88%. The contraction and expansion of the clusters is in accordance with the results of earlier research (Owens, 1971; Severson & Erickson, 1990; Szabo, 1985).

With the weather warming after a period of cold, we noticed the expansion of the clusters in both box hives and cylinders. In the warmer weather of late winter (February, March), the clusters in the cylinders expanded and did not contract much even when the

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weather subsequently cooled. By contrast, the clusters in the hives also expanded but contracted once more again when the weather became colder. Thus, the bees in the cylinders were less affected than those in the hives by a fall in the outside temperature and the bee clusters, having expanded up to the round walls, were protected from cold by the walls of the cylinders surrounding them. So, in the second half of the winter, the honey bee colonies in the cylindrical cavities were more able to avoid heat losses and thereby reduced food consumption. The same was confirmed by higher temperatures by the inner walls of the cylinders in freezing weather, though to a smaller degree. Warmer areas moved a little upward over the winter, indicating the upward movement of the winter clusters toward honey stores as food was consumed. Earlier studies have revealed that the clusters become less compact and their volumes gradually increase in the second half of winter (Owens, 1971). This was observed well in the cylinders. Possibly, expansion of the clusters in warmer weather enables the bees to occupy and warm up new combs of honey.

Temperatures were low in the hives in cold weather. The change in hive temperature has been shown to lag behind the outside temperature by 1–2 h (Owens, 1971), depending on the insulation. But if colonies had been packed as a group of four and well insulated, with the outside temperature falling down to −38.8 °C, the lowest mean temperature registered inside the box hives was −14 °C (Szabo, 1985). Thus, bees can avoid the heat losses more easily if the nest is well insulated.

Szabo (1988) observed that bees can raise the temperature at the top entrance to 35 °C at an ambient temperature of −8.8 °C so that frost melted and the bees appeared at the top entrance. Only slight temperature changes were observed in other parts of the hive and cluster (Szabo, 1988). Such a pattern of behavior, and expansion and contraction of the winter cluster, its unusual motion in various directions, falls and rises in temperature inside the cluster as well show that bees in the cluster probably take coordinated action rather than have individualistic behavior. This accords with the superorganism model of the honey bees (Moritz & Southwick, 1992).

When the cylinders were demolished at the end of the study, it was discovered that all of them were lined inside with a layer of propolis and wax. This is in accordance with research results published earlier, which showed that bees cover the walls, floor, and ceiling of a cavity with propolis (Seeley & Morse, 1976).

In both winters, there were fewer dead bees on the floors of the cylinders than the hives, but the numbers of bees flown out in winter was slightly but not significantly more in the cylinders. It shows that, in the cylinders where the temperatures are more comfortable for bees, most of the bees eager to fly out managed to leave the hive and did not leave any dead bees on the bottom. The total bee mortality did not differ significantly in cylinders and hives. The collection of dead bees on the bottom is a natural process both in a hive and natural nest, not a phenomenon caused by unsuitable living conditions in hive where the quadrangular nest space is not in accordance with the shape of the cluster.

The numbers of bees flown out in winter made up a large part of the total bee mortality. Thus, the flight out of the hive in winter can be regarded as the endeavor of bees to avoid the collection of dead bees on the bottom disturbing the colony; however, bees rarely die in the nest in summer as well. Winter flights also reduce the amount of defecation in the hive and help to eliminate nosema-diseased bees by chilling them in flight (Moeller, 1972).

Food consumption was considerably lower in the cylinders than in the hives. The cluster of bees was more protected from cold inside round walls, and higher temperatures dominated in the cavity. In the cylindrical cavity, winter clusters always stayed closer to the walls than in the box hives and thus heat losses were smaller. But in the hive, combs and frames uncovered by bees conducted more heat away from the cluster. In the round cavity only a single wall surrounds the cluster, but in the hive there are four walls with cold corners. Thus, we can assume that, in natural tree cavities, food consumption is probably also less than in box hives. However, although food consumption was less in the cylinders than in the hives, it was not as much as twice or thrice less, but only 13.5% less on average. With cold weather, the winter cluster of honey bees contracts and though keeping more in contact with the front wall than in a box hive, it doesn't cling to it and most of the surface of the cluster is exposed to the influx of cold air. Consequently, the cluster in the cylinder has more options for reduction in heat losses in warm weather or spring while it has already expanded enough. In a box hive, walls, particularly the back wall, but also the corners are situated too far from the cluster and hence the cooling process is more intense. This was indicated by comparing temperatures in the hives and the cylinders. Farrar suggested that the cluster does not heat the unoccupied space in the hive. During a protracted cold period, the temperature in this space will become almost as low in a well-insulated hive as in one with no protection (Farrar, 1973). However, in our experiments it was warmer both inside the hive and the cylinder, areas not covered by bees included, than outside. It demonstrates well that, although the cluster retains most of its heat in its core, some is lost from the surface and the more it does, the greater is the gap between the temperature on the surface and that of the surrounding ambience. It is in accordance with findings that at least 8% of weakly endothermic bees were found on surfaces of winter clusters (Strabentheiner et al., 2003). So, hive insulation plays a significant role in reducing heat loss.

In conclusion, we can say that, although there were more beneficial thermal conditions for overwintering for honey bees and, respectively, lower metabolism level and food consumption in the cylinders, it is not expedient to construct hives with round nest space or honey combs attached to side walls. This would be costly and make practical work with bees much harder. The energy saving would not be enough to justify reconstruction of hives. On the other hand, design and construction of hives has an impact not only on internal temperatures, but also on air flow and moisture inside the hive. Thus, it can affect, for instance, development of chalkbrood and nosemosis. So it still remains of vital importance to advance such a hive that would contribute to maintenance of optimal microclimate inside the hive and save more energy of bees in winter.

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Disclosure statement

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CURRICULUM VITAE

First name:	Kalle
Surname:	Toomemaa
Citizenship:	Estonian
Employment:	Beekeeper, entrepreneur
Date of birth:	11.04.1955
Academic degree:	Master of Science in entomology "Wintering of honey bees and factors influencing it", Estonian University of Life Sciences, 1999

Education:

2015 – 2016	Estonian University of Life Sciences, doctoral studies, extern
1997-1999	Estonian University of Life Sciences, master studies, entomology
1984 – 1986	Institut of Beekeeping, Rõbnoye, Rjazanskaya obl., Russia
1977 – 1982	Estonian Academy of Agriculture, veterinary

Foreign languages:

English (intermediate level)
Russian (high level)

Employment:

2002 -	Beekeeper beginning from 1988
1989 – 2001	Estonian Society of Biodynamics, consultant
1982 – 1983	Collective farm of L. Pärn, beekeeping

Research interests:
Biosciences and Environment, Agricultural Sciences. Biology and ecology of honeybees, its wintering and diseases (varroosis and brood diseases).

Participation in research projects:

2012-2015	ESF grant No 9450:"Impact of pesticide residues on the foraging behaviour and physiology of pollinators".
2006-2008	TF project SF0172655s04: "Development of environmentally friendly plant protection II".
2015-2020	Institutional Research Funding IUT 36-2.

ELULOOKIRJELDUS

Nimi:	Kalle Toomemaa
Kodakondsus:	Eesti
Sünniaeg:	11.04.1955
Töökoht:	Füüsilisest isikust ettevõtja, mesindus
E-mail:	ktoomemaa@gmail.com
Teaduskraad:	Loodusteaduste magister (M.Sc.) entomoloogia erialal, väitekiri „ Meemesilaste talvitumine ja seda mõjutavad tegurid“, EMÜ, 1999.

Hariduskäik:

2015-2016	EMÜ, doktorantuur, eksternina
1997-1999	EPMÜ, magistrantuur, rakendusentomoloogia
1984-1986	Venemaa Mesinduse Täiendusinstituut, Rõbnoje, Rjazani oblast
1977-1982	Eesti Põllumajanduse Akadeemia, veterinaaria

Keelteoskus: Inglise keel (kesktase)
Vene keel (kõrgtase)

Teenistuskäik:

2002 -	Füüsilisest isikust ettevõtja, mesinik alates 1988
1989 – 2001	Eesti Biodünaamika Ühing, konsulent
1982 – 1983	L. Pärna nim. kolhoos, mesindus

Teadustöö põhisuunad:
Bio- ja keskkonnateadused, põllumajandusteadus. Meemesilaste bioloogia, talvitumise ökoloogia ja haigused (varroatoos, haudmehaigused)

Osalemine uurimisprojektides:

2012-2015	ETF grant nr 9450: “Pestitsiidijääkide mõju tolmeldajate korjekäitumisele ja füsioloogiale”.
2006-2008	Sihtfinantseeritav teema SF0172655s04: “Keskkonnasäästliku taimekaitsetehnoloogia arendamine II”.
2015-2020	Institutsionaalne uurimistoetus IUT 36-2.

LIST OF PUBLICATIONS

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1. Toomemaa, K., Martin A.-J., Williams, I.H. 2010. The efffect of different concentrations of oxalic acid in aqueous and sucrose solution on *Varroa* mites and honey bees. Apidologie 41: 643-653.
2. Toomemaa, K., Martin, A.-J., Mänd, M., Williams, I.H. 2013. Determining the amount of water condensed above and below the winter cluster of honey bees in a North-European climate. 2013. Journal of Apicultural Research 52(2):81-87.
3. Toomemaa, K., Mänd, M., Williams, I.H. Wintering of honey bee colonies in cylindrical nest cavities versus oblong box-hives in a North-European climate. Journal of Apicultural Research 54 (published online: 11 May 2016).

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1. Toomemaa, K., Martin, A.J. 2005. Comparison of varroatosis control with vaporized oxalic acid in hives and hermetically closed boxes. Proceedings of the Nordic-Baltic Apicultural Research Symposium, Helsinki, 5. Febr., p. 20-23.
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1. Toomemaa, K., Martin, A.-J., Mitt, S. 1999. Mõningate häirivate tegurite mõjust mesilaste talvitumisele. EPMÜ. Teaadustööde kogumik 203, Agronoomia, Tartu, lk. 173-179.

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1. Toomemaa, K., Martin, A.-J., Williams, I.H. 2011. L'effetto di diverse concentrazioni di acido ossalico in soluzione acquosa zuccherina sulla Varroa e sulle api. 2011. l'Apicoltore italiano 1: 3-9.
2. Toomemaa, K. 2012. Vaikus mesilas ehk mesilasperede hukkumine. Tallinn, Eesti Mesinike Liit.
3. Toomemaa, K., 1999. Mahemesindus – mesilase ja inimese koostöö. Elav Maa. Mahepõllumajanduse leht 14, sept.: 1-4.
4. Toomemaa, K. 2007. Mahemesindus. Eesti Mahepõllumajanduse Sihtasutus. Põllumajndusministeerium, 14 lk.
5. Toomemaa, K. 2001. Mesilasperede talvitumine. Maamajandus 12: 22-24.
6. Toomemaa, K. 2002. Mesilasperede talvitumine. Maamajandus 1: 14-16.
7. Toomemaa, K. 2002. Mesilasperede talvitumine. Maamajandus 2: 37-40.
8. Toomemaa, K. 2002. Mesilasperede talvitumine. Maamajandus 3: 38-40.
9. Toomemaa, K. 2002. Mesiniku aasta algab sügisel. Maamajandus 9: 25-27.
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