FINAL REPORT FOR PROJECT PH0506 – ANNEX 2

Objective 3.1. Review literature to assess efficacy of available Integrated Pest Management practices for *Varroa*

Milestone 9: Complete literature review of available Varroa control methods

Varroa destructor: A review of its biology and control methods

Introduction

The honey bee, *Apis mellifera* L., is critical not only for honey production, but also for crop pollination. From an economic standpoint, the value of crops that require pollination by honey bees, in the United States, is estimated at nearly $24 billion each year and the added value to U.S. crops from honey bee pollination at $14.6 billion (Morse and Calderone 2000). Gibbs and Muirhead (1998) quote $1.2 billion as the value of crop pollination provided by honey bees in Australia (commercial and feral bees) and Gordon and Davis (2003) quote $1.7 billion. Within the United Kingdom, recent estimates suggest that for both agricultural and horticultural crops grown commercially are in the region of £200 million per annum (Carreck and Williams, 1998; Temple *et al*., 2001; Wilkins *et al*., 2007). Many important UK horticultural crops, such as apples, may cease to be economically viable if it were not for bee pollination (Cuthbertson and Brown, 2006). The millennium ecosystem assessment project estimates the global annual monetary value of pollination to be in the order of many hundreds of billions of dollars (MEA, 2005). Therefore, the control of pests and diseases that affect honey bees is off utmost importance to ensure the continuous supply of pollinators for the worlds food crops (Buchmann and Nabhan, 1997; Cuthbertson and Brown, 2009; De la Rua *et al*., 2009; Cuthbertson *et al*., 2008, 2010).

Varroa jacobsoni was first described by Oudemans in 1904 as a natural ectoparasitic mite of the Eastern honey bee, *Apis cerana* F. Recently, Anderson and Trueman (2000) reported that *V. jacobsoni* is a complex of 2 different species that parasitize *A. cerana*. The original species, *V. jacobsoni*, encompasses 9 haplotypes that infest *A. cerana* in the Malaysia-Indonesia region. In contrast, the newly described species, *Varroa destructor*, includes 6 haplotypes that infest *A. cerana* on mainland Asia. Adult females of *V. destructor* are larger and less spherical than females of *V. jacobsoni*, and the 2 species are reproductively isolated (Anderson and Trueman 2000).

Varroa destructor Anderson & Trueman, the ectoparasitic mite now on *Apis mellifera* L., has caused widespread damage to the beekeeping industry worldwide and if not controlled can cause 100% losses of the bee colonies within a few weeks (Ball, 1994; deJong 1997; Calderone, 1999; Wilkins *et al*., 2007: Sammataro *et al*. 1998; Damiani *et al*., 2009). Originally from eastern Asia, by 1975, *V. destructor* was found in continental Europe, northern Africa and South America (de Jong *et al*. 1982) and was first detected in the United States in 1987 (Chandler *et al.* 2001). Varroa infestations have been largely responsible for the almost complete elimination of feral colonies in the U.S.A (Rinderer *et al*. 2001).

Varroa destructor is closely linked to its honey bee host and lacks a free living stage. There are two distinct phases in the life cycle of *V. destructor* females: A phoretic phase on adult bees and a reproductive phase within the sealed drone and worker brood cells. Males and nymphal stages of the mite are short lived and can only be found within the sealed brood cells. On the adult bees the Varroa females are transported to brood cells for their reproduction or spread by foraging and swarming bees (Kuenen and Calderone, 1997). On the adult bees the Varroa female usually is hidden under the sternites of the bee (Fernandez *et al*., 1993). The mites suck substantial amounts of hemolymph from both the adult bees and from the preimaginal host stages within the sealed brood cells.

Varroasis, the disease caused by this mite, starts when the fertilized *V. destructor* female abandons the adult bee that it has parasitized and penetrates into a bee breeding cell about to be sealed. Mite oviposition starts two days later, the female lays between 3 and 12 eggs and after 48 h the nymphs are born which start to feed on the hemolymph of the forming bee. These mature into adults within 5 to 8 days. The mating of the mites occurs in the cell before the bee emerges (Harbo and Harris, 1999; Llorente, 2003). The *V. destructor* female looks for the soft zones of the adult bees to perforate and suck their hemolymph, causing physical damage by decreasing protein content, and infectious toxin due to the transmission of microorganisms causing viral and bacterial diseases (Chandler *et al*., 2001; Nordström, 2003).

Mite infested colonies die from parasitism within a few years unless mite control methods are implemented (Fries et al. 1994). However, little experimental data is available to substantiate the assumption that varroa mites directly cause colony mortality (Ball 1993). Although mites do damage their parasitized hosts, a growing body of evidence suggests that the viruses vectored and/or activated by mites have a larger negative impact on their hosts than the mites themselves. One virus commonly associated with varroa mites is deformed wing virus (DWV). Before the widespread dispersal of varroa mites, this virus was considered benign. However, in association with varroa mites, DWV causes bee mortality and can lead to colony death (Bowen-Walker *et al*. 1999; Nordström 2003; Chen *et al*. 2004; Shen *et al*. 2005; Yang and Cox-Foster 2005, 2007). Given the combined impact of mites and viruses, effective varroa mite control strategies should not only consider the impact on mite populations but also the effect on virus levels. Until now, control of this pest has been based on chemical acaricides which in turn affect production costs, increase toxic residue content and resistance development (Melathopoulos *et al*., 2000). Combining several methods of control within an Integrated Pest Management (IPM) approach may offer the best means of mite control (Delaplane *et al*., 2005; Rosenkranz *et al*., 2010).

Biological control

Several non-chemical strategies can be utilised as potential control measures against varroa. These include (as listed in Delaplane *et al.,* 2005): (1) eliminating the mites from the colony. This can be achieved by grooming behaviour in the bees (Peng, 1992), various brood trapping techniques (Dung *et al*., 1995), and dusts applied in the hive (Fakhimzadeh, 2000). (2) slowing the rate of mite population growth. This is weighted toward honey stocks that display genetic varroa resistance (Spivak, 1996; Harbo and Harris, 1999; Harbo and Hoopingarner, 1997; Rinderer *et al*., 1997), but also include apiary isolation (Sakofski *et al*., 1990), apiary exposure to sun (Rinderer *et al*., 2004) and screen hive floors that reduce colony mite levels (Pettis and Shimanuki, 1999), apparently by decreasing the rate at which foundress mites invade brood cells (Harbo and Harris, 2004).

A partial or total alternative to the use of chemical acaricides is the biological control of varroa which could form part of an IPM system against the pest. Natural enemies of the *varroa* mite are few and, until recently, included no records of fungal pathogens (Chandler *et al*., 2001). Research of natural enemies against mites phylogenetically related with varroa point out the use of entomopathogenic fungi as a promising alternative (Chandler *et al*., 2001; Rodríguez *et al*., 2009a). Indeed, Chandler *et al*. (2000) reported the susceptibility of *V. destructor* to infection by *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschn.) Sorokin fungi when the mites were placed upon these colonies. Several species of entomopathogenic fungi have also been found to infect *varroa* mites in the laboratory (Shaw *et al.* 2002) and have been tested in the field (Kanga *et al.* 2003). Kanga *et al*. (2003) and Rodríguez *et al*., (2009b) found a highly pathogenic *M. anisopliae* isolate for *V. destructor*, reaching control levels in field assays similar to those obtained with fluvolinate acaricide 42 days after its application. Meikle *et al*., (2008) and James (2009) also showed that varroa mites were highly susceptible to infection by a range of entomopathogenic fungi, including *B. bassiana*.

Many entomopathogenic fungi have a ubiquitous distribution and a wide host range, therefore it might be expected that foraging bees could frequently carry fungus conidia into the bee hive from their environment. The lack of observed natural infections of varroa mites by these fungi could be due to a combination of hygienic behaviour of worker bees and the harsh environmental conditions in bee colonies. Nevertheless, Meikle et al. (2006) isolated *B. bassiana* from approximately 0.2% of varroa mites collected from a number of apiaries in southern France and documented that natural infections could indeed be found. *Beauveria bassiana* has also been reported from varroa mites in southern Spain (García-Fernández et al., 2008). Although the fungi present the advantages of easy manipulation, adaptation to different environments, specificity, and penetration directly through the tegument of the host (Lecuona *et al*., 1996), to achieve successful results, environmental conditions to which they will be exposed must be considered since these can influence germination and subsequent host colonization by the pathogen (Alves, 1998). One of the most important abiotic factors for the entomopathogenic fungi is temperature since it affects its metabolism by altering the production processes of enzymes, toxins, spore germination, development of the germinative tube, penetration, colonization, and reproduction (Alves, 1998).

Chemical control

Many chemicals have been used to reduce or eliminate the damages of the *Varroa* mite throughout the world (Genç and Aksoy, 1992; Milani, 1993; Goodwin and Eaton, 2001; Baggio *et al.*, 2004; Akyol and Korkmaz, 2006). Methods to control varroasis are based on the use of synthetic acaricides such as tau-fluvalinate, flumetrin, and coumaphos. However, it is possible to find resistance to these products (Harbo and Harris, 1999; Floris *et al*., 2001) and their residues in honey and wax (Calderone, 1999; Wallner, 1999; Pérez *et al*., 2000). Another alternative control is the use of oils and organic acids such as formic acid, oxalic acid, and thymol, which have been intensively studied in Europe and Asia. In spite of their effectiveness in controlling varroa, damaging effects have been reported on bees, such as alteration in the recognition of the queen's oviposition, bee mortality, and caustic effects on the skin of the handler (Calderone, 1999; Kanga *et al*., 2003; Floris *et al*., 2004). Apart from the latter concerns of residues in wax and honey, mite populations resistant to the most common chemical pesticides, fluvalinate and coumaphos, have been observed (Elzen *et al.* 1998, Milani 1999, Elzen and Westervelt, 2002). Efficacy of most of the chemicals has decreased, as in many ecosystems, because of the development of resistant mites (Colin, 1990; Gerson *et al.*, 1991; Milani, 1995; Imdorf *et al.*, 2003).

Due to the increasing problems of chemical residues (Wallner, 1999), research on the use of natural products, such as organic acids and components of essential oils (for example thymol), has been intensified for the control of *Varroa* mite (Colin, 1990; Calderone *et al.*, 1991; Chiesa, 1991; Rickli *et al.*, 1991; Imdorf and Carriere, 1996; Baggio *et al.*, 2004). *Varroa*cidal activity of thymol was shown in both laboratory assays and in field studies in Europe and in North America (Imdorf *et al.*, 1999; Mattila and Otis, 1999; Whittington *et al.*, 2000; Ellis *et al.*, 2001; Melathopoulos and Gates, 2003). Thymovar has also been recommended for controlling *Varroa* in honey bee colonies (Chiesa, 1991; Rickli *et al.*, 1991; Imdorf and Carriere, 1996; Goodwin and Eaton, 2001)

Formic acid fumigation has received considerable attention as a mite control product (Calderone and Nasr 1999, Kochansky and Shimanuki 1999, Calderone 2000, Hood and McCreadie 2001, Underwood and Currie, 2004). One major advantage of formic acid fumigation is that it provides control for other honey bee parasites, including the honey bee tracheal mite, *Acarapis woodi* (Rennie) (Wilson *et al.* 1993, van- Engelsdorp and Otis 2001), *Tropilaelaps clareae* Delpnado et Baker (Hoppe *et al*. 1989, Sharma *et al*. 2003) and possibly nosema disease (*Nosema apis* Zander) (Hoppe *et al.* 1989, Sharma *et al*. 2003, Underwood and Currie 2004). Formic acid is the only acaricide able to kill mites within the sealed brood cells (Fries, 1991). New products based on formic acid are being developed for mite control, for example Mite-Away-II^{TM}, however it may not always provide an adequate level of contol on its own (Calderone, 2010). Work has shown that formic acid in gel matrix is effective for Varroa control (Eguaras *et al*., 2003).

Currently, 8 chemical treatments are registered in the United States to control *Varroa* mites: 1) Apistan® (fluvalinate), 2) CheckMite+® (coumaphos), 3) Mite-Away II® (formic acid), 4) Apicure® (formic acid), 5) Apiguard® (thymol), 6) ApiLife-Var® (blend of thymol, eucalyptol, menthol, and camphor), 7) Sucrocide® (sucrose octanoate) and 8) Hivastan® (fenpyroximate). Apistan® has been used extensively for *Varroa* control, achieving nearly 100% efficacy in susceptible mite populations (Faucon *et al.* 1995). Apistan® is sold in plastic strips impregnated with fluvalinate that are suspended between frames in the brood chamber for 6-8 weeks. They require contact with the cluster of bees to be effective. Apistan® was first registered in Nebraska in 1990 and reports of resistant mite populations emerged between 1996 and 1997. Fluvalinate resistance however, has been widely reported in the United States (Eischen 1995, 1998, Elzen *et al*. 1998, 1999, Macedo *et al*. 2002,) and Europe (Milani 1994, Lodesani *et al.* 1995, Thompson *et al*. 2002). Today, many beekeepers have discontinued the use of Apistan® because its effectiveness has significantly decreased.

Natural organic acids such as formic acid, oxalic acid and lactic acid and essential oils such as thymol, eucalyptus oil, and citrus oil (Gregorc and Jelenc, 1996) have been used for the control of the mite (Rickli et al., 1991; Imdorft and Carriee, 1996). These substances are used increasingly because they are generally inexpensive and have fewer helath hazards to both man and honey bees (Isman, 2000). Essential oils have recently been tested for the control of different honey bee pathologies (Eguaras *et al*., 2005). Most investigations suggest that they may be a useful alternative in maintaining lower mite infestation rates in beehives (Imdorf et al., 1999). Some essential oils have a considerable potential for controlling American Foulbrood disease caused by a sporulated bacteria *Paenibacillus* larvae (Gende *et al*. 2009), chalkbrood disease originated by *Ascosphaera apis* (Dellacasa *et al*. 2003) and the parasitic mites, *Acarapis woodi* and *V. destructor* (Calderone *et al*. 1997). Investigations using lavender essential oil have showed good mite mortality (Ardeshir *et al.* 2002) moreover, affecting mite reproduction; but it has been seen that higher oil concentrations disturbed bee behaviour (Imdorf *et al.* 1999). Also, lavender, laurel and thyme essential oils were found to have repellent actions against the Varroa mite (Imdorf *et al*. 1999). Colin et al. (1999) hypothesized that long-term repellency may reduce female mite fecundity. *Laurus nobilis* essential oil has in its composition 1,8-cineole as the major constituent, Imdorf *et al.* (2006) and Ruffinengo et al. (2007) showed that 1,8-cineole caused high mite mortality. Lee *et al.* (2008) concluded that the activity of different essential oils was associated with molecular structure, indicating that the types of functional groups rather than hydrophobicity or vapor pressure parameters seem to play a role in determining the toxicities against insects.

Studies by Gashout and Guzmán-Novoa (2009) concluded that besides thymol (which is an already registered varroacide) origanum oil, clove oil and menthol are potential candidate products for further testing against *V. destructor*. All products were effective at killing mites.

Calderone and Spivak (1995) concluded that numerous factors contributed to the overall efficacy of any acaricide or essential oil compound, these being; the concentration of the compounds involved, the length of treatment, the delivery method, the colony environment (whether or not brood is present), the apiary environment and the ambient temperature. These can all influence the effectiveness of a given treatment. Adamczyk *et al.* (2005) concluded that the presence of residues of essential oil components in honey samples does not represent a sanitary risk or a risk for human health, they may however change the taste of the honey.

Oxalic acid is an organic acid; it is a natural constituent of honey and very effective against the *Varroa* mite. Uses of oxalic acid for the control of *Varroa* have been increasing in recent years (Charrière and Imdorf, 2002; Aliano and Ellis, 2009). Oxalic acid is safe to use, has no residue problems and a 5% dose of it can be tolerated by bees (Rademacher and Harz, 2006; Bogdanov et al., 2002). It has been used by the beekeepers in the USA and Europe (Charrière and Imdorf, 2002) and the EU regulations permit its use in biological beekeeping (EU Council Regulation, No. 1804/1999: Charrière and Imdorf, 2002). It is simple to use, cheap, and safe for beekeepers; no case of honey bee toxicity has been

reported and it is very effective especially in broodless colonies (Rademacher and Harz, 2006; Mutinelli *et al*., 1997). Akyol and Yeninar (2009) showed that a single application of oxalic acid could reduce *Varroa* populations by 84%.

The organic substance rotenone, a well-known natural active ingredient taken from the roots of *Derris elliptica* and *Lonchocarpus utilis*, has been used for 150 years to control chewing insects and to kill unwanted fish. More recently, rotenone has been used for parasite control in livestock production and also for the control of *Varroa* infestations (Higes, 1999; Gregorc and Poklukar, 2003). Eguaras *et al.* (2005) showed that rotenone treatments caused a significant reduction in varroa mite infestation, both during spring and autumn.

Conclusion

In the development of IPM schemes against *V. destructor*, the main aim is to reduce or eliminate beekeepers' reliance on synthetic acaricides. The development of non-chemical means is a rapidly developing area of research. However, even with the promising IPM tools suggested within the literature large-scale uptake of IPM has not been realised in many bee keeping areas of the world. Within the IPM strategies suggested the control of *V. destructor* populations in honey bee colonies requires that treatments show an acceptable acaricidal activity without side effect on honey bees and that they leave no or minimal residues in honey and wax within safety margins to the customer.

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