Swarm prevention and spring treatments against *Varroa destructor* in honey bee colonies (*Apis mellifera*)

Bram Cornelissen & Lonne Gerritsen
Applied Plant Research (PPO), Bee Unit, PO Box 69, 6700 AB Wageningen, The Netherlands, E-mail: Bram.Cornelissen@wur.nl

In 2004 and 2005 experiments were carried out to test the efficacy and efficiency of *Varroa* control combined with swarm prevention methods in spring. Honey bee colonies were split in an artificial swarm and a brood carrier. Hereafter the swarms were treated with oxalic acid and the brood carriers either with formic acid (2004) or Thymovar (2005). Both the oxalic acid and the formic acid were very effective, resulting in an average efficacy of 97% and 96%, respectively. There was some worker bee mortality in both treatments. Thymovar was less effective (71%), but did not cause any worker bee mortality. The results show that the combination of *Varroa* control and swarm prevention can effectively be used in spring.

Keywords: Varroa destructor, Apis mellifera, honey bees, spring treatment, swarm prevention, formic acid, oxalic acid, Thymovar

For more than 25 years the parasitic mite *Varroa destructor* (Anderson & Trueman 2000) has had a major impact on honey bees (*Apis mellifera* L.) and beekeeping in Europe. Without effective control, colonies infested with *V. destructor* collapse within two years (Martin 2001). Secondary infections, like Deformed Wing Virus benefit from the presence of *V. destructor*, and contribute to the decline of colonies (Sumpter & Martin 2004).

Populations of *V. destructor* reach a peak at the end of summer, when brood rearing in honey bee colonies decreases. At that time 'winter bees', needed for the survival of the winter are formed. Research shows that the production of healthy winter bees is critical for the survival of colonies during the winter (Korpela *et al.* 1992). *V. destructor* reproduce in sealed honeybee brood, the pupal stage of honey bees. As a consequence, pupae infested with *V. destructor* show physical deformations (Amdam *et al.* 2004). Common characteristics are a shorter abdomen and weight reduction. Also the longevity of honey bees is affected. Winter bees that normally survive until spring may die early. Because most control methods used against *V. destructor* are timed during or after the development of winter bees, chances are that colonies might not survive until spring. Treatments earlier in the season could prevent autumn infestations to reach alarming rates and thus make sure that winter bees are healthy and more vital.

There are several substances available for *Varroa* control. Thymovar is a product based on the active agent thymol which can effectively kill *V. destructor* (Floris *et al.* 2004). Because of its easy application it is gaining interest. It is made out of a viscose sponge containing 15 grams of thymol crystals. It can only be used in colonies with broodright conditions.

Formic acid is also very effective, but only in colonies with broodright conditions (Ostermann & Currie 2004, Calderone 1999). It is widely used throughout Europe. Normally a solution is made and then vaporized using special equipment installed in the hive.

It has been demonstrated that oxalic acid is highly effective in broodless colonies in winter (Nanetti *et al.* 2003). It can be applied by either spraying or trickling as a solution, or sublimated by heating. Yet it is not commonly used in the Netherlands. There is not much known about the application of oxalic acid in broodless colonies in spring or summer, when broodless periods occur for instance during swarm prevention (Brødsgaard *et al.* 1999).

Swarm prevention methods are a common feature in Dutch beekeeping. It takes place in spring or early summer, around the time when queen cell development is initiated by worker-bees. By actively taking away a part of the colony swarming can be prevented. Generally, 3 to 6 frames covered with bees and the queen are taken out of a colony and shaken into a new hive. Frames with honey and pollen are added. This part is called the artificial swarm.

In the remaining colony, called the brood carrier, the workers start to make several queen cells from cells containing small larvae and after 13 days the young queens are fully developed. If more than one queen emerges and there are ample bees, swarming might still take place. By removing all queen cells but one, or by releasing one queen into the hive, a beekeeper is able to control the process.

Calis *et al.* (1999) illustrated that biotechnical control methods against *V. destructor* could effectively be combined with swarm-prevention techniques. But because the method is time-consuming and success is greatly influenced by weather conditions, it did not find widespread use. In this paper a similar approach is used combining different varroacides with swarm prevention. In 2004 and 2005 hives were split in two, and separately treated with varroacides. The goal was to create a method that can easily be applied during the spring or early summer. The efficacy of the method against *V. destructor* and its effect on bees was tested.

MATERIALS AND METHOD

Preparations

In May 2004, 28 honey bee colonies containing twelve or more frames occupied with bees were selected for this experiment. The average daily mite fall before experiments started was 2.5 mites. This represents the natural mortality of *V. destructor*. The colonies were placed in two locations.

On day 0, 27 honey bee colonies were divided in two, creating 27 brood free artificial swarms and 27 'brood carriers'. One colony showed to be queenless before the experiment started, and was excluded from the experiment.

Swarms

The swarms were made by collecting 0.8 to 1.5 kg of bees (on average 1.2 kg) from the original hive. The bees were put in a plastic box and weighed. Of these swarms, 19 were treated with a 3% oxalic acid solution. The solution was sprayed on the bees. An average of 52 ml (min-max: 44-68) was used. As a control, eight swarms were treated with water (on average 52 ml) instead of the oxalic acid solution. Immediately after the treatment the queen was reintroduced and together with the bees put in a 10 frame hive. The frames consisted only of wax foundations. The swarms were given food in the form of sugar-patties or a sugar solution. The hives were then moved to another site at least 4 km from the original location.

When the treatment period ended, the colonies were weighed again. To evaluate the efficacy of oxalic acid, a perizin treatment was carried out, nine days after the first treatment. Perizin (Bayer Co.) is a coumaphos based varroacide. It has a known efficacy of 95% and is generally used to evaluate acaricidal effects of treatments against *V. destructor*. For the analysis the data of 2004 and 2005 (see below) were combined.

Brood carriers

The brood carrier consisted of the remaining bees and brood. This part remained on site. To make sure young queens would hatch at approximately the same time, all queen cells that were found on day 0 were removed. On day 13 the hives were once again checked for queen cells. This time one or two young emerging queens per hive were introduced. The remaining queen cells were destroyed.

Twenty brood carriers were treated with a short term formic acid treatment. A viscose sponge was placed on the bottom-board of the hive. A 60% formic acid solution was then spread out over the sponge. Per storey 30 ml was used. The treatment was repeated three times with intervals of 2 to 3 days. In total 180 ml formic acid solution was added. As a control, eight hives were treated with water using the same method. The treatments took place between day 14 and day 21 and mite-fall was recorded until day 28. Again, a perizin treatment was used to evaluate the acaricidal effect.

Bee-traps

At the start of the treatments bee traps (type: Muenstertrap, Illies *et al.* 2002), were placed in front of the hives to collect dead worker bees. The traps are designed to restrain worker bees from removing dead bees from a hive. They have to fly through a gaze screen and in the process drop dead bees in a container at the bottom of the trap. The number of dead worker bees was counted during the treatments.

Experiment in 2005

In 2005 the trials were repeated with a slightly different setup. Nineteen brood carriers were treated with Thymovar in stead of formic acid and bee traps were placed six days before the experiment started. Thymovar treatments started on day 0 and lasted until day 21, unlike formic acid treatment, which started after young queens hatched on day 14. Also three colonies were added to the control-group and the experiments were carried out on different locations

RESULTS

In 2004 and 2005 a total start population of 39 colonies (excl. control treatment) was used, out of which 77 colonies were made and treated against *V. destructor*. At the end of the experiment 58 (an increase of 49% of the start population) brood- and queenright colonies remained.

Swarms

The oxalic acid treatments were very effective against *V. destructor* (Table 1), and were significantly more effective than the control treatment ($p < 0.001$).

Of the 38 swarms that were made and treated with oxalic acid, 28 remained at the end of the experiment (74%). Two swarms left the hive just after they were placed on the location, one swarm was too small to survive and in seven swarms (18%) the queen died during the treatment period. In the control group only one colony of a total of 19 swarms lost its queen.

The average number of worker bees that died during the treatment was low, but significantly higher when compared with the control group ($p < 0.001$). On average colonies treated with oxalic acid had a weight reduction of 398.4 g, while colonies in the control group had an average weight reduction of 240.6 g Due to the large variance in the weight reduction, no clear difference could be found between the groups ($p=0.12$).

Brood carriers

Formic acid

The treatment with formic acid in the brood carriers showed a high and significant effect ($p < 0.001$) against *V. destructor* compared to the control treatment (Table 1). Bee mortality during the treatment period differed from mortality in the control group ($p=0.02$). At the end of the experiment five colonies treated with formic acid were queenless (25%), however in the control group four were queenless (50%).

Table 1. The effect of different treatments on mites and bees (mean ± standard error)

	Efficacy against *V. destructor* (%)	Worker bee Mortality (n)	Weight reduction (g)
Oxalic acid (n=35)*	96.7 ± 2.8	203.8 ± 17.2	398.4 ± 59.2
Control (n=19)	4.7 ± 5.7	56.4 ± 11.5	240.6 ± 80.4
Formic acid (n=20)	95.7 ± 0.8	118.9 ± 21.8	-
Control (n=8)	8.1 ± 3.1	45.3 ± 23.4	-
Thymovar (n=18)**	70.7 ± 2.6	414.8 ± 29.5	-
Control (n=8)***	17.6 ± 3.4	489.1 ± 51.2	-

* Two swarms that left the hive and one swarm that was too small were excluded from data analysis. ** One brood carrier that swarmed during treatment period was excluded from data analysis. *** Two brood carriers with symptoms of other secondary infections or other diseases and one brood carrier that swarmed during treatment period was excluded from data analysis.

Thymovar

Thymovar treatments proved to be less effective than the formic acid treatments, but showed a significant effect ($p < 0.001$) when compared with the control treatment (Table 1). There was no effect of Thymovar on bee mortality ($p=0.815$). When the experiment ended, five colonies were queenless and 14 out of 19 colonies, initially treated with Thymovar remained. One colony swarmed for a second time during the treatments.

Only one colony in the control group was queenless at the end of the experiment. Another control colony swarmed during the treatment period, and two control colonies showed symptoms of secondary infections caused by *V. destructor*, or other diseases.

DISCUSSION

The results show that an oxalic acid treatment in broodless swarms can be used very effectively. Although the numbers of dead worker bees collected in the bee traps were significantly higher than in the control group, no alarming amounts were reached.

There is an indication that the number of dead worker bees correlates with the total number of mites found in the colony ($R^2 = 0.68$). This suggests that when more mites are present in a colony, the tolerance of honey bees towards varroacides might be lower.

There is some concern about the number of queens that died during the treatment. This is probably caused by the treatment itself, since only one queen was lost in the control group. A possible explanation might be that the dosage used was too high. Generally 30 to 50 ml of the suspension is used for effective *Varroa* control, while the dose used in the experiment ranged from 44 to 69 ml.

There was a large variance in weight reduction in the swarms during the treatment period. There were even a few swarms that showed an increase in weight. This suggests that bees started drifting after the swarms were placed on a new location. A factor causing drifting activities could be the bee trap. It might hinder honey bees' ability to recognize their colony.

Formic acid proved to be very effective in this experiment. This confirms other studies (Ostermann & Currie 2004). Research shows an increase of formic acid residues when applied in spring. Bogdanov *et al.* (2002) even state that the use of formic acid in spring should be avoided, because formic acid residues might exceed taste thresholds. Donders *et al.* (2006) confirmed that, but in addition showed that when applied during a pre-honeyflow period the residues in honey can be limited, and only in some cases marginally exceed the taste threshold. The timing of the method described here is after the first honey- harvest, in the Netherlands generally related to fruit pollination activities, and before the harvest of summer honey, in July. Considering that the amount of formic acid in honey decreases in time (Bogdanov *et al.* 2002), the treatment should not cause problematic levels of residues in summer honey.

Worker bee mortality was significant when formic acid was used, but because of the low number of dead bees, the effect on colonies is minimal.

The loss of queens in five treated (25%) and four control (50%) colonies was probably caused by the bee traps. It is assumed that some young queens when initiating or returning from a mating flight experienced difficulties flying through the gaze of the bee trap in front of the hive entrance.

This might also play a role in the Thymovar treatments in 2005, but Thymovar itself might also have an effect on queen loss, presumably caused by the paralleled timing of the treatment and queen development in the larval and pupal stage. Research has shown that a negative effect of thymol based products on bee brood can be observed (Floris *et al.* 2004). However the effect of bee traps, as explained above, must not be overlooked.

The Thymovar treatment was less effective compared to treatments in autumn. This is probably caused by the fact that there was no queen in the colonies when the treatment started and consequently brood rearing ceased. This decreases the ambient temperature inside the hive, which has a negative effect on the fumigation of thymol and thus its efficacy against *V. destructor* (J.J.M. van der Steen, personal communication).

The results show that a combination of different spring treatment and swarm prevention can effectively and efficiently be realized. This enhances the possibilities for beekeepers to control *V. destructor* and increases the chance for honey bee colonies to develop a healthy bee population.

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