TO PROTECT BEES
FIGHTING VARROA MITES WORLDWIDE TO PROTECT BEES
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FIGHTING VARROA MITES WORLDWIDE TO PROTECT BEES

Varroa mites are the number one problem for today’s beekeepers, and certainly bees. This collection of recent articles and BUZZ releases gathers the best and brightest information on the biology and control of this pest.

Our only long-term solution is to produce honey bees immune to the beast, or bees that have learned to live with it – and both of these are covered.

In the short term, beekeeper intervention is required when Varroa infestations reach very low levels, and we have fouled our nest with chemicals trying to do just that. Organic acids, formic and oxalic, are both safe for bees and beeswax and we examine them closely here. Know about Varroa, or quit keeping bees. You’ll learn about it here.

The first few articles discuss the Varroa mite, how it enters the hive, its reproduction and its link to the deformed wing virus. Several articles discuss ways to fight the Varroa, and the series ends with good news for Australia.

Let’s start with how those cunning little creatures get into the hive.
VARROA MITES MIMIC BEE SMELL
If there were an international smelling bee, a deadly mite would be a favorite to win.

New research has revealed that Varroa mites, the most serious threat to honeybees worldwide, are infiltrating hives by smelling like bees.

The Michigan State University-led study, appearing in an issue of Biology Letters, shows that being able to smell like their hostess reduces the chance that the parasite is found and killed.

The parasites were originally found on Asian honeybees. The invasive species, however, revealed their versatility when they began infesting and killing European honeybees.

“The mites from Asian honeybees, or the original host, are more efficient in mimicking both Asian and European honeybees,” said Zachary Huang, MSU entomologist and one of the papers’ lead authors. “This remarkable adaptability may explain their relatively recent host shift from Asian to European honeybees.”

Chemical camouflage isn’t a new weapon in insects’ arsenals. Bolas spiders, for example, emit not one but three chemicals to emulate a sex pheromone to attract moths to eat. However, fooling socially sophisticated insects, such as honeybees, requires the faux scents to be spot-on.

That’s because the complex society of bees comprises tens of thousands of individuals divided by a sophisticated caste system. So, the mites aren’t simply tricking a solitary bee collecting pollen from a flower; they’re fooling an entire society. The stealthy mites do this by not only by being able to smell like bees, but also by effectively emitting the specific scents of small, individual colonies.

“They are essentially getting through the door and reaching the inner sanctum by using bees’ own complex communication codes against them,” Huang said.

The codes in which they communicate are hydrocarbons, the simplest of organic compounds. By tweaking the proportions of these chemical colognes, the mites give off the correct scents to fool their enemies.

Specifically, it’s the cuticular hydrocarbons, compounds released from hair-shaft glands, that emit scents that differentiate queens from fertile and infertile workers; it’s the smell that invokes acceptance or triggers aggression.

Huang and his team showed that mites are able to change their surface chemicals to an entirely different species of honeybees. Further, they also revealed that the mites were able to make these changes rather quickly – adapting in days rather than evolving over generations.

“Our study challenged the mites’ ability to modify their hydrocarbons,” Huang said. “Conversely, bees are adapting to detect these invaders. Our results give a clear illustration of an arms race between the parasites and the host bees based on chemical mimicry and its detection.”

Additional researchers contributing to this study include scientists from the French National Institute for Agriculture Research, Honeybee Research Institute (China) and the Research Institute for the Biology of Insect (University of Tours, France).

Huang’s research is supported in part by MSU AgBioResearch. He gives a bit of background on how the project unfolded:

“The story goes back to 2004, when I invited a friend and colleague, Dr. Yves LeConte, from France to visit China. Prof. Zhijiang Zeng hosted our visit. Yves and I stayed in the same hotel for about 13 nights (April 10-22).

“I had in mind to see how far mites can go to change their surface chemicals — the chemicals that insects often use for recognition. Honey bees, for example, use these chemicals to distinguish who are their hive mates. In the case of honey bee workers,
it seems the smell is a combination of genetics (from father and mother) but also smells from the environment, forming a “Gestalt” of odor.

“In the case of the notorious Varroa mites, we know that they can change their surface chemicals to match the developmental stage of their hosts; a mite will smell more like a pupae if the host is at the pupae stage, and more like an adult bee when the bee is ready to emerge.

“I thought perhaps we can challenge the mites to see if they could smell like a honey bee of an entirely different species. Nobody has done this before and this can be challenging. For one thing, it took me several years to find Varroa mites on the Asian honey bee, Apis cerana (Ac). So we thought we will just transfer mites from A. mellifera (European honey bees, the only species introduced in North America, Am) to Ac and see if mites will change their smell to the new host. We got lucky and found mites on A. cerana! So we did both transfers: Ac to Am and Am to Ac.

Of course we need to have controls, so some mites remained on their original hosts, Ac or Am, but still transferred to control the handling and experimental conditions.

Once Varroa are in the hive, they pick the best bees to bite.

VARROA FEED ON BEES IN NURSE PHASE
New insights into the reproductive secrets of one of the world’s tiniest and most destructive parasites – the Varroa mite – has scientists edging closer to regulating them.

“If you know your enemies better, you can come up with new ways of controlling them,” said Michigan State University entomologist Zachary Huang, whose research explores the fertility of the notorious mite, a pest that is devastating honeybee populations worldwide. The mite sucks the blood of honey bees and transmits deadly viruses.

The Varroa mite’s lifecycle consists of two phases: one where they feed on adult bees, called the phoretic phase, and a reproductive phase that takes place within a sealed honeycomb cell, where the mites lay eggs on a developing bee larva.

The MSU-led study, published in the current issue of Scientific Reports, shows that the mites clearly prefer to infest adult bees at mid-age, or during the nurse phase of a bee’s lifecycle when they take care of larvae, rather than during the younger (newly emerged) or older (forager) phases of an adult bee. The study also found that the physiological type of a host bee had significant effects on the mite’s reproductive fitness and success later on.

“Varroa mites feed on a honey bee larva. New MSU research is exploring the fertility of the notorious mite, a pest that is devastating honeybee populations worldwide. Credit: Zachary Huang, Michigan State University

Our study clearly demonstrated that Varroa mites preferred nurses over the older and younger bees,” said Huang, the study’s lead author. “Further, we showed that feeding on different hosts gave them different reproductive outputs.”

Mites chose bees in the nurse phase of their lifecycle – the nutritional prime of bee life – over their older and younger counterparts at significantly higher rates. Also, those that fed on nurses had the highest reproductive success rates and the lowest infertility rates.
Previous studies have shown that the mites can easily choose their reproductive hosts, but Huang’s study shows that they can go one step further: the mites can correctly pick the most nutritious bees to suck blood from.

“This might seem very smart for the mites because they do not realize the reproductive advantage right away, but under natural selection this is rather easy to achieve,” Huang said. “The mites who made the correct choice will have more babies and their genes will become more dominant over time.”

The recent results have helped researchers zero in on mite reproductive and nutritional preferences and are a significant step in understanding the mysterious, parasitic relationship between the Varroa mite and the honey bee.

“This is an important step in understanding mite reproductive biology,” Huang said. “We can utilize this information as a step toward finding ways to regulate them.”

In future research, Huang will look to identify what precise factors the mites are relying on for their reproductive success.

“If they require a certain factor to have babies we can regulate that factor without affecting the bees – only the mites – and reduce their reproduction,” Huang said. “Instead of killing them with a chemical, this could eventually lead to a more natural way of mite control and a better outlook for honeybees.”

In trying to determine any factors that lead to Varroa mite reproductive success, the timing of the processes during this stage have been examined.

VARROA MITE REPRODUCTION PROCESS
Reproducing Varroa females lay the first egg in the brood cell approximately 70 hours after host cell capping.

The life cycle of the female Varroa mite is subdivided into a phoretic phase in which she lives on adult bees and a reproductive phase occurring within worker or drone brood cells. The reproductive phase is initiated when the female mite leaves the adult host and enters a brood cell with a fifth instar larva shortly before the cell is capped. This foundress female passes between the larva and the cell wall to the bottom of the cell and becomes stuck within the larval food (larval jelly).

Approximately five hours after cell capping, the bee larva has consumed the rest of the larval food, which frees the mite (Ifantidis 1988). At that time the female mite has already started oogenesis (creation of eggs) in the terminal oocyte (Steiner et al. 1994; Garrido et al. 2000).

After leaving the larval food, the mite begins feeding on hemolymph of the bee prepupa. The Varroa mother prepares a feeding site by making a wound in the prepupa cuticle, which is used by all mite offspring including the male. This feeding site is critical for the survival of all developmental stages because their mouthparts are not strong enough to pierce the soft cuticle of the bee pupae.

The infesting mother mite also forms a rendezvous site with her feces on the cell wall on which all mobile individuals aggregate and on which matings preferentially occur (Donzé and Guerin 1994). In cells infested by more than one Varroa foundress mite, no aggressiveness between them has been observed and the members of the different families construct and cohabit the feeding punctures and fecal accumulations. The increased number of progeny in such cells does, however, leads to competition at the feeding site.

Female mites may invade worker or drone brood cells when worker bees bring them in close contact with brood cells. The attractive period of drone brood cells is two to three times longer than that of worker brood cells. The attractiveness of brood cells is related to the distance between the larva and the cell rim and the age of the larva.
The moment of invasion of the mite into a brood cell is not related to the duration of its stay on adult bees. The fraction of the phoretic mites that invade brood cells is determined by the ratio of the number of suitable brood cells and the size of the colony. The distribution of mites over worker and drone brood in a colony is determined by the specific rates of invasion and the numbers of both brood cells (Beetsma et al. 1999).

Garrido et al. (2000) determined the moment of activation of oocyte growth in Varroa females. Ovaries of the mites were dissected and stained with toluidine blue. The coloration of the terminal oocyte indicates the uptake of euplasmatic and/or yolk material and therefore, the initiation of the reproductive phase.

In phoretic mites removed from adult bees, no staining of the ovary was detected. Females artificially introduced into freshly capped brood cells and removed for dissection six hours later already showed clear blue staining of the terminal oocyte. The ovaries of female mites introduced 14 hours after capping of the brood cell, however, remained uncolored after incubation in toluidine blue. In phoretic mites, oogenesis is apparently arrested in a previtellogenic phase.

Immediately after invasion of the brood cell, reproduction is activated by some factor. This factor is present in freshly capped brood cells but not in brood cells 14 hours after capping. Oocyte growth in reproductive mites depends on the consumption of hemolymph from freshly sealed larvae (Donzé and Guerin 1994; Tewarson and Engels 1982).

Reproducing Varroa females lay the first egg in the brood cell approximately 70 hours after host cell capping (Ifantidis 1983; Steiner et al. 1994). This egg is unfertilized and develops into a male, while the three to four subsequent eggs that are laid at approximately 30-hour intervals are fertilized and develop into female offspring (Rehm and Ritter 1989; Martin 1994). However, the last eggs laid will usually not reach maturity, because the developmental time of the immature bee in the capped cell is too short to allow completion of mite development.

Since the capped stage of drone cells is about two days longer than that of worker cells (Jay 1963), drone cells are in principle more rewarding in terms of mite reproduction than worker cells because more young mites can reach maturity. In the European honey bee, mites produce on average two to three viable female offspring in drone cells and one or two viable female offspring in worker cells (Schulz 1984; Fuchs and Langenbach 1989).

The mite larva develops within the egg during the first hours after oviposition. During the period of time from egg hatch until adult molt, the mite offspring pass through protonymphal and deutonymphal stages. The total development time is about 5.8 and 6.6 days for female and male mites, respectively (Donzé and Guerin 1994; Martin 1994; Rehm and Ritter 1989).

Using transfer experiments, Garrido and Ronskranz (2003) examined whether the sequence of sexes (first egg unfertilized, followed by fertilized eggs) in the brood cell is triggered by a host stimulus. When reproducing Varroa females were transferred from white-eyed worker pupae into freshly capped worker brood cells, 77% of the fertile mites after the transfer began a new reproductive cycle by laying an
unfertilized egg. The proportion of brood cells with male offspring was similar to naturally infested brood cells.

Varroa females transferred into brood cells with young pupae reproduced, but only 6% of the fertile mites after the transfer produced male offspring. This was significantly different from male production in naturally reproducing Varroa females and those transferred into freshly capped brood cells. They concluded that a host stimulus present in freshly capped brood cells triggers both the start of reproduction and the sequence of sexes.

The reproductive cycle of the Varroa mite is closely linked to the development of the honey bee host larvae. Using a within colony approach, phoretic Varroa females were introduced into brood cells of different ages in order to analyze the capacity of certain stages of the honey bee larva to either activate or interrupt the reproduction of Varroa females (Frey et al. 2013).

Only larvae within 18 hours (worker) and 36 hours (drones), respectively, after cell capping were able to stimulate the mite’s oogenesis. Stage specific volatiles of the larval cuticle are at least part of these activation signals. This is confirmed by the successful stimulation of presumably non-reproducing mites to oviposition by the application of a larval extract into the sealed brood cells. Preliminary quantitative gas chromatography-mass spectrometry analyses suggest certain fatty acid ethyl esters which make up brood pheromone, as candidate compounds. If Varroa females that have just started egg formation are transferred to brood cells containing host larvae of an elder stage, two-thirds of these mites stopped their oogenesis.

This confirms the presence of an additional signal in the host larvae allowing the reproducing mites to adjust their own reproductive cycle to the ontogenetic development of the host. From an adaptive point of view, that sort of a stop signal enables the female mite to save resources for a next reproductive cycle if their own egg development is not sufficiently synchronized with the development of the host.

The reproduction of Varroa mites during successive honey bee brood cycles was investigated (de Ruijter 1987). Newly capped worker brood cells were identified and into each cell an adult female mite was introduced. After 10 days the cells were opened and the contents examined. Those females still present and alive were once again introduced into newly capped brood cells and so on. Varroa mites were capable of reproducing up to seven times under these experimental conditions. The maximum number of eggs laid was 30 per female. Females that produced only male offspring because they were unmated kept doing so in subsequent brood cycles. Though in contact with adult males several times, no successful mother mite matings occurred. Probably only young females mate successfully.

The male mates with the female offspring of the mother mite in the brood cell and only the mother and daughter females emerge from the cell. Protandry (appearance of males prior to females) in Varroa enables the fertilization of a maximum number of daughters within the limited time span of the capped brood. To be successful, however, the newly emerged adult daughters must encounter a male. However, adult males are scarce, occurring in only 60% of single infested cells due to developmental mortality (Fuchs and Langenbach 1989).

The mating of Varroa daughters occurs after ecdysis (molting) and as soon as they arrive on the fecal accumulation prepared by the mother mite (Donzé et al. 1996). Such females are remated for as long as no other freshly molted daughter arrives on the fecal accumulation. The number of spermatozoa stored in the mite’s spermatheca increases with remating, a strong indication for sperm mixing when brood cells contain more than one Varroa foundress.

The number of daughters per infesting mother decreases at higher rates of infestation per
cell, but the proportion of such daughters with a mate rises sharply due to the higher probability of finding a male within multi-infested cells. The number of mated daughters per mother is maximal in cells with two foundress Varroa females.

Martin (1995) investigated the developmental times and mortality of Varroa in drone cells. The position and time of capping of 2,671 naturally infested drone cells were recorded. Six hours after the cell was capped, 90% of the mites were free from the brood food to start feeding on the developing drone. The developmental time of the mite’s first three female offspring (133±3 hours) and the male offspring (150 hours) and the intervals between egg laying (20-32 hours) were similar to those found in worker cells. However, the mortality of the offspring was much lower in drone cells than worker cells.

The mode number of eggs laid were six and five in drone and worker cells, respectively. All offspring had ample time to develop fully in drone cells, with the sixth offspring reaching maturity approximately one day before the drone bee emerged.

Normal mites (those which lay five or six viable eggs) produced on average four female adult offspring. But since only approximately 55% of the mite population produced viable offspring, the mean number of viable adult female offspring per total number of mother mites was two to 2.2 in drone cells.

Within any mite population, large numbers of mites fail to produce fertile female offspring despite entering a suitable host cell. These can be classed into those that do not lay eggs, those that lay non-viable eggs and those that only produce viable male offspring.

Another cause which leads to the production of non-fertile females is the premature death of the male offspring before it is able to mate with its sisters. This situation arises because female mites only produce a single male during each reproductive cycle and this male needs to fertilize all of his sisters (Martin et al. 1997).

THE ENEMY IS IN SIGHT
Blood-sucking varroa mites (Varroa destructor) are considered the No. 1 enemy of beekeepers. In powerful numbers and weakened colonies, they can overwhelm and collapse a hive.

We remember seeing a Varroa mite attached to a foraging honey bee one warm summer day in our pollinator garden. The mite was feeding off the bee and the bee was feeding on the nectar of a lavender blossom.

Didn’t seem fair.
Take a close look at stunning photos by Allan Jones here.

Now that you’ve seen the Varroa mite on the bee, it’s time to examine the phoretic stage of the mite’s life.

VARROA MITE ORIENTATION
Female Varroa mites parasitize both adult bees and bee brood, but only reproduce in capped brood cells. Therefore, the mites have to leave the adult bees and invade the brood cells. Between reproduction periods in capped brood cells, female mites are phoretic (an association between two species in which one transports the other) on adult bees for a variable period
lasting a few days to several weeks (Boot et al. 1991).

Both worker and drone cells are invaded by the mites, but in drone cells reproductive success is higher than in worker cells (Fuchs 1992; Boot et al. 1995a). More adult offspring are produced in drone cells compared to worker cells.

Varroa mites selectively parasitize honey bee larvae within a narrow time window: 15-20 hours before brood cells are capped for pupation for worker larvae, and 40-50 hours for drones (Boot et al. 1992). The mites also prefer to infest drone brood cells, which are eight to 12 times more readily parasitized than worker brood cells (Fuchs 1990; Boot et al. 1995b). In addition, female mites exhibit preferences for adult bees of a specific age as mites readily abandon newly emerged bees and move to nurse-aged workers.

Drone cell preference is partly influenced by the properties of the brood cells. Larger cells contain higher numbers of mites. Cells protruding over the comb surface either naturally (DeJong and Morse 1988) or induced by partly filling them with melted wax (DeRuijter and Calis 1988) were shown to contain increased numbers of mites.

Varroa mite infestation levels on worker larvae reared in elevated individual cells was 6-fold higher than in the adjacent six cells surrounding them (Kuenen and Calderone 2000). This differential infestation rate is similar to published values of higher mite infestations of drone cells compared to worker cells. Infestation levels in control cells were the same as in the surrounding cells.

In contrast to infestation of these individually raised cells, mites invaded worker larvae in raised cells along the perimeter of a patch of raised cells (10 by 21 rows) 2.5 times more often than surrounding unraised cells, and similarly ca. 2.5 times more often than in the remaining raised cells (interior) of this patch.

In similarly prepared frames of drone comb, mites invaded individually raised drone cells 3.3-fold more often than the adjacent surrounding cells and control cells. On the other hand, mites infested drone larvae in the interior of the raised-patch area as often as drones in raised cells along the perimeter of the raised-patch, and this rate was ca. 2.5-fold higher than for drone larvae in unraised cells surrounding the raised-patch and drone larvae in control cells.

Rather than the shape of the cell, the time and construction effort needed for capping might be the relevant factor in determining the degree of mite infestation. In addition, stimuli from the larvae are involved. Drone comb preference was not influenced by the number of infesting mites or the absolute number of available cells (Fuchs 1990).

Invasion of Varroa mites into honey bee brood cells was studied in an observation hive, using combs with cell openings at one side only. The cell bottoms had been replaced by a transparent sheet, through which mites were clearly visible after invasion into a cell. Mites invaded worker cells from 15-20 hours preceding cell capping, whereas they invaded drone cells from 40-50 hours preceding capping (Boot et al. 1992). The larger number of mites generally found in drone cells, when compared to worker cells, may be partly due to the longer period of mite invasion into drone brood.

Invasion of mites into drone cells of honey bees was studied in colonies without worker brood. The probability for a mite to invade was dependent on the brood/bees ratio, which is defined as the number of drone brood cells capped per kg of bees. When compared with invasion in colonies with exclusively worker cells, Varroa mites invaded drone cells 11.6 times more frequently.

This suggests that the biased distribution of mites over drone and worker cells in colonies with both types of brood cells results predominantly from the higher rate of invasion into a drone cell per se, when compared to that into
a worker cell (Boot et al. 1995b).

Since the rate of invasion is high in drone cells, a trapping method using drone combs may be very effective in controlling Varroa mites. When no other brood is present, 462 drone cells are estimated to be sufficient to trap 95% of the mites in a colony of 1 kg of bees.

In colonies of Apis mellifera carnica infested with Varroa jacobsoni, the invasion of worker brood cells and drone brood cells by reproductive female Varroa mites were studied (Fuchs 1990). In 68 choices between brood combs of both cell types, the infestation of mites per cell was, on average, 8.3 times higher in drone brood.

This drone cell preference was not affected by the infestation level. It was more marked if drone brood was rare and it decreased toward the end of the drone rearing season.

Studies of Varroa destructor orientation to honey bees were undertaken to isolate discrete chemical compounds that elicit host-finding activity. Petri dish bioassays were used to study cues that evoked invasion behavior into simulated brood cells and a Y-tube olfactometer was used to evaluate Varroa orientation to olfactory volatiles. In Petri dish bioassays, mites were highly attracted to live fifth instar worker larvae and to live and freshly freeze-killed nurse bees.

Olfactometer bioassays indicated olfactory orientation to the same type of hosts, however, mites were not attracted to the odor produced by live pollen foragers. The odor of forager hexane extracts also interfered with the ability of mites to localize and infest a restrained nurse bee host. Varroa mites oriented to the odor produced by newly emerged bees (<16 hours old) when choosing against a clean airstream. However, in choices between the odors of newly emerged workers and nurses, mites readily oriented to nurses when newly emerged workers were present.

Varroa mites may detect relative concentrations of these compounds in order to discriminate between adult bee hosts and preferentially parasitize nurse bees over older workers in honey bee colonies. The volatile profile of newly emerged worker bees also may serve as an initial stimulus for mites to disperse before being guided by allomonal cues produced by older workers to locate nurses. Allomones are chemical substances produced and released by an individual of one species that affect the behavior of another species to the benefit of the originator but not the receiver.

Fatty acid esters, previously identified as kairomones for Varroa, proved to be inactive in both types of bioassays. Kairomones are chemical substances emitted by an organism which mediates interspecific interactions in a way that benefits an individual of another species which receives it and harms the emitter.

Several bioassays have been used to test the orientation behavior of the mites to semiochemicals. The activity of contact-chemo-receptive compounds has been examined using simple Petri dish or glass plate assays (Kraus 1993, 1994; Rosenkranz 1993; Zetlmeisl and Rosenkranz 1994; LeDoux et al. 2000; Nazi et al. 2001; Calderone and Lin 2001; Aumeier et al. 2002; Calderone et al. 2002).

These studies have established that mites orient to the stage-specific odor differences of live hosts, and that their movement can be arrested by blends of host cuticular compounds or larval food.

Other researchers have employed a semipermeable membrane as a bioassay arena to evaluate the locomotory behavior of Varroa (Rickli et al. 1994; Donze' et al. 1998), revealing that mites are readily arrested by combinations of straight-chain hydrocarbons or primary aliphatic alcohols and aldehydes derived from extracts of larvae or cocoons.

The detection of airborne host volatiles by the mite also has been examined using sev-
eral techniques. Le Conte et al. (1989) used a four-arm olfactometer to show that Varroa preferred to orient to the odor of live drone larvae, drone extracts and the fatty acid esters methyl palmitate, ethyl palmitate and methyl linolenate. Rickli et al. (1992) found that mites on a servosphere walked in straight paths confined to airstreams containing the odor of live larvae, adults, larval extracts or palmitic acid, but exhibited only a weak response to methyl palmitate.

Chemical components of honey bee pheromones also influence the host-finding behavior of Varroa mites. Using wax tube choice tests and a Y-tube wind channel, Kraus (1990) demonstrated that the odor produced by honey bee sting glands, and most of the individual components of alarm pheromone itself, were highly repellent to mites.

Hoppe and Ritter (1988) used simultaneous choice tests to show that the preference of Varroa for different ages of adult bees might be explained by the repulsion of mites to Nasonov gland odor or one of its principal components, geraniol.

Varroa mites are attracted to its major prey, drone larvae, by methyl and ethyl esters of straight-chain fatty acids, in particular methyl palmitate. These esters were extracted from drone larvae with n-hexane and were identified by gas chromatography-mass spectrometry. Their behavioral effect was evaluated with the use of a four-arm airflow olfactometer (Le Conte et al. 1989).

Varroa mites exhibit preferences for adult bees of a specific age as mites readily abandon newly emerged bees and transfer to nurse-aged workers, generally three to 12 days old, over older foragers (Kraus et al. 1986; Le Conte and Arnold 1987; Kuenen and Calderone 1997).

With no known optical system (Bruce 1997), Varroa must rely on non-visual stimuli for orientation to specific larval and adult hosts. Within the environment of a honey bee colony, semiochemicals appear to be likely candidates for these cues.

One alternative for controlling Varroa may be through the use of semiochemicals that either disrupt the normal host-finding behavior of the mite or to attract and trap a portion of the mite population within a colony. The high degree of host specificity exhibited by Varroa suggests that kairomones are used by mites to locate and parasitize larval and adult hosts (Pernal et al. 2005).

Varroa mites are not the only enemy of bees. The deformed wing virus also has been under scrutiny for the damage it causes. And the two may be related. At least one study has examined this parasite-pathogen partnership.

**VARROA AND DEFORMED WING VIRUS**

Honey bee colony losses concern beekeepers and agriculturists alike, as *Apis mellifera* are important crop pollinators. A pair of factors that affect the health of honey bee colonies are the mite, *Varroa destructor*—which parasitizes honey bee larvae—and the pathogenic deformed wing virus (DWV), which *V. destructor* can transmit.

Scientists have long tried to understand the details of the mite-virus-bee ecology. It was already known that DWV benefitted from its association with the mite, as the parasite helps the virus spread. But it had been less clear whether the mite gained anything from serving as a viral vector.

It turns out that mites show more reproductive successes when parasitizing honey bees with active DWV infections, according to a study published today (March 7) in *PNAS*.

“We provide evidence supporting the occurrence of a mutualistic symbiosis, unrecognized so far, which accounts for the severe impact of this parasite-pathogen association on honeybee health,” study coauthors Francesco Nazzi of the University of Udine and Francesco Pennacchio of the University of Naples Federico II, both in Italy, told The Scientist in an email.
“Nobody was expecting this,” said University of Salford ecologist Stephen Martin, who was not involved in the work.

A mother V. destructor mite parasitizes a larval honey bee by entering its brood cell before it is capped, poking a hole in the insect’s abdomen through which to feed, and reproducing within the sealed brood cell.

“It was believed that the virus was just passed mechanically,” Martin said. The mites, it seemed, “were effectively syringes” for the virus. It now appears that the mite benefits from spreading the virus.

Nazzi, Pennacchio, and colleagues previously suggested that DWV weakens the bee immune system by affecting proteins of the NF-κB family of immune-response genes. The researchers hypothesized that the bees’ weakened immune systems might make them more tolerant of mite parasitization.

For the present study, the researchers probed the correlation between the bees’ DWV levels and the strength of their immune responses. The team tested the bees’ immune responses by testing their abilities to coat a nylon thread in melanin and other materials, as they would react to most other pathogens. The researchers found that bees infected with more copies of DWV were less able to melanize the thread effectively.

The researchers then explored the relationship between DWV infection and genes involved in bee immunity, including members of the NF-κB signaling pathway. The researchers used RNA interference (RNAi) to knock down expression of NF-κB-family member Dorsal-1A, finding that levels of Amel\102—the bee homolog of a butterfly gene involved in melanization—were reduced.

This suggested that Amel\102 expression is influenced by that of NF-κB. In bees with higher levels of the virus, Amel\102 levels were decreased, while levels of a gene that impairs NF-κB activation—Amel\LRR—were increased. These results, the authors suggest, indicated that DWV impairs the honey bee immune system by blocking NF-κB signaling.

The team also examined reproductive success of mites infesting DWV-infected bees. The proportion of reproducing mites increased in those infected with more copies of DWV, the researchers found.

“Their work to show that there’s a correlation between immune genes and DWV levels [is] interesting, but I don’t think that correlation is necessarily the same as indicating a direct causation,” said Diana Cox-Foster of the U.S. Department of Agriculture’s pollinating insects unit, who was not involved in the work.

“I still question whether it’s a symbiotic relationship, per se,” she added.

Together DWV and the honey bee-parasitizing mite present a powerful threat to honey bee health worldwide, noted Norman Carreck of the University of Sussex, U.K., who was not involved in the study.

“Colony losses around the world are very commonly associated with deformed wing virus,” he told The Scientist. “In my view, Varroa remains the main threat to beekeeping worldwide.”

A better understanding of their ecology, Nazi and Pennacchio told The Scientist, “will support the definition of new strategies to prevent colony losses.”

Now that the Varroa mite reproductive life cycle has been examined, the following articles provide information for assisting in controlling the Varroa population in bee hives, with research to back up the recommendations. First, get the guide to help detect and control Varroa mites.
VARROA MITE GUIDE
The Honey Bee Health Coalition, a diverse coalition dedicated to improving the health of honey bees and other pollinators, has a guide aimed at helping beekeepers strengthen hive health by controlling the Varroa mite (Varroa destructor).

This parasitic mite undermines honey bee health by literally draining the life from honey bees, spreading viral diseases, and wiping out vast numbers of hives along with the pollination services these bees provide. As a result, these tiny mites are one of the biggest threats to honey bees and global food production.

“Varroa mites are one of the most serious threats to honey bee health and hives across North America,” said Bob Sears, president of the Eastern Missouri Beekeepers Association. “This straightforward guide, compiled using the best available scientific and commercial information, will equip beekeepers—from hobbyists to commercial—with effective and environmentally sensitive approaches to monitoring as well as control techniques to ensure their colonies can endure.”

“These problematic parasites have demonstrated a startling resiliency and ability to spread to other honey bee colonies,” said Christi Heintz, executive director of Project Apis m. “This guide, developed by leading honey bee health experts, will ensure beekeepers can more easily confront the problem of Varroa mite infestations, better protect their own bees, and mitigate the parasites’ abilities to move into other nearby apiaries.”

The Honey Bee Health Coalition worked with Dr. Dewey Caron, emeritus professor of Entomology and Wildlife Ecology at the University of Delaware and affiliate professor at Oregon State University’s Department of Horticulture, to gather input from leading experts on Varroa mite control. The resulting guide identifies straightforward, proactive and flexible monitoring methods and guidelines to help beekeepers detect and control Varroa mites.

The guide lays out an Integrated Pest Management (IPM) strategy for managing Varroa mite infestations, including how to monitor mite levels, chemical and non-chemical methods to control the mites, and methods to determine which treatment is appropriate for a beekeeper to use at different phases in a colony’s life cycle.

The Honey Bee Health Coalition brings together beekeepers, growers, researchers, government agencies, agribusinesses, conservation groups, manufacturers and brands, and other key partners to improve the health of honey bees and other pollinators in North America. Its mission is to collaboratively implement solutions that will help to achieve a healthy population of honey bees while also supporting healthy populations of native and managed pollinators in the context of productive agricultural systems and thriving ecosystems.

The Coalition is focusing on accelerating collective impact to improve honey bee health in four key areas: forage and nutrition, hive management, crop pest management, and communications, outreach and education.

In addition to the Coalition’s guide, there are ways to control Varroa mites with and without chemicals. This breeding method may help the bees in their fight against the mites.

BREEDING MITE-BITING BEES
Despite the general recognition among bee
Keepers and bee researchers that Varroa mites are the number one risk factor for honey bee colony mortality, a look at the Bee Informed Partnership national surveys tells us that most beekeepers are hobbyists and most of them do nothing to control for Varroa mites in any given year, and those who do not control mites have much higher colony losses (2010-2014).

There are some non-chemical practices that beekeepers use that help control mite levels such as introducing a break in the brood cycle by splitting colonies and re-queening, or the use of screened bottom boards. There are also some commonly used mite control practices that research has shown are ineffective, for example the use of comb with small cell sizes (Zhou et al. 2001; Taylor et al. 2008; Ellis et al. 2009; Berry et al. 2010; Coffey et al. 2010; Seeley and Griffin 2011). One important non-chemical strategy for sustainable beekeeping is use of mite-tolerant honey bee stocks.

Progress in selecting for resistance to Varroa has been slow but there is evidence the bees have begun their own fight against the mites. Some queen breeders are trying to help bees in this fight by incorporating lines of bees that have been subjected to natural selection by surviving without miticide treatment, such as Russian bees imported to North America by the USDA (Rinderer et al. 2010).

Another approach is to select for specific traits that are effective at lowering mite populations. A study in Europe found that colonies with low mite populations had damaged mites falling from the bees (Moosbeekhofer 1992) and other studies have suggested that grooming behavior is important for resisting mite infestation, as it is in the mites’ original host, the Asian honey bee (Peng et al. 1987; Boeking and Spivak 1999; Mondragon et al. 2005). However, the benefit of using the proportions of damaged mites as selection criteria has been questioned (Rosenkranz et al. 1997).

Varroa-sensitive hygiene (VSH) was discovered as an important mite resistance mechanism by measuring the growth of Varroa populations in many colonies with queens that came from different sources (Harbo and Harris 1999; 2005). The VSH trait has been effectively incorporated into breeding lines and VSH queens are commercially available (Rinderer et al. 2010).

A similar study with another set of queens showed that in those colonies grooming behavior was the trait that was most closely associated with reduced mite levels. Higher-grooming colonies also were more likely to bite the mites (Arechavaleta-Velasco and Guzmán-Novoa 2001).

At least two other studies also showed a link between grooming behavior and the proportion of chewed mites falling from colonies. Cages of bees that removed a higher proportion of mites from themselves in a lab assay had fewer mites left on the bees, and the proportion of mites removed correlated with the proportion of damaged mites on sticky boards from the source colonies (Andino and Hunt 2011).

Another study looked at four pairs of allegedly mite-tolerant and mite-susceptible lines from different populations. In each comparison, the more resistant stocks had more vigorous and effective individual grooming behavior when a mite was put on worker bees, and had higher proportions of chewed mites falling from the
source colonies (Guzman-Novoa et al. 2012). This means that when bees are selected for low mite population growth they tend to be better groomers.

Genetic studies identified regions of honey bee chromosomes and candidate genes that influence both of these complementary resistance traits but using DNA markers to select for good resistance genes is not very practical in our opinion (Tsuruda et al. 2012; Arechavaleta-Velasco et al. 2012).

Even after confirming an individual gene’s effect on a trait, the value of selecting based on DNA or protein markers would be limited because other unknown genes also influence these traits, so you would only be increasing the frequency of some of the “good genes.” It seems that at least for now the best way forward is to select based on the trait itself. We conducted a breeding program to select for “mite biters.”

Here we describe some of the selection methods, correlations between measures of grooming behavior and mite levels, and the results of a beekeeper stock evaluation.

Methods

Breeding population and selection. The breeding population was established in 1997 from diverse sources, including queens from commercial queen breeders: some Carniolans from California breeders and one each of VSH and Russian colonies from Glen Apiaries. But many of the colonies in the population were those that had survived for years without miticide treatments. Each year the population consisted of about 100 colonies. It was not a closed breeding population. Occasionally, queens from other Midwest queen producers or feral colonies were introduced.

Queens were all marked with paint and records kept of supercedure events. Initially, breeder queens were either instrumentally inseminated or open-mated. Daughter queens were open-mated in two mating yards one mile apart and isolated from all but a few other beehives by two miles. They contained selected drone-producing colonies with one or two frames of drone comb. From 1997-2006 breeder colonies were selected based only on low mite population growth as measured by two to three counts of mite fall on sticky-board sampling sheets during the spring and summer.

Beginning in 2007 we began selecting for mite-grooming behavior. For the early years we treated colonies with miticide if Varroa levels were too high (usually >100 mites falling in a day late in the season). For the past six years no mite controls have been used and we do not split the colonies very often so there are minimal breaks in the brood cycle, which would reduce mite levels. Breeder queens were selected based on the proportion of mites that had damaged legs or an apparent bite in the body (the idiosoma) of the mite.

To measure the proportion of chewed mites, plastic sampling sheets were sprayed with vegetable oil and slid underneath colonies that had screened bottom boards (Country Rubes, Grass Valley, California) and left for two or three days. Using enough vegetable oil makes it fatal for the mites, and also for any ants that might try to feed on them.
Mites were carefully removed from the sample board using a small paintbrush and placed belly up (ventral side) in rows on microscope slides. If fewer than 10 mites were present, the data was recorded but not used for selection decisions. The number of mites on sticky boards was recorded, slides were examined with a microscope (15X), and the number of mites missing legs or leg parts or showing mutilation of the idiosoma was counted.

Pale immature mites were not examined because these could have fallen as bees emerge from brood cells and may be more susceptible to damage unrelated to grooming behavior. Sometimes empty shells – the idiosoma with virtually no contents – were observed. These were not counted because we do not know their cause.

The relative severity of mutilations was also scored as low, moderate or high, meaning most mites had multiple legs chewed and bites to the idiosoma were seen.

Selection was hierarchical, which means that we first selected colonies with the highest proportion of chewed mites that were highly mutilated. We secondarily selected for low mite population growth and colony strength over the season. Colonies were re-queened if they had high mites or had chalkbrood or other brood diseases. A hygienic behavior test was usually performed on potential breeder queen colonies, which were required to show at least 95% hygienic removal of freeze-killed brood within 24 h (Spivak and Downey 1998).

In 2009 selection was based on the results of laboratory grooming assays for mite removal as well as the proportion of chewed mites in a colony (Andino and Hunt 2011). Beginning in 2010, we tested all of the colonies at least two or three times per season for the proportion of chewed mites, and each breeder queen was instrumentally inseminated with semen from multiple drones from one or two selected hives.

During 2013 and 2014, we tested for correlations between mite drop and the proportion of chewed mites. Because of a mistake that was made in 2014, the total number of mites was not counted in colonies that had more than 70 mites falling on the sampling sheets but the proportion of chewed mites was recorded for a sample of 70 mites. There were seven of 56 and 19 of 63 colonies in this category for May and August measurements, respectively.

**Beekeeper stock evaluation.** In late June 2014 we initiated a beekeeper stock evaluation program by providing marked commercial-source queens and “IN mite biters” from the Purdue breeding program to beekeepers. This was a blind study; beekeepers received marked queens to identify the sources but were not told which ones were mite-biters. Beekeepers were asked not to treat to control Varroa mites. We purchased queens from three Western queen breeders (two Carniolan and one Italian). We chose two IN mite-biter grafting sources to test. Participants were asked to de-queen a colony and split it so that one commercial-source and one IN queen could be introduced into each half, presumably starting with equal mite loads. Beekeepers were asked not to treat colonies to control mites and to report whether they survived for a full year. Some beekeepers also provided data on mite levels, honey production, personal observations and preference for one queen source over another.

**Results And Discussion**

**The proportion of chewed mites is a heritable colony trait:** It was possible to increase the proportion of chewed mites in the breeding population, even though we selected from a base population of only about 100 colonies. These results show that the trait is heritable. Starting with an average of 3% chewed mites, the proportion of chewed mites increased steadily (Figure 1).

There no doubt is experimental variation because different observers scored the mite damage, but only one individual scored mites in any given year. We have also observed VSH activity in some colonies in the breeding population...
One difficulty in finding a relationship between mite-biting and mite levels in our colonies may be that they are not uniform in size and in colony history. For example, mite populations decline when a colony is re-queened because there is a break in the brood cycle and mite levels are higher in colonies that have a lot of brood.

We also do not know how much VSH behavior varies in the colonies. One of our grafting sources in 2014 exhibited this trait by removing mite-infested brood within 48 hours (Fig 2). It maintained low mite populations and had a high proportion of chewed mites dropping on the sticky board. But in general the 2014 results suggest that grooming is effective at reducing mite levels, at least when the mite population is not too high.

There appears to be good reasons for bees to bite mites. Bites from worker bees can remove legs, which interferes with the mite’s ability to move and hold on to bees, and also opens them up to dessication. It was recently shown just that 2-heptanone from worker mandibular glands, long thought to act as an alarm pheromone, actually is an anesthetic to invertebrates. A worker bite to a small wax moth larvae or the application of only 0.061 microliters of 2-heptanone to the back of Varroa mites causes temporary immobilization. This may be the main function of this chemical for the bees (Papachristoforou et al. 2014).

Not enough is known about different variables that influence the trait “proportion of chewed mites.” Repeated testing of colonies shows it varies much more than we would like, which means it is influenced by environmental effects. Perhaps when a large patch of adults emerge from the brood, more mites fall passively.

Another difficulty is that colonies that are effective in reducing mite populations often have insufficient mites on the sampling sheet, especially in the spring. This is a good problem to have! But it necessitates choosing the spring grafting sources depending on data from tests.
done the previous year.

Better screening methods are needed that can be performed on colonies even if they have few mites. We are considering making initial selections based on the proportion of chewed mites and then using a secondary lab assay to observe grooming behavior of individuals or groups of workers to choose grafting source and drone colonies. We are also considering more regular testing for VSH.

**Community stock evaluation:** We distributed 102 queens to 39 beekeepers in 2014. We received data from 23 beekeepers from Illinois, Indiana and Ohio that successfully introduced both of the queens into splits that came from one hive and evaluated them for a year, which represents 54 queens or 27 side-by-side comparisons of the two types of stock.

Beekeepers were given a single pair of queens to compare, except for one beekeeper that had four pairs of queens and another that had two pairs. The three commercial queen sources from Western states did not differ from one another in survival or mite levels, so they were combined into one “commercial” stock for beekeeper evaluation. Likewise, the two Indiana queen sources did not differ from one another and were combined into one “IN” stock for evaluation.

Most colony mortality occurred during the winter; some of this was starvation and some was probably mite-related. A few colonies died before the winter from unknown causes, perhaps queen failure. By March 2015, only six of 27 commercial colonies (22%) were surviving. In contrast most of the IN colonies (15 of 27 or 55%) were still alive.

Honey yields were estimated based on reports from 14 beekeepers over both years. Making the assumption that a medium depth super yields 30 lb. of honey and a shallow yields 20 lb., commercial source colonies produced an average of 11.7 lb. compared to 52.1 lb. for hives with IN queens, a 40.4 lb. difference.

Most colonies did not produce surplus honey the first year, so most of the yield difference was caused by differences in survival. But there were also some differences in colony strength. Relative colony strength was reported in 12 cases; hives with IN queens were rated stronger for eight, weaker for two and equal strength for the other two.

Eight beekeepers reported on Varroa mite levels for both types of queens during 2014 or 2015 using either powdered sugar shakes, alcohol washes or sticky board sampling. One of these beekeepers reported a lower mite count in the commercial source hive (two thirds of the IN mite-biter level on a sticky board a month after introducing queens). Two beekeepers reported no mites in the IN colonies but found either six mites (powdered sugar shake) or 147 (sticky board) in the commercial colonies. The other seven beekeepers reported that the commercial source colonies averaged threefold higher mite levels compared to those with mite-biter queens (2.7-fold higher for the eight comparisons).
Of the 11 beekeepers that stated a preference, 10 chose IN mite-biter queens over commercial-source queens in this blind comparison. One of those that preferred the mite-biters was comparing four pairs of queens. Two beekeepers mentioned that the colonies with IN queens were more aggressive. One of these two said that the IN hive was slightly more aggressive than the other hives but that he preferred it because it was more productive. The other said that the commercial-source hive had very high mite counts (tested with alcohol wash) and was dead by Christmas.

On the other hand, the IN hive had lower mite levels in the fall, which were further reduced after winter, but was “extremely defensive” and had 20 frames of bees by July of 2015.

This experiment was limited in scope because it only compared daughter queens from five mother queens. We also did not compare the “mite-biter” queens to other Midwestern stocks, which may have similar or better survival. We have seen other stocks that have relatively high proportions of chewed mites and believe this trait can be selected for in any genetically diverse population of bees. We do not know if this trait shows genetic dominance.

One test of this would be to take virgin queens of low-biting stock and let them fly in our mating yard to see how their progeny do.

There may also be environmental effects that influenced survival that had nothing to do with genotype or that interacted with genotype, such as exposure of queens to Nosema or virus, or the mating conditions. So we can’t make any strong conclusions but the large difference in winter survival and the beekeepers’ preference suggest that breeding for mite-grooming behavior in local stocks of bees will make beekeeping more sustainable in the North Central U.S.

Stocks from this breeding program are being made available through the Heartland Honey Bee Breeders Cooperative. We think that it is important that queen breeders select for both VSH and grooming behavior in bees that have survived northern winters. Splits are another method of controlling Varroa that also produce more colonies and new queens.

SPLITS TO CONTROL VARROA
At a recent beekeeping meeting the question was asked if a split can be made to help control Varroa. The answer is yes, and the reason is Varroa’s dependence on developing bee larvae.

Varroa can only reproduce by entering a cell containing a mature larva ready to be capped. Once the cell is capped, Varroa begin to reproduce. They feed on the developing pupa’s hemolymph vectoring viruses and ultimately weakening the emerging bee. This cycle then repeats for as long as there are larvae for Varroa to infest.

Since more than one mature Varroa will emerge with a single adult bee, eventually the Varroa population can overrun the bee population and the colony’s viral load will cause extensive disease and ultimate collapse. Depending on the initial level of infestation, this can happen in as short an interval as a single season.

There are many ways to make splits and almost an equal amount of reasons why beekeepers make them. This article discusses splits made with the intent to control Varroa using manipulations to temporarily stop the colony from...
producing bee larvae and therefore providing control of the Varroa population.

This means the split must go through a period when no eggs are being laid either because it’s in transition to a new queen or the existing queen is being restricted from laying. This is referred to as a brood break, or broodless period, and must be of sufficient duration to deny mites the opportunity to reproduce for an extended period.

During the broodless period all the reproducing mites, already under capped cells, will emerge and with no available larvae to continue reproduction some will die and others will be groomed off. Since the population is diminished, once the brood cycle resumes the emerging bee population can grow faster than the Varroa population. This has been described as a forever-young colony where periodic brood breaks allow bee populations to naturally out-run their pathogens and pests. The model in nature is the African honey bee that constantly swarms and absconds re-starting their brood cycle each time.

With a split, accompanied by a brood break, there are a few important considerations. The colony loses a great deal of population and the split must be timed to allow recovery before the end of the season. Also, facilitating a successful brood break requires understanding the brood cycle. The splits must be re-queened, but egg laying must be delayed. How you introduce a queen will depend on the resources you have, or can obtain, at the time of the split. Here are some examples of how to re-queen and at the same time facilitate a brood break.

- A walkaway split where a colony raises an emergency queen.
- Timing the introduction of a virgin queen using a ripe queen cell.
- Timing the introduction of a purchased fertile queen.

Let’s look at each one.

**Walkaways**

Using walkaway splits will depend on having brood frames with eggs so the splits can draw queen cells and raise emergency queens. If all goes smoothly, raising a new egg-laying queen will take about 27 days. It will take another 21 days, or about seven weeks total, before the colony has an emerging population of new bees.

For the first nine to 10 days after the split, Varroa can still find open cells with mature larvae to reproduce but after that all existing larvae will be capped. While the current brood cycle finishes, Varroa will continue to emerge with adult bees but are denied larvae to reproduce until about day 34. At that point the new queen’s larvae will mature and Varroa can resume reproduction. The 25-day period, between day nine and day 34, is the start of the Varroa population decline that will become evident in future Varroa measurements.

The main advantage of a walkaway split is that it can be done without the timing restrictions of acquiring new queens or queen cells. This option works best around swarm season with good nectar flow and plenty of available drones.

A walkaway also allows time for additional Varroa controls when all the mites are out of cells and on bees. That window is difficult to predict, but can occur starting around day 24 and can...
Varroa entering a cell

last about 10 days. That’s the sweet spot for optional additional treatments. Inspections can help determine exactly when all the old brood has emerged and when new brood is first available to mites.

**Queen Cells**

A delayed introduction of a ripe queen cell provides a similar brood break. With this method the queen in the cell is only a day or two from emerging. Assuming the split is eggless, with no opportunity for emergency queen development, you can leave the split queenless for about 10 days then install the ripe cell. A few days later the virgin emerges and a few days after that she begins mating flights.

By delaying the introduction of the queen cell, the events inside the colony follow the timing of a walkaway split with all the same benefits.

The advantage of using a queen cell is your choice of queens. If you’re doing this during swarm season, you can use your own swarm cells from colonies that have characteristics you want, or you can purchase desired cells from local queen breeders.

Since the queen-cell splits must be ready before the cells arrive, timing is critical. You want your splits done and waiting for about 10 days previous to the arrival of your ripe queen cell. During the 10-day period prior to introduction of your queen cell it’s critical there are no emergency queen cells in production by the colony. Emergency cells are normally started within a day or two.

Check a few days after the split and if you encounter emergency queen cells you must remove the frame, shake or brush off the bees, destroy all the emergency cells and put the frame back. Then before installing the ripe queen cell check for emergency cells again and repeat the procedure above if needed. It’s imperative that you’re only allowing the queen you want to emerge.

**New Queens You Purchase**

Unlike the parent colony queen, new queens must be introduced to the colony slowly, allowing their pheromone to permeate and facilitate acceptance. The technique is the same, meaning you must delay the introduction long enough to facilitate a brood break.

Just like with ripe queen cells, there are the same critical timing issues and colony behaviors to consider. If the split had eggs, the colony will start emergency cells almost immediately. Those will need to be removed as explained previously. If no emergency cells are present, you can simply leave the split queenless for few days, then introduce the new queen in her cage with the cork in place denying access to the candy plug normally used to release the queen.

After a few days you can remove the cork and expose the candy plug. It takes about three to five days until she’s released and another five, or more, until she starts laying.

**The Parent Colony Queen**

In all cases, the parent colony queen will continue to lay without a brood break unless you intervene. One way to accomplish a brood break is to capture her and leave her in the colony but restrict her laying to a very small area under a “push in cage.” These cages are generally about four inches square made from #8 hardware cloth and deep enough so the queen has room and can be attended by workers.
Another way is to install a commercially available queen release frame and leave her in the frame. Either way her physical presence in the colony will maintain some order and you can leave her caged until all the existing brood has emerged.

Emergency cells are removed in splits where a new queen is introduced, but allowed to emerge during a walkaway split.

Which Option is Right for You?
To help decide, start with an inspection and determine the number of splits you can make based on the quantity of nurse bees, brood and food frames that are available. Choosing an option, like queen cells, or new fertile queens will depend on connecting with a supplier and timing the splits with delivery.

During swarm season you can use your own swarm cells and, as mentioned, a walkaway can be done almost spontaneously as long as drones are available to mate the virgin queen.

Other Considerations
All the options require inspections to determine if the queen is accepted and laying. When re-queening doesn’t happen according to plan, you must intervene to get the colony on a path to becoming queenright.

Leaving a colony without a queen, for an extended period, will result in worker bees developing their ovaries and when that happens workers will start laying eggs. A colony of laying workers produces all drones and the colony is basically doomed. Also, if your virgin queens from queen cells or walkway splits are not mated correctly, they can also lay all drones and must be replaced.

The weather plays a major role in the mating of virgin queens and can also delay your planned date for doing splits. Queens will emerge from cells on their schedule regardless of weather, so you may need to think about how you would care for virgin queens in case weather prevents you from getting cells into colonies before they emerge.

Notes:
Since these splits are for the purpose of Varroa control, monitoring mite populations before and after is critical to understanding efficacy. If your ultimate goal is limited treatment, your requeening choice should favor queens with hygienic behavior that resist Varroa or, conversely, bees that can tolerate higher levels of infestation.

When using walkaway splits or ripe queen cells, area drones will determine part of the genes passed on to the splits. The hygienic quality of locally mated queens can only be determined as you monitor Varroa counts. Also, you will lose queens that you purchased for their hygienic traits if they swarm and her daughter queens may produce workers with different traits because they will mate with local area queens of unknown genetics.

A realistic goal is to achieve a balance between infestation and a healthy colony. Realizing it will not be possible to eliminate all Varroa, or to ignore them hoping the bees will deal with them on their own, is the first step toward successful Varroa control.

Critical Dates Post Split – Approximate
Day 9 – All existing open brood are capped.
Day 12 – New queens emerge from various options.
Day 24 – All brood and all mites have emerged.
Day 24 – All mites are phoretic until 7-8 days after the new queen starts laying.
Critical Dates Based on the New Queen – Plus or Minus Five days
Day 27 – New queen starts laying.
Day 33 – Last day that all mites are phoretic.
Day 34 – Mites resume reproduction in new brood.
Day 48 – New mites begin to emerge.

Now, take a look at some chemical treatments for controlling Varroa mites.

OXALIC ACID REGISTERED FOR VARROA MITE CONTROL
More than 250 letters were received by the Environmental Protection Agency (EPA) when beekeeper input was sought. The response was overwhelmingly positive and EPA, working with Canada’s Regulatory organization, feels Oxalic Acid, when used in accordance with label instructions, is safe for bees, beekeepers and the environment. It will be available to treat packages, colonies as an additive to sugar syrup or as a fumigant. Read the entire report here.

USING OXALIC ACID
The toxicity of oxalic acid (OA) to Varroa has been known for nearly three decades based on experiments conducted in Asia and later in Europe. The initial tests compared spraying weak OA solutions on bees, trickling OA sugar syrups into honey bee colonies and OA fumigation (vaporization). All of these methods demonstrated very high efficacy and were quickly adopted by beekeepers.

Early use of OA by European beekeepers involved spraying each comb of bees in broodless colonies with a 2%-3% OA water solution. The technique was effective but time consuming since each comb covered with bees had to be removed and sprayed on both sides for control. The treatment was later modified to make the application more efficient by adding the OA into sugar syrup and trickling 5-6 ml on the bees clustered between combs.

The role of sugar is unclear since minimal OA syrup is consumed by the bees and mite mortality is from contact with the acid. It is thought that the sugar solution adheres to bees better or the sucrose makes the solution more hygroscopic.

During the 1990s European bee researchers tested the effects of different OA concentrations and syrup solutions on both the mite and bees via the trickle method. Researchers found optimal Varroa control at OA concentrations between 2.1% and 4.2% with concentrations of 2.8% and 3.5% the best regarding mite mortality and minimal damage to bees.

Researchers also compared Varroa mortality in OA treated hives when brood was present vs. absent. Varroa mortality in hives with brood was 25% and 39.2% at the 2.9% and 4.2% OA concentrations and 97% and 99.4% respectively under broodless conditions. Experiments indicate that increasing the dosage of OA above 3.5% does not improve efficacy and high doses of OA aren’t used due to bee toxicity.

Research clearly demonstrates that OA is most effective in broodless colonies for both the trickle and fumigation techniques. The application of OA via trickle or vaporization in late fall/early winter affords beekeepers and excellent Varroa cleanup for colonies with residual mite populations that rebound after late summer Varroa treatments and/or hives that become reinfested by robbing hives undergoing Varroa collapse.

Trickle Treatments
As is the case with other registered Varroa controls, researchers have also identified issues related to the OA trickle treatment. For example:
1. When brood is present, repeated applications of OA can result in higher queen and adult bee mortality and a reduction in the amount of brood that can last for two months.
2. The midguts of honey bees fed OA sugar syrup have an elevated level of cell death but under field conditions bees tend to avoid consuming syrup with OA. (This may explain why a recent report stated that bees treated with OA have a lower nosema incidence.)
3. In some instances, bees show poor tol-
erance to OA trickle applications. Colonies receiving an overdose (excessive amounts, short-term repeated applications or excessive concentrations) can be weak in spring or die during winter. Some studies have found that certain colonies do not tolerate the OA trickle even at normal doses.

4. Low temperatures can diminish the efficacy of OA treatment.

5. There may be a correlation with increased bee mortality when applied during periods of high humidity. Further research regarding high environmental humidity is needed.

6. Prolonged storage of OA syrup solutions results in a change of color to brown. Analysis indicates a high increase in hydroxymethylfurfural (HMF) that may be toxic to the bees if ingested. As a precaution, it is recommended that beekeepers administer freshly prepared solutions of OA syrup or use premixed solutions that are stored in the refrigerator. Solutions can be stored for a maximum of six months at a storage temperature of 59 degrees.

Following are European recommendations and remarks for OA trickle treatment or the “Solution Method” as it is called on the U.S. EPA label. Some of these recommendations appear on the U.S. Oxalic Acid Dihydrate label.

• Trickle 5ml of OA solution directly on the bees clustered between the frames (occupied bee space) in each hive body.
• The maximum dose of OA solution is 50ml. (i.e., 30ml for a small colony, 40ml for a medium-sized colony, and 50ml for a large colony).
• Use only in late fall or early spring when little or no brood is present. The European recommendation: carry out one treatment in broodless hives only in autumn (November-December).
• Treat with lukewarm solution.
• Apply treatment at an outdoor temperature above freezing (32 degrees).
• Use only freshly made solutions or those stored for no more than six months at a maximum of 59 degrees.
• Do not use when honey supers are in place.
• Apply only when monitoring indicates treatment is required.
• Wear gloves, safety goggles and respiratory protection during treatment.

**Vapor Treatments**

There is much less published literature regarding OA heat vaporization (sublimation) in comparison to the OA trickle method. Research and reports from beekeepers indicate that the vaporization technique does have some advantages. Research suggests it is less detrimental to adult bees, brood and hive strength following application and from the beekeeper’s perspective, there is no need to unwrap, open hives and disturb the cluster during winter. The risk to the applicator, however, is somewhat greater due to the potential of inhalation of the OA fumes.
European research indicates that when brood is present, vaporization three to four times at weekly intervals in spring is an effective Varroa control. However, the U.S. OA label does not address this strategy and European recommendations prescribe treatments during broodless periods at temperatures between 35-61 degrees.

There are two methods used to vaporize (sublimate) OA, passive and active. The passive method involves placing the prescribed amount of OA (1 gram/hive body) onto a mini battery-powered heat plate that is inserted into the hive’s entrance. After insertion, the hive entrance is closed with foam or a piece of cloth and the electricity is applied. The crystals melt and sublime into smaller crystals that disperse within the hive covering the bees and hive interior.

All other entrances and openings such as cracks must be closed or taped shut so the fumes don’t escape and reduce treatment efficacy. It takes approximately three minutes for the OA to sublime and it is recommended that the hives remain closed off for 10-15 minutes after treatment.

There are several passive Varroa vaporizers on the market. Examples include the Varrox-vaporizer from Switzerland, Heilyser Technology vaporizer from Canada, Varroa cleaner from Serbia and Kiwi Vaporizer from New Zealand. There are other homemade vaporizer designs marketed.

The other method of OA vaporization is the active method in which the OA crystals are heated within a container until sublimation occurs outside the hive. After sublimation, vapor is blown into the hive entrance. These gizmos typically require a heat gun, source of electricity and air compressor for some of the designs. Lega bee supply from Italy markets one of the designs.

For some entertainment, do a Google search on commercially available and homemade OA vaporizing contraptions. Some of the designs featured on YouTube do not appear to be safe or effective!

As is the case with the OA trickle application, the efficacy of OA vaporization may be reduced when applied in cold temperatures when bees are in tight cluster since the crystals do not penetrate into the clustered bees. Likewise, high humidity during treatment may reduce treatment efficacy. Active methods of vaporization are said to work better than passive vaporization when bees are in tight cluster.

It is imperative for beekeepers to read the Oxalic Acid Dihydrate label prior to use and follow the directions. Although OA is considered an organic mite control, it has a “Danger-Poison” signal word on the label, meaning it is highly toxic and corrosive. Beekeepers need to adhere to the personal protection label requirements and the personal protection equipment (PPE) statements. Do not apply OA to hives of bees with supers in place so honey is not contaminated with this toxic substance.

Late fall or early winter Varroa treatment with oxalic acid is a valuable component for honey bee pest management. The use, efficacy and safety are well-documented in Europe and elsewhere when used properly in accordance with label instructions.

With time and experience, beekeepers will find that OA treatment fills an important need as a Varroa “clean-up” that will ensure healthy hives in spring. Please read and follow the label. Remember, “The label is the law.”
While oxalic acid is successful against Varroa, the application method also makes a difference. Here’s a case for using sublimation.

**SUBLIMATION USING OXALIC ACID**

**Summary**

Research by LASI (Laboratory of Apiculture and Social Insects) shows that of the three application methods used by beekeepers (trickling, spraying, sublimation) to control Varroa with oxalic acid, sublimation is the best in all respects. Sublimation is effective at lower doses, causes no harm to the bees, and results in colonies with more brood in spring. Spraying significantly reduced colony survival. Application of 2.25g oxalic acid via sublimation to broodless hives in winter killed 97% of the Varroa.

**Introduction**

Pests and diseases are a challenge to all beekeepers. One of the most serious is Varroa, the mite Varroa destructor, which originates from East Asia and is now found in all continents except Australia. Varroa was first detected in the USA in 1987 and is now found throughout North America.

Varroa mites harm colonies directly, through the harm they do to pupal worker bees in sealed cells where the female mites lay their eggs, and where the mother mite and her offspring feed on the blood of the pupa. Worker bees parasitized in this way as pupae have reduced lifespans. However, the greatest harm is caused by Varroa spreading virus diseases, such as deformed wing virus. Colonies with relatively low numbers of Varroa can die if virus is also present, especially in winter.

For many years beekeepers could easily control Varroa with Apistan strips, which slowly release a synthetic chemical (fluvinate) that is highly toxic to Varroa.

However, resistance has evolved. In a test at the Laboratory of Apiculture and Social Insects at the University of Sussex, we found that Apistan treatment killed only about half the Varroa in a colony. When Apistan was first introduced, and Varroa were non-resistant, the kill was nearly 100%.

For some diseases, particularly American foulbrood, beekeepers try to keep the level in their hives at zero. It is practical to do this by regular inspections and elimination of infected hives and equipment.

However, it is not practical for beekeepers to eliminate all the Varroa in their beekeeping operation. What is needed is a way of keeping the Varroa populations in colonies under control, so there are insufficient Varroa to cause harm.

Many control methods have been tried against Varroa. Our research at LASI has focused on hygienic behaviour, oxalic acid, and trapping in drone brood. Our results indicate that the first two methods are effective, but that trapping is not very effective.

In this article we describe a two-year research project on the effectiveness of oxalic acid and which was published (Al Toufailia et al. 2015) in the *Journal of Apicultural Research*. The original article is available here.

**Why study oxalic acid?**

LASI research on Varroa control falls within our wider project, the Sussex Plan for Honey Bee Health and Well Being. The Sussex Plan focuses on two of the major challenges faced by honey
bees and beekeepers: 1) Controlling pests and diseases; 2) Improving the bee food supply. In the Sussex Plan we have been trying to carry out research with clear practical benefits. Before starting we talked to beekeepers. It was clear that they considered Varroa to be a major problem, and this matched our understanding and experience as scientists.

Oxalic acid has been used to control Varroa for several decades and is known to be effective. So why was further research needed? The reason is that the previous research was incomplete. In particular, different application methods and doses had not been compared side by side to determine how effective they were at killing Varroa, and the effects they had on the bees. In addition, previous research had usually determined the numbers of mites killed rather than the proportion killed.

What LASI did

We treated 100 hives with oxalic acid on 12 January 2013. A further 10 were untreated controls, making 110 hives in total. The hives were in 10 apiaries in Sussex, southern England, 11 per apiary. The hives were all in a single Commercial box (11 frames, volume 56 litres, about the size of two medium-depth Langstroth boxes), with a wooden bottom board with mesh floor, inner cover and telescopic outer cover, and were similar to the hives being overwintered by beekeepers in terms of management and numbers of bees. Hives had approximately five to 10 thousand workers.

The hives did not have any capped brood when treated. This is important. Varroa can occur in two locations in a hive:

1. In brood cells, where the adult female mites lay their eggs and the young mites develop by feeding on the blood of the pupa;
2. Phoretic, clinging to the body of an adult bee.

Oxalic acid only kills the phoretic mites. In December and early January, when we did our study, 90% of the hives were naturally broodless.

All the hives had been checked a few weeks before applying oxalic acid, and any brood was scraped out with a honey uncapping fork. Care was taken to minimise any disturbance to the bees, and without breaking the cluster by shaking bees off the frames as is usually done during a hive inspection in warm weather.

The hives were also checked one day before each of the two samples of bees were collected to confirm that there was no capped brood. As a result, we could be sure that all Varroa were phoretic.

Our application of oxalic acid (technically, oxalic acid dihydrate) followed methods already being used by beekeepers. This was because our aim was not to develop new methods, but to compare existing methods. In the trickling/dribbling and spraying methods, we applied 50 ml of sugar solution (1 kg sugar dissolved in 1 litre of water) with oxalic acid, made 12-18 hours previously, to each colony.

In the dribbling method, the lid of the hive is removed and the solution is poured onto the top bars and gaps between the top bars where the bees were clustered, although there was never a tight cluster. In the spraying method, the frames are briefly removed from the hive and the bees sprayed with the solution.

The sublimation/vaporisation method uses oxalic acid crystals. These were placed into the small cup at the end of the electrically heated applicator, which was inserted into the centre of the hive below the frames. The heat causes the crystals to sublimate, that is, to turn directly from solid to gas. We used a Varrox® M3080 sublimator powered by a 12-volt lead acid battery.

The doses followed existing methods. In all three methods we used doses of 0.56, 1.125, and 2.25 grams per hive. For sublimation we also used a dose of 4.45 grams. In total, there were 10 treatment groups and one control group.

To eliminate any bias due to possible apiary ef-
fects, there was one hive per group in each of the 10 apiaries. The winter weather was quite cold for England, maximum 5°C, on the day of oxalic acid application with an average maximum of 3°C over the following 10 days. It is recommended to apply oxalic acid at temperatures of 4-16°C.

To determine Varroa mortality we took two samples of worker bees (mean = 267 bees per sample) from each colony. The first was taken just before oxalic acid treatment and the second 10 days later, when the mortality caused by the oxalic acid was over but before any capped brood was present. The samples were frozen and analysed later.

The dead bees were placed into a double-mesh honey strainer. A jet of warm water from a hose nozzle was used to wash the Varroa off the bees. The Varroa passed through the first mesh and were trapped in the second, finer, mesh. We had previously checked this method, examining washed bees under a microscope, and had found that it extracted all the Varroa. We then counted the Varroa and bees from each sample. If, for example, the first sample had 10 mites per 100 bees and the second had 0.5 mites per 100 bees, then the mortality was (10 – 0.5)/10 = 0.95 = 95%.

We also monitored the fall of honey bees and mites for eight days before and 10 days after oxalic acid application, the survival and strength of colonies in spring (four months after application), and if they had a queen.

**Results**

**Initial Varroa levels**

In the samples of bees collected immediately before the first oxalic acid treatment, the average level of Varroa was 9.8 per 100 bees, range two to 29, across the 110 hives. This is quite a high level and meant that we had plenty of Varroa to study, and to ensure adequate data for statistical analyses.

**Varroa mortality**

Figure 1 shows that all methods gave high

<table>
<thead>
<tr>
<th>Application Method &amp; Dose of Oxalic Acid (g)</th>
<th>0.56</th>
<th>1.125</th>
<th>2.25</th>
<th>4.5g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trickling</td>
<td>50.4</td>
<td>50.6</td>
<td>51.3</td>
<td>51.3</td>
</tr>
<tr>
<td>Spraying</td>
<td>24.9</td>
<td>23.2</td>
<td>19.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Sublimation</td>
<td>81.7</td>
<td>85.7</td>
<td>85.1</td>
<td>86.2</td>
</tr>
</tbody>
</table>

Figure 1. Varroa mortality as determined by extracting phoretic mites from samples of worker bees taken immediately before and 10 days after oxalic acid treatment. Figures 1-4 are based on those from Toufailia et al. 2015. Journal of Apicultural Research. Vol 54(2), are copyright of the International Bee Research Association, and are reproduced by permission of the editors of the Journal of Apicultural Research. The original article is available open access at http://dx.doi.org/10.1080/00218839.2015.1106777.
Bee mortality at the time of application
The number of bees falling onto the bottom board and into the dead bee trap did not increase after application for sublimation, but did increase for trickling and spraying (Figure 2). In particular, spraying with the highest dose, 2.25g per hive, resulted in a 10-fold increase in the number of dead bees per day. However, as the hives contained five to 10 thousand bees, even this method killed only 1-2% of the bees at or soon after application.

Colony mortality after four months
Figure 3 shows the number of hives out of 10 that survived until 3 May 2013, and if they were queenright. Of the 10 untreated control hives, eight (80%) had survived. Of the hives treated with oxalic acid, survival was: sublimation, 38/40 = 95%; trickling, 25/30 = 83%; spraying, 19/30, 63%. Sublimation gave the highest survival, and was significantly better than spraying. Of the 10 hives treated with the highest dose of oxalic acid by spraying, 2.25g, only 4/10 survived.

Colony strength after four months
The surviving control colonies had an average of 4.1 frames with brood (counting 0.5 per side with brood present) (Figure 4). This was slightly higher than in hives treated with oxalic acid via trickling (3.6-3.9) or spraying (3.3-3.7). However, the hives treated via sublimation had significantly more brood (4.4-5.0 frames), and was an average of 21% more than the control hives for the three highest doses (1.125, 2.25, 4.5 g).

Checking the Results
Based on the results above from year one, we concluded that we could advise beekeepers that the best method was to treat hives with 2.25g of oxalic acid via sublimation. Although 1.125g via sublimation also gave high Varroa mortality, the highest dose, 4.45g, did not cause any harm to the bees or colonies. Therefore, it seemed reasonable to recommend the middle dose to allow a margin for error, for example in case a colony had more or less bees than average and so received a larger or smaller dose per bee.

However, we first wanted to double check our results. Therefore, in mid-December 2013 we treated 89 broodless hives with 2.25g of oxalic acid via sublimation, using the same methods.
as before. The average Varroa level per hive was 14.5 per 100 bees. Most hives, 87 (98%), survived until spring. Varroa mortality was 97.6%. This convinced us that we could recommend 2.25g via sublimation, and be confident that this dose would kill most of the Varroa without harming the colony.

Sublimation Equipment. Top left – Varrox sublimator, 12 volts and 150 watts. Lower left – Close up of the heated cup at the end of the sublimator into which the oxalic acid crystals are placed. Top right – Lead-acid battery, 115 amp hours, capable of powering the sublimator for approximately nine hours. Lower right – Half teaspoon measures are cheap to buy and are a convenient way of dispensing oxalic acid crystals for sublimation as half a teaspoon, 2.5ml, of oxalic acid crystals is almost exactly 2.25g.

Conclusions
Our conclusions are simple. The sublimation method is the best in all respects. It results in Varroa mortality that is as high as the trickling and spraying methods, but at lower oxalic acid doses. It gives the highest colony survival four months later, in spring, and results in colonies with significantly more brood than untreated control colonies or colonies treated by trickling or spraying.

Based on these results we recommend that beekeepers do not use the trickling or spraying methods. In particular, the spraying method harms colonies and results in significantly lower colony survival over the next four months compared to sublimation. A bonus of the sublimation method is that the hive does not need to be opened for application, and it is generally the quickest method, taking about three minutes per hive. Most of the time is taken waiting for all the oxalic acid to sublimate, which takes a few minutes for 2.25g.

Our results showed that colonies treated via sublimation had more brood in spring than control colonies or colonies treated via trickling or spraying. We do not know why. However, a likely reason is that sublimation-treated colonies were healthier than control colonies, as most of the Varroa had been killed, and so built up faster.

There was quite a high level of Varroa before treatment, 9.8 per 100 bees on average, and these would not have been killed in the control hives. Although trickling and spraying also kill Varroa, the harm they do could have cancelled out the benefit of killing the Varroa. Oxalic acid treatment via sublimation requires the beekeeper to buy or borrow an applicator. Most are heated electrically and need a 12-volt supply. We used a 12-volt, 115 amp hour lead-acid “leisure” battery, of the type used in a caravan and effectively the same as a normal car or truck battery.

The Varrox® M3080 sublimator we used was rated at 150 watts, meaning that it draws a current of 150/12 = 12.5 amps. A fully charged 115 amp hour battery would be able to power the applicator for 115/12.5 = 9.2 hours. At three minutes per hive this would be enough to treat up to (60/3) x 9.2 = 184 hives.

In other words, a large battery would be enough for a full day’s work treating hives. Although we did not try it, a petrol generator could also be used. However, it would seem to be less convenient than a battery and the noise and vibration might annoy the bees.

Oxalic acid is a natural chemical, and is found in honey and in many vegetables. Carrots contain 0.5g oxalic acid per 100g (information from Wikipedia). Therefore, a 1lb (= 0.45kg) bag of carrots would contain 2.25g oxalic acid, or enough to treat one hive. The lethal dose for humans is 0.6g per kg (information from Wikipedia) meaning that a beekeeper weighing 165 pounds (75kg) would need to swallow
45g (enough to treat 20 hives) to have a 50% chance of dying.

Oxalic acid is harmful to the eyes and mucous membranes. It is important not to breathe in oxalic acid, both the powder and the fumes. We found that it took only a few seconds to place the oxalic acid crystals into the applicator cup and to insert the applicator into the hive. As a result, we found that even if the applicator was already under power and hot it could be inserted into the hive before oxalic acid fumes were produced, so that all the fumes were confined to the hive.

We temporarily sealed the hive entrance using pieces of foam so that the vapour was confined within the hive. It is recommended to keep the entrance sealed for up to 10-15 minutes after the applicator is removed. As we applied oxalic acid in winter on cool days, there was no foraging activity to disrupt by temporarily closing the entrance.

The administrative position of using oxalic acid to treat hives to control Varroa varies from country to country. In Britain, a registered oxalic acid product, Api-bioxal, was approved in 2015 by the Veterinary Medicines Directorate. Api-bioxal consists of oxalic acid dihydrate (88.6% by weight), plus small amounts of silica gel and glucose. The latter materials would seem not to be toxic to Varroa. The official UK recommendation, for sublimation, is to use 2.3g to treat one hive. This corresponds to 2.3 x 0.886 = 2.04 g of active ingredient. Our research used almost exactly this amount.

The British VMD approval document states that a protective mask conforming to European Standard EN149 (type FFP2) must be used when handling oxalic acid. In the U.S., the EPA (Environmental Protection Agency) approved the use of oxalic acid to control Varroa in March 2015. The EPA stated that “In addition to the standard beekeeping suit (veil, long-sleeved shirt, long pants and gloves) as personal protective equipment, a respirator and goggles are required.”

It is possible to obtain a wide variety of masks with different levels of face protection and a range of filters. Some filters that conform to the required European standard, which applies specifically to dust inhalation, also provide protection against organic chemicals and acids. If using the sublimation method, it would seem sensible to use such a dual-purpose filter in order to provide full protection during both the handling of the oxalic crystals and the sublimation. U.S. beekeepers might test some of the masks available in the USA that conform to these standards and report back on how they compare.

Because oxalic acid only kills phoretic mites, for maximum effectiveness it is necessary to treat broodless hives. By means of hive inspections in late autumn and winter, beekeepers can determine when the natural minimum brood period occurs in their area.

However, brood rearing may vary year by year. In the winter of 2015/6 we found that brood rearing in Sussex continued longer into December than usual, and resulted in our delaying oxalic acid treatment until January and having a lower proportion of broodless hives. This was probably due to the very mild autumn, with weather warm enough for foraging throughout December, plus prolonged flowering of ivy, the main autumn flower source, into early December.
What we do is check hives immediately or a few days before oxalic acid application and scrape out any small patches of capped brood. Although it is extra work, it is worth doing as even small areas of capped brood will allow many adult female Varroa to escape the oxalic acid. Our results show 97% Varroa mortality apply to broodless hives.

The U.S. is a large country with many different climates, and beekeepers in different regions may need to work out the best method and time of applying oxalic acid to broodless hives. In areas with warm winters there may be no full natural winter break in brood rearing, and in northern and mountain areas it may be too cold in winter to open hives.

Package bees provide a very good opportunity for applying oxalic acid, as the colony will not have capped brood for approximately eight days after hiving. Beekeepers are practical people and can figure out suitable methods.

The EPA document states, “The solution method and the vaporized applications are made in the late fall to early spring, when little brood is present.” But even a little brood can protect a lot of Varroa from the oxalic acid, as many Varroa will be breeding in capped cells. At LASI, we determined the amounts of capped brood in hives in different months and the proportion of Varroa in brood cells. This varies from c. 70% to 10% in brood cells, in total, with the minimum occurring in December.

How useful is killing, say, 50% or 75% of the Varroa versus the 97% kill that can be achieved using oxalic acid in a broodless colony? It may seem that these kills are half or three-quarters as good as 97%. However, when we look at the surviving proportions of Varroa, 50% and 75% versus 3%, it is clear that a 97% kill in broodless hives is much more effective than a 75-80% kill in hives with small patches of capped brood.

After a 97% kill, the Varroa population would have to double slightly more than five times (3 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 96) to get back to where it was. For a 75% kill it only has to double twice, and for a 50% kill just once.

(The populations of all living organisms have the ability to grow in this “geometric” manner, 2-4-8-16-32 etc., when not overcrowded.)

At LASI, we carried out a study in which we determined Varroa populations in 42 hives at an interval of one year (Al Toufailia et al. 2014). In that year, the Varroa populations increased on average by 40 times per hive, equivalent to slightly more than five doublings. This means that killing 75% of the Varroa in a colony slows the population by the equivalent of approximately two-fifths of a year and killing 50% by just one fifth of a year. By contrast, killing 97% was the equivalent to the control of a year’s Varroa population growth.

It is good to have results worth sharing with beekeepers, especially as the Sussex Plan for Honey Bee Health & Well Being is aimed at providing practical information. When we started our research on Varroa control, we never imagined that we would be able to make such a clear and simple recommendation. That is, to have solid evidence that one application method was the best in all respects: killing Varroa, not harming the bees, resulting in stronger colonies, and in being quick and easy to apply. That method is sublimation.

It is fortuitous that we are publishing our results soon after oxalic acid has been approved for use to control Varroa in both the U.K. and U.S. Although the EPA has approved the use of oxalic acid via trickling, spraying, and sublimation, sublimation is the best.

Now, compare these two treatments for Varroa with different chemicals.
FORMIC ACID VERSUS OXALIC ACID
Two methods for treating Varroa mites (Varroa destructor) were tested during this study. One method of treatment was vaporized oxalic acid applied once per week for three weeks. This method did prove beneficial in reducing mite loads in honey bee (Apis mellifera) colonies.

The second method of treatment was the use of one formic acid pad, MiteAWay Quick Strip (MAQS). The use of formic acid slightly reduced the mite load in the tested colonies.

While there are a number of available treatment options, not all treatment options are recommended in all temperature situations. Formic acid, one of the more commonly used mite treatments, has limited usefulness in the climate in which this comparison was performed. Summer temperatures in geographic location Zone 8 exceed the manufacturer's high temperature limit. Another commonly used method of treating Varroa mites is oxalic acid, which has the extra benefit of no temperature restrictions.

Location and Conditions
Our sample study to compare the effectiveness of formic acid with oxalic acid in hives with brood present consisted of 90 hives. These hives were located in three different yards with 30 hives in each yard. Within each yard 10 hives were designated as control hives, 10 hives were treated with formic acid and 10 hives were treated with oxalic acid. The hives were numbered, labeled and grouped by treatment method.

In an attempt to control variables and conditions to the extent possible, all three yards were located in Dooly County, Georgia, on similar sites. The yards were within a radius of five miles and separated by at least two miles. As the study began each yard was experiencing a good nectar flow and had easy access to water. All hives were 10-frame, Langstroth hives with bottom screen boards. The yards and groups were balanced as to hive size and sun exposure.

Materials and Methods
Control Hives were left untreated.

Hives in the formic acid group were treated with MAQS by placing one strip inside the hive on frames containing brood where it remained for one week before being removed. The use of one strip per hive was a deviation from the manufacturer's recommended product use of two strips per hive. This approach to treatment was based on conversations with beekeeping professionals coupled with articles such as the one written by Robyn M. Underwood, and Robert W Currie appearing in the Journal of Economic Entomology in December 2005 where they conclude formic acid in “high concentrations can cause queen mortality.”
Hives in the oxalic acid group were treated in seven-day increments for a total of three times by vaporizing two grams of product (1/4 tsp) into each hive in the group for three minutes. A commercial vaporizer was used to treat four hives at a time.

Samples were taken from each hive on April 2 before treatment had begun, on April 23 after completion of all treatments and on May 16 (the third sampling). The samples were placed in an alcohol wash in jars labeled by hive and group. The resulting mite load per hundred was noted on the graph and spread sheet.

**Results and Observations**

The results of the three samplings areas follows:

All hives experienced consistent colony growth and nectar collection during the course of this study.

The **control group** was not treated and saw a steady increase in mites from the first to second sampling. The mite count dropped some from the second to the third sampling. The decrease from the second to the third sampling could be because some of the more heavily mite load-ed hives swarmed and this took their larger mite load numbers out of the count.

The **formic acid** treatments (MAQS) were applied once and left on the hive for one week. The total mite count for the group increased from 76.8 mites to 90.4 mites from the first mite sampling to the second. The third sampling showed the mites count decreased to 72.9 mites. This decrease might be due to the mite count already being fairly low at the beginning of the study. The experienced mite drop with this treatment was not consistent with expected results. R. W. Currie and P. Gatien 2006 said that “formic acid provided consistent control of V. destructor in spring applications.”

The application of MAQS strips only takes about two minutes per hive and another one minute to remove the strip the next week. However, the cost of formic acid using MAQS is significantly more than that of two grams of oxalic acid.

It is also worth noting that, even though the treatment used was one half of the manufacturer’s recommendation, this group experienced more hive loss than either of the other groups, suggesting that formic acid is a factor in queen mortality.

The **oxalic acid** treatment was applied for three weeks and resulted in a reduction in the mite count from 4.06 average mites per hive to 2.24 average mites per hive for the group. The reduction of mites is consistent with previous studies by Rademacher 2006. The time required to treat each hive with oxalic acid is greater than with MAQS. The oxalic acid application takes approximately seven plus minutes for four hives. Although the cost of the actual oxalic acid is minimal per hive, the application apparatus requires a significant investment of approximately $600.00 for the four vaporizers.

**Conclusion**

Two conclusions can be drawn from this study:

The oxalic acid applied three weeks in a row is an effective treatment for reducing mite load. The hives treated with oxalic acid saw a significant drop in mite count.

The formic acid treatment was not dramatically effective as a mite treatment at the dosage of one strip at this time of the year. An additional study would be needed during the heat of the summer month at this same dosage, which could show improved results because of the heat increasing the speed of the formic acid release. At lower temperatures such as experienced during this testing period, two strips may have improved the mite drop.

**WHEN USED RIGHT, FORMIC ACID IS SUCCESSFUL**

Every year in early spring, beekeepers inspect their hives to find out if and how their bees have made it through the winter (Figure 1).
And every year, many of these beekeepers are in for a rather unpleasant surprise when they discover that a number of their colonies have not survived the cold season.

Varroa destructor remains one of the most significant causes of these colony losses, especially when the winter is exceptionally mild. Keeping your mite counts in check in late summer and treating your bees with MAQS® at the right time can prevent these losses.

Here’s why the late season Varroa treatment is the most important treatment in the year, and why formic acid is the weapon of choice against Varroa.

Honey bees (Apis mellifera) are fascinating insects in many different ways, but to me, one of the most interesting aspects of Honey bee life is the difference between summer bees and winter bees. Worker bees that hatch in spring or summer have an average life expectancy of 30-40 days, whereas their sisters born in September or October are built to remain alive during the broodless period and throughout the winter until the following spring.

When the cooler weather arrives along with shorter days, the queen slows and eventually stops laying eggs completely. The colony shrinks from 20,000-60,000 bees in July to a modest 5,000 bees that stay in the hive during the cool season. These workers all attend to the queen, feeding her and keeping her warm until the following spring.

This overwintering process of Honey bee colonies has become much more vulnerable since the Varroa mite (Varroa destructor) began parasitizing colonies more than two decades ago. Research studies from Canada and Germany conclude that Varroa mites are the main cause of colony loss and severely reduced colony strength in northern climates.

To understand why Varroa pose such a threat to Honey bee colonies in the overwintering phase, let’s look at the correlation between Honey bee and Varroa mite populations over a full beekeeping year.

The first thing we will notice is that the Honey bee population and the Varroa population are not in synch.

The graph shows how the bee population starts to build up slowly in late winter, then the build-up gains more speed in spring until it peaks in June and July (Figure 2). In July, the number of workers begins to decline, and, depending on the climate the queen slows and eventually stops laying eggs altogether in November/December. The Varroa population lags behind the number of worker bees in the hive, because mites need the bee brood to reproduce.

If the previous year’s treatment for Varroa was successful and the brood break sufficient, Varroa counts should be low. As the bees are expanding their brood cluster, the Varroa populations grow too. Mites then have access to a dramatic increase in worker and drone brood cells for reproduction, and a steady supply of Honey bee haemolymph, or bee blood, a Varroa staple.

But there is one significant difference between the seasonal development of bee population and that of the Varroa population: The mites reproduce exponentially – as long as there is brood in the hive. More specifically, the Varroa population doubles around every three weeks between springtime and the onset of the broodless period.

With such a sharp increase in the Varroa population over the season, you might ask yourself...
how to realistically stay below the threshold of serious colony damage until August or September. The answer is drone brood removal throughout the season, and treatment during or in between honey flows. MAQS® Beehive Strips are an authorized Varroa treatment during honey flow.

If you choose not to treat during honey flow, apply MAQS® in between two flows. Just make sure that your colonies have enough access to fresh air during the seven-day treatment period by fully opening your entrances, and pay attention to the required maximum daytime temperature range (between 10°C and 29.5°C).

But what about the late summer treatment between late July and early October? The exact timing of the treatment depends on various factors such as the Varroa levels in your hives, the length of the season and the weather conditions. To determine when you should treat in your zone, consult the Varroa calculator, a helpful tool provided by the National Bee Unit.

With a late summer or early autumn treatment, you want to eliminate as many Varroa as possible to protect your winter bees. Remember, they have a (relatively) long bee life ahead of them, and hand over the colony to the next generation of summer bees the following spring. Looking at our graph again, we can see that the mite population increases even when the Honey bee population decreases. This means more Varroa for fewer bees.

A high Varroa load during the cold season will also increase the virus load in the Honey bee colony. This can lead to a shorter lifespan for winter bees, reflected in pitiful springtime finds by the beekeeper of small groups of leftover bees in the hive, or complete colony losses.

Formic acid, the active ingredient of MAQS®, is the best candidate for Varroa treatment in late summer. First and foremost, it is the only available substance that targets mites under the brood cap where they reproduce. Second, mite resistance against Formic Acid is presently unknown and also quite unlikely.

And finally, the formic acid in MAQS Beehive Strips is an organic acid that is not soluble in wax or honey, and vaporizes over time. MAQS® combines all of these advantages in a smart and easy-to-use dosage form, which offers a safe and much shorter treatment period compared to treatments with liquid formic acid.

In early October 2009, the Ontario Beekeepers’ Association tested the long-term efficacy of MAQS and compared it to the efficacy of Apistan. In this trial, colonies treated in late summer with the full dosage of MAQS® (two strips per hive) had a mean mite count of 1.3 per 100 bees the following April. In those hives treated with the half dosage (one strip per hive), 2.9 mites were found on a sample of 100 bees. The Apistan-treated hives showed an infection rate of five mites per 100 bees (Figure 311).
Pre-treatment infection levels for all three groups in this trial were above 20 Varroa per 100 bees. Moreover, the group of colonies treated with the full MAQS® dosage was also the group with the lowest winter mortality (9%), whereas 42% of the Apistan-treated control group were reduced to drone laying workers, or had died off completely.

This test shows clearly how important the late summer or early autumn treatment is for the health and survival of your colonies over the winter. To treat against Varroa successfully, it is crucial that you also monitor the treatment success after the treatment has been finalized. To see the full efficacy of MAQS® under the brood cap, we advise to check the treatment success after day 21 (three weeks after the application of the strips). At this time, all bees that were still under the cap during treatment will have emerged, and the dead Varroa from their brood cells will have fallen.

If your colonies have suffered from a particularly high mite load before the treatment and you want to repeat it, you should wait at least a month between your MAQS® applications.

Lastly, while the Varroa mite has been found in Australia, it is not the Varroa destructor, but the Varroa jacobsoni.

**VARROA DESTRUCTOR NOT IN AUSTRALIA**

Australia imposed a regional quarantine after Varroa mites were found in northern Queensland and they may have been there for years.

Confirmation from the federal and Queensland governments and industry sources that the two mites in a feral Asian honey bee hive in Townsville, 830 miles north of Brisbane, were jacobsoni means Australia has again dodged the Varroa bullet.

Varroa jacobsoni parasitizes Asian honey bees (Apis cerana). The more damaging Varroa destructor, only identified as a separate species in 2000, is a parasitic mite that attacks both Apis cerana and Apis mellifera.

In an update, Biosecurity Queensland Chief Plant Health Manager Mike Ashton says the Commonwealth Scientific and Industrial Research Organization (CSIRO) has confirmed the mites were Varroa jacobsoni.

“Asian honey bees are the natural host of this species of Varroa mite,” he said in a statement. “However, a recent report by the CSIRO has shown for the first time this species reproducing on European honey bee and it is this strain that we are most concerned about. This strain is known to be widespread in Papua New Guinea.

“Asian honey bee is not known to be established in Townsville and to date, no further feral Asian honey bee hives have been found in the area where this hive was located and then destroyed.”
Ashton says Biosecurity Queensland’s quarantine and surveillance program includes surveillance of managed and feral hives, and the setting of traps to attract bees to check for the presence of Varroa mite.

“We are keen to examine a number of managed hives in the Townsville area to ensure they are not infested with Varroa mite,” he said. “We are also asking the public to report feral hives so they can be sampled and destroyed to prevent any spread of the mite.”

The Brisbane Department of Agriculture said that based on expert advice, the 5,000-strong colony of Asian honey bees where the Varroa mites were found was potentially up to two years old.

“During this time the bees could have swarmed and spread from the site of detection,” a quarantine order signed by department Director General Elizabeth Woods stated.

Australian Honey Bee Industry Council Executive Director Trevor Weatherhead posted a statement he said had been approved for circulation to all industry members.

“Scientific analysis of the bees has confirmed that two Varroa mites (Varroa jacobsoni) were present on two of the bees,” it says.

“A check of the surrounding area has found no further Asian honey bees or their hives,” it said. “Traps and sentinel hives that are already in place around the port as part of the National Bee Pest Surveillance Program have not collected any exotic bees or mite pests over the past two years.”

The council statement said testing will be done to see if these bees have any relationship to the Asian honey bee that is already present in areas of Far North Queensland, or bees that were associated with previous detections at the Townsville port – with the last detection in 2014.

“While Asian honey bees are established in areas of Far North Queensland, Varroa mites are not known to be present in that population,” the statement said.

The national Consultative Committee on Emergency Plant Pests met July 1 to confirm the identification of the pests, and discuss the required response activities for Varroa jacobsoni.

It has previously been determined that Asian honey bees cannot be eradicated from Australia, so response activities are only focused on the Varroa mites.

“Australia has well-established arrangements in place for responding to exotic pests, such as Varroa,” the council said. “This is a nationally significant pest that will see all efforts put in place to prevent it from establishing in Australia.”

Australia is, for now, the only major honey-producing country where the bee-killing Varroa has not established itself.

Woods said the Asian honey bee hive was detected in the Port of Townsville on June 27 and was immediately destroyed.

The dead bees and comb were sent to the department’s Operational Science Program in Brisbane, which found Varroa.

Varroa jacobsoni was then confirmed morphologically three days later by the Commonwealth Scientific and Industrial Research Organization in Canberra.

Woods says surveillance work since June 27 has not resulted in further detections of Asian honey bees in the Townsville area.

The quarantine, or movement control order as the state government calls it, is intended to assist in the eradication of Varroa by restricting the movement of mite carriers and allow surveillance and control measures.

“I consider it necessary to make a movement control order as I am satisfied, on reasonable grounds, that Varroa mite (Varroa jacobsoni) poses a serious biosecurity risk,” Woods said in the order.
The quarantine order covers 6.2 miles (10 kilometers) around the Port of Townsville.

Under the order, a person must not move a Varroa mite carrier out of the area without a biosecurity permit. People within the movement control area must allow an inspector or a person under the direction of an inspector to inspect or test any Varroa mite or a Varroa mite carrier, to treat or destroy any Varroa mite or Varroa mite carrier and to clean or disinfect any place, including any structure or thing at a place.

The movement control order will stay in effect for three months unless earlier revoked.

Meantime, the Australian Vegetable and Potato Growers Federation said with Varroa a nationally significant pest, all efforts will be put in place to prevent it from establishing in Australia.

“Professional and amateur beekeepers need to be vigilant in inspecting their hives for signs of Varroa mite and other exotic pests,” Jessica Lye, federation scientific affairs national manager, said. “It is vital that any suspected sightings of these pests are reported to state or territory’s department of agriculture or biosecurity early so that all measures can be taken to ensure they are dealt with in a timely and effective manner.”

The federation statement quoted Weatherhead as saying while there are no expected domestic or international trade issues at this time, it is important that all industry stakeholders work together to ensure rigorous surveillance of Varroa is maintained.

“It is currently believed that these forms of Varroa mites do not readily transfer between host species – that is, if the mite is found on Asian honey bees, it does not readily move to European honey bees,” Weatherhead said.

To see the original articles online, please use the links below.

Varroa Mites Pick The Best Bees To Bite: from Michigan State University
Varroa Mite Reproduction: by Clarence Collison
When Varroa Mites Hitch A Ride: by Kathy Keatley Garvey
Varroa Mite Orientation: by Clarence Collison
Varroa And Deformed Wing Virus: by Ashley P. Taylor
The Honey Bee Health Coalition’s Guide To Controlling Varroa: from the Honey Bee Health Coalition
Breeding Mite Biting Bees: by Greg Hunt, J Krispn Given, Jennifer M. Tsuruda, Gladys K. Andino
Splits & Varroa: More colonies, fewer mites, new queens – what could be better? by William Hesbach
Oxalic Acid Registered For Varroa
Using Oxalic Acid: By Tony Jadczak
Sublimation – The Best Way To Kill Varroa: by Frances L.W. Ratnieks, Luciano Scandian, Hasan Al Toufailia
Comparing Mite Treatments: Formic Acid and Oxalic Acid: by Shearer Turton
Winter Bees And Formic Acid: by Ulrike Lampe
Australian Varroa find is jacobsoni, not destructor: by Alan Harman

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