Keeping bees healthy requires beekeepers to address mite problems through management techniques such as breeding, colony manipulation, or treatment with miticides. While breeding and manipulation are the first and most important lines of defense, many beekeepers still need to use chemical treatments to keep their colonies alive. No beekeeper enjoys applying chemicals to their hive because it is expensive, time consuming, and may adversely affect bees. However, many beekeepers apply miticides once or twice a year to reduce mite infestation, often without first checking to see if the mite level is high enough to warrant treatment. Reducing treatments to only those necessary is imperative to keeping costs down, reducing hive treatments to only those necessary is important lines of defense, many beekeepers still need to use chemical treatments to keep their colonies alive. No beekeeper enjoys applying chemicals to their hive because it is expensive, time consuming, and may adversely affect bees. However, many beekeepers apply miticides once or twice a year to reduce mite infestation, often without first checking to see if the mite level is high enough to warrant treatment. Reducing treatments to only those necessary is imperative to keeping costs down, reducing hive contamination, and slowing the development of mite resistance to new miticides.

Here we describe an efficient sampling method for Varroa. This method will allow beekeepers to make treatment decisions based on knowledge of actual infestation levels of mites on adult bees and worker brood in an individual colony or entire apiary. Many sampling methods have been developed previously and include dislodging the mites from adult bees with alcohol, powdered sugar, or ether; monitoring the natural mite fall with a sticky board; or sampling drone brood or examining individual worker pupae. These methods have typically been used to determine if mites are present or absent. If these methods are used to quantify mite levels, it is often unclear how the number of mites in the sample translates into actual numbers of mites in a colony or apiary.

To develop an easy and standardized way of sampling adult bees for mites, we addressed two questions. First, what is the best method of sampling adult bees to determine mite infestation? Second, can the colony infestation level (i.e., mites on both adult bees and pupae) be estimated from a sample of adult bees? We chose to focus on sampling adult bees because sampling worker brood is cumbersome, sampling drone brood has wide variability, and the use of sticky boards requires two trips to the apiary, special equipment, and at least several days to get a good estimate of natural mite fall. In this article, we describe a sampling plan for beekeepers. A method suitable for researchers, with detailed sampling statistics, is published online in the Journal of Economic Entomology.

**Sampling to Determine Mite Infestation of Adult Bees**

To determine the most efficient way for beekeepers to sample adult bees for Varroa, we needed to understand the distribution of mites within a colony and an apiary. To obtain these data, we sampled a total of 954 colonies in 31 apiaries owned by five commercial migratory beekeepers. The operations were sampled in Minnesota, North Dakota, California, and Texas. The sizes of the five operations ranged from 1,000 to 20,000 colonies, and the number of colonies at sampled apiaries ranged from 24 to 84. Sampling was done in March (TX and CA) in 2006, May-June and August-September (MN and ND) in 2005, 2006, and 2007. We collected approximately 35 adult bees in alcohol from each frame in each colony and recorded the following information for each sample: date, beekeeper, yard, pallet, colony, frame location, and comb contents (e.g., open brood, sealed brood, pollen, nectar/honey, or empty). The 35-bee samples were taken back to lab, where we counted number of bees and mites in each. In 142 of the colonies, one sample of approximately 300 adult bees was taken to compare the mite infestation to the multiple 35-bee samples. We found no difference between the infestation of the large sample and the combined 35-bee samples from the same colony, indicating a single large sample is adequate to estimate adult bee infestation.

Using data from the 954 commercial colonies, we wanted to know how many adult bees needed to be sampled to accurately estimate mite infestation on all adult bees? We found that a sample size of 300 bees per colony is adequate to determine the mite infestation level of adult bees in a colony. The recommendation of sampling 300 bees confirms previous recommendations, but this is the first time this number of bees has been associated with a precision level.

To develop an apiary level sampling plan, we used a computer program to determine the number of adult bees in a colony and colonies in an apiary to sample. We first combined different numbers of the 35-bee samples within each colony to achieve different sample-unit sizes (i.e., bees to sample per colony, ranging from 35 bees to 280 bees). To estimate the infestation in an apiary, the computer program randomly selects the inputted sample-unit sizes for each apiary until it reaches the set precision for the number of colonies per apiary to sample. Thus, if 35 bees are sampled per colony, 16 colonies would need to be sampled in an apiary, and if 280 bees are sampled, only 8 colonies would need to be sampled. Since it is easier to sample more bees per colony than fewer bees from more colonies, we recommend sampling 300 bees from each of 8 colonies to estimate apiary infestation. We chose 300 bees (rather than 280) to err on the side of obtaining a better estimation.
We next determined if mites congregated on brood frames (frames with eggs, larvae, or sealed pupal cells). We found that mites on brood had significantly more mites than non-brood frames, with 2.4 mites per 100 bees on frames with brood comb and 1.8 per 100 bees on frames without brood. We recommend beekeepers sample from a frame with brood.

Then, we wanted to examine how mites are spatially distributed among colonies in an apiary. We were not surprised to find that some colonies had higher mite levels than others. However, we wanted to know if direction of the hive entrance or location in an apiary (e.g. colonies on pallets at the end of a row in an apiary compared to colonies in the middle) contributed to higher mite loads. Our analyses suggested that mite levels were independent of colony direction or location.

Mite levels were sometimes highly variable among apiaries in beekeeper operations sampled at the same time of year. This means that beekeepers should make treatment decisions on an apiary-by-apiary basis and should not assume that all apiaries have similar levels of mite infestations.

**Relationship Between Mites on Adult Bees and Mites in Brood**

We developed a simple “correction factor” to account for the proportion of total mites in the colony that are on pupae (i.e. sealed brood) by intensively sampling brood in 62 colonies from two commercial beekeepers in MN and ND. The colonies were sampled in May-June or August-September. These are times of year when many beekeepers normally treat for *Varroa*. In each colony, we estimated the population of adult bees and sealed worker brood, and the mite infestation of adult bees and sealed worker brood. We also estimated the number of drone pupae and mite infestation on drone pupae in seven intensely sampled University of Minnesota colonies.

Drones were not included in the correction factor because the number of mites in drone brood was dwarfed by the number of mites found on adult bees or in worker pupae. In this study, an average colony had 24,500 adult bees and 14,000 worker pupae. Drone brood comprised, on average, only 3.2% of the total pupae. An average 6.8% of all mites were on drone pupae, while an average of 45.6% were on worker pupae, and the remaining 47.7% were on adult bees. These results suggest that unless there is an abnormally high amount of drone brood in the colony, the number of mites in drone brood contribute little to the total number of mites in the colony.

We examined the relationship between the adult bee infestation and the colony infestation (density of mites on adult bees and worker pupae). We included two factors that we predicted would influence the relationship: the time of year the colony was sampled and the ratio of worker pupae to adult bees. Although both factors can affect the relationship between adult bee infestation and colony infestation, the statistics indicated only adult bees need to be sampled, and a correction factor applied, to estimate the total colony mite density. We calculated this correction factor by plotting the adult bee infestation against colony infestation (mites on adults and in brood) to find the slope of the line, which was 1.8. Thus, the number of mites on adult bees can be multiplied by 1.8 to correct for the number of mites in worker brood. To simplify and err conservatively on the side of over-estimation, we recommend using a correction factor of 2, or doubling the adult bee infestation level to estimate the mite infestation in a colony. If there is no brood, then no correction factor is needed. If there is an abnormally high amount of worker or drone brood relative to adult bees, there is a possibility the correction factor could lead to an underestimate of total mite load.

**Sampling Plan Recommendations for Beekeepers**

Based on our results, we provide the following recommendations for beekeepers to estimate the mite infestation level:

1. **Colony**
   - Sample 300 adult bees from one frame containing brood (i.e. eggs, larvae or pupae).
2. **Use Table 1** to apply the correction factor to convert the number of mites on adult bees to the colony infestation level (i.e. total mites on adult worker bees and in worker pupae). Or divide the number of mites found in a sample of 300 bees by 3 and multiply the result by 2 to estimate colony infestation level.

**How to Sample Adult Bees**

Counting out 300 bees for each sample is impractical, but there are a few ways to sample by volume since 300 live bees occupy about 0.42 cups or 100 ml. We realize that 0.42 cups of bees is a strange volume, however bees are small so small variations in the volume can mean large variations in the number of bees in a sample. For example, 1/3 cup averages just under 200 bees, 0.4 cups is about 275 bees, and 1/2 cup is just under 400 bees. It is important to accurately measure out the correct volume to sample 300 bees.

To make your own measuring cup, add 0.42 cups of water (1/3 cup + 1 tablespoon + 1 ¼ teaspoon) to a cup that preferably has a smaller diameter relative to height, and make a mark at the water line. Add a handle to the cup to make sampling easier. To sample, rap bees off of a brood frame into a 5 gallon bucket or plastic wash-dish container.

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### Table 1. Number of mites found in a sample of 300 adult bees and the corresponding colony mite density after the correction factor is applied.

<table>
<thead>
<tr>
<th>Apiary</th>
<th>Colony infestation</th>
<th>#Mites per 8 300 adult bee samples</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1%</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>1%</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>2%</td>
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<td>3%</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>4%</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
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<tr>
<td>8</td>
<td>5%</td>
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<td>9</td>
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<td>72</td>
</tr>
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<td>10</td>
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<td>88</td>
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</tr>
<tr>
<td>15</td>
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<tr>
<td>16</td>
<td>11%</td>
<td>128</td>
</tr>
<tr>
<td>17</td>
<td>11%</td>
<td>136</td>
</tr>
<tr>
<td>18</td>
<td>12%</td>
<td>144</td>
</tr>
</tbody>
</table>

**American Bee Journal**
then use the marked cup to scoop out 300 bees (Figure 1). Rap the cup on a hard surface to make sure the bees are at the 0.42 cup line (add or subtract bees as needed). Keep the bucket or wash-dish from becoming coated in nectar, since mites can stick to the nectar. If your cup is rectangular (such as those that come in some powdered laundry detergent boxes), then you can use the marked cup by running it gently over the backs of bees, causing them to tumble down into the cup (Figure 2). Again, be sure to measure the bees at the 0.42 line. One further sampling method is to use a device called “Gizmo” that was designed by Gary Reuter to measure 300 bees (Figure 3). Gizmo is sold by the Walter T. Kelley Beekeeping Company, or you can make your own using the plans online at the University of Minnesota Bee Lab website (www.extension.umn.edu/honeybees). The Gizmo device can be more accurate, but if you consistently measure bee at the 0.42 line, then the cup method works just as well.

Once the bees are measured, we recommend dislodging mites from the adult bees using the powdered sugar method12 (Figure 4). It is quick, easy, and gives a adequate estimate of the mites in the sample. Dump the 300 bee sample into a jar with a size 8 hardware mesh top and add about 2 Tablespoons (or a hive tool scoop) of powdered sugar. Add more sugar if the bees don’t look ghostly. Let the jar set at least one minute in the shade so the bees don’t overheat, then shake vigorously for one minute into a white dish. Be sure to shake hard. Some bees may lose a leg or two, but you’ll want to get as many mites off the bees as possible. After shaking, add a touch of water to the dish to dissolve the powdered sugar, and count the mites. Replace the sugar-coated bees to their colony where they will be groomed by nestmates. In areas with high humidity, the powdered sugar may not work well because the sugar clumps in the jar so that some mites are not dislodged from the bees. Dislodging mites using alcohol and then straining them is more accurate13, but it kills the bees. If you prefer the alcohol wash, Dr. Medhat Nasr made a handy device (discussed in the American Bee Journal, August 2010) or you can make a strainer with size 8 hardware cloth to separate the bees and mites.

**Treatment Decisions**

Once you sample a colony or an apiary to determine the mite infestation level, how can you use the information to help make a treatment decision?

Stationary colonies (e.g. beekeepers that keep their colonies in one location year round)

Researchers have found treatment thresholds for colonies in a stationary apiary to be 10-12% colony mite infestation in autumn14, 15, 10. However, the threshold may be different in different regions, so these thresholds may not apply to other locations. There are many factors that can influence the density of mites a honey bee colony can tolerate, including number of neighboring colonies, length of brood rearing season, nutrition, hygienic behavior, and disease and parasite levels. We highly recommend that ALL beekeepers sample their colonies for mites in early spring and late summer, and compare mite levels with other beekeepers in the same area. It would be very beneficial for groups of beekeepers to keep records of mite levels in their regions. In this way, regional patterns could emerge and show the level of mite infestation that warrants treatment to keep colonies alive, and what level does not warrant treatment. While other factors (i.e. colony strength, presence of diseases) affect colony survival, having ongoing records of mite levels, before and after treatment, in different regions at different times of year would be extremely useful for developing regional treatment thresholds.
Transported colonies (e.g., commercial migratory beekeepers)

There are no reported estimates of treatment thresholds for migratory beekeepers, as the thresholds will vary depending on region, season, and migratory path. However, the same principle holds that ALL migratory beekeepers should sample their apiaries for mites prior to “treatment windows,” or periods of time that treatments can be safely applied. A treatment window could be in the spring before honey supers are placed on colonies, or in late summer just after the honey supers are removed. The idea is to treat ONLY if your bees will not survive until the next treatment window. Keeping records of mite levels before and after treatment, over several years, will help beekeepers understand the mite levels that colonies can tolerate before the next treatment window. A few things that could lower the treatment threshold are if a beekeeper has many colonies situated in areas dense with other beekeepers within flight range of the bees, moves and feeds colonies to stimulate continued brood rearing for much of the year, and if the bees have high virus and disease levels. There will not be a single threshold for all beekeepers. Again, monitoring colonies and keeping good records can help beekeepers to find the infestation level that requires treatment in their specific operation. Since the method to find the infestation levels is standardized, beekeepers can share their levels with each other in a meaningful way and potentially help control the mite levels in the surrounding area.

With sampling, beekeepers have the potential to decrease the use of miticides, reduce chemical contamination in the hive, and save time and money. Importantly, monitoring mite levels is an important tool in the selection of colonies with few mites for breeding. Breeding queens from colonies whose bees have lower mite levels compared to colonies around them can increase the prevalence of natural mite resistance.

References


