Results of Research: Using Essential Oils for Honey Bee Mite Control Jim Amrine, Bob Noel, Harry Mallow, Terry Stasny, Robert Skidmore (Last Updated: December 30, 1996)

We have found that several essential oils can either kill, or adversely affect varroa mites.

Essential Oils have Two Modes of Action:

1) Toxicity by direct contact:

When varroa mites contact essential oils such as wintergreen, patchouli, tea tree oil, et al., mixed into oil or grease, they are killed on contact--usually within a few minutes.

2) Impaired reproduction via feeding syrups containing essential oils:

When varroa mites feed on larvae that contain essential oils, their reproduction is interrupted. If the oil is strong enough, the females are unable to lay eggs. If the oils are in lower concentration, eggs are layed, but development of immature mites is delayed; young mites do not reach maturity before the bees emerge from the cell; consequently, the immature mites die. Involvement of Essential Oils in Impaired Reproduction of Varroa Mites: Syrup containing the essential oils is fed at the hive entrance or in the broodnest. Many bees feed on the syrup and pass the essential oils around by trophalaxis (adult bees sharing their food reserves). The syrup and essential oil is ingested by nurse bees and enters the communal food in the crop and passes into the milk glands. When the nurse bees feed larvae, the essential oils are in the bee milk and communal food and are ingested by the larvae. Thus, when female varroa mites feed on treated larvae or larval food at the bottom of the cell, they ingest the essential oils which adversely affect their reproduction. The probable mechanism is interference with enzymes in the complex gestation (especially in the production of nutrients and new proteins) of the oocyte and embryo-larva of the varroa mite. Research needs to be conducted to verify this hypothesis and to verify the presence of the essential oils in bee larvae and ultimately, in the female varroa mites.

Impaired reproduction is not observed when canola oil, mineral oil, or shortening (eg., Crisco, a vegetable lard) containing essential oils are delivered to the hives. The fats and greases do not enter the food chain as readily as syrups, and the amounts of essential oils ending up in larval food or in the larvae themselves are inconsequential. Thus, there is no interruption of the development of mite eggs or of immature varroa mites. The mites that directly contact these materials rapidly die; but others are able to escape the essential oils in grease or canola oil by entering cells of mature larvae that are about to be capped, or by moving onto displaced nurse bees (see below, "Recent Findings") near the top of the colony, where the grease patties and tracking strips are not placed. We found that putting paper towels soaked in canola + essential oils in the tops of colonies from July to September, kills the varroa mites residing on the displaced nurse bees which congregate in the upper supers of large colonies. Feeding of sugar syrup with essential oils at the entrance, or in the brood nest, places the essential oils into the food chain and prevents oviposition by female mites or retards the

development of immature mites in capped larval/pupal cells.

We had several colonies that were treated with tracking strips and grease patties only, and we saw resurgence of varroa mites, especially when bee populations were at their peak, lots of brood was present, and when the bees occupied many supers as well as two brood chambers. However, we also had several colonies that were treated with the tracking strips and grease

patties, and were continually fed syrup + essential oils at the entrance; in these colonies very few varroa mites were found. Those few that were found appeared to have come into the colonies on drifting bees.

Revised Dosages Used in Experimental Treatments:

- 1). Syrup: 25 drops (1 cc) of wintergreen or spearmint is added to one pint of honey (or two cups of sugar (about one pound or 453.6 grams)) in a quart jar (0.95 liter); hot water is added to fill the jar. We found that more of the essential oil goes into solution in honey than in sugar syrup; there may be a natural emulsifier in honey that helps essential oils to stay in solution. When making sugar syrup, we found that we must add the oil to the granulated sugar then add the very warm water (not too hot or else the oils will evaporate). We feed the bees as much syrup as they will take; Bob uses 1/2 gallon jars on his entrance feeders. We have had good results with wintergreen, spearmint, rosemary and peppermint oils. We plan to conduct experiments this fall and winter combining the essential oils with fumadil in syrup to see if the treatments are compatible. See diagram below for making entrance cleats to eliminate robbing. Problem: oils mix poorly with water; we have had some reports of a few bees being killed when they feed on the last dregs of the syrup, above which lie pools of undissolved, pure oil;—they become completely wetted by the remaining oil. We are looking for a food based emulsifier which will allow all of the 1 cc or more of essential oil to go into solution in the syrup without harming the bees.
- 2). Grease patties: are made with four cups of granulated sugar, two cups of shortening (lard of vegetable origin--used for making pastries and frying fish and chips, etc., eg., Crisco) and one of the following: 21 cc of wintergreen oil, 21 cc of tea tree oil or 21 cc of patchouli oil; or 11.5 cc of each of any two oils (eg., wintergreen + tea tree). (This rate is 1/4 oz. (=7.4 cc) of essential oils per pound of sugar and grease). The components are thoroughly mixed (wear gloves or use a mixer or large spoon, as wintergreen oil in such concentrations may be toxic; patchouli oil and tea tree oil are not toxic through the skin). The mixture is then made up into 4 ounce patties (like a small, 3.5" hamburger); each patty is then divided and placed on top of each brood box (about one half pound or 8 ozs. of grease patties per hive; one batch treats 5.8 hives). We plan to make patties this fall and winter that also contain teramycin (TM25 or TM50) to determine whether medications can be combined for both mites and foul brood.
- 3). Tracking strips: are made by cutting sheets or pieces of 1/8 in. plexiglass into strips 3" wide by 14" long (7.5 cm by 35.5 cm), and used as a base for holding a treated slurry containing essential oil. The slurry is made as follows: 17.5 ozs. (2 and 1/5 cups) of canola oil (synonym for rapeseed oil) are mixed with 6.5 ozs. (slightly more than 4/5 cup) of melted beeswax (ratio is 4:1.5), stirred and set on a hot plate. To this liquid we add 24 cc's of wintergreen oil (or 24 cc's of tea tree oil or patchouli oil or 12 cc's of any two oils). We stir the liquid well and pour it into three 8 oz. plastic cups. When cooled, the slurry has a shoe-polish or salve-like consistency. Then, 2 to 3 teaspoons of the slurry are applied to the tracking strip which is placed lengthwise just inside the front entrance of each colony. The bees must track through this slurry when they enter or exit the hive; they then attempt to clean off the slurry and get it onto each other. When the slurry contacts varroa mites, it kills them. Treatments are repeated after 5 days or so: the old slurry, dead mites and dead, deformed bees are scraped off and new slurry added. The tracking strips also serve as small mite sampling strips: if varroa mites are present, dead mites (the number reflecting the size of the population on adult bees) can be found on the strips about 24 to 48 hours after treatment. The number of dead mites on the strips is significantly greater than what it would be due to natural mite mortality. Plexiglass is used for tracking strips because it

has a very smooth finish allowing an even coverage and it is too hard for the bees to chew up or remove; masonite or other similar material could be used just as well.

4). Paper inserts at top of hive: For control of varroa mite on displaced nurse bees (see comments above), we use 5.5 cc's of wintergreen per cup of canola oil; we put this solution into a honey bear (used as a squeeze bottle) and apply several lines of wintergreen oil mixture in both directions on a paper towel so that the towel is saturated; the towel is then placed under the inner cover or in the upper supers where displaced nurse bees congregate. The bees chew it up and remove it in a week or so. These are replaced as needed to treat the <u>varroa mites trying to avoid the other treatments</u>. Be careful! Do not get the oil-wintergreen mixture directly onto the queen.

Notes About Grease Patties:

- 1) a recent author stated that grease patties had no adverse effect on tracheal mites when administered to his colonies; the paper stated that the patties were purchased commercially. Be certain that the grease patties you make or buy contain plenty of grease or vegetable oil. It is the free oil or grease that appears to suffocate tracheal mites and probably also, varroa mites. We believe that this physical suffocating function is enhanced or synergized by the essential oils.
- 2). Several beekeepers have stated that they have used powdered or confectioner's sugar in place of granular sugar, or even replaced sugar with honey; some have also added canola oil to the patties to make them softer and easier to apply with an ice cream scoop. The bees take these softer patties more rapidly; and, if made with honey, the essential oils may enter into the food chain of the bee larvae and thus impair development of the varroa mites. We plan to try to examine this question in next year's research.

Conversion Factors: (go to the attached file called "measures")

About Essential Oils: (go to the attached files, "oils" and "chemical")

Safety in Using Essential Oils: (go the the attached file, "safety")

Varroa Mite Assessment: You can place a detector board on the bottom of the hive in combination with patties and a tracking strip to obtain a count of varroa mites within 24 to 48 hours. In our tests the essential oils killed more mites than Apistan.

We make detector boards by making light wooden frames, a little smaller that the bottom board area, by stapling thin wooden molding strips at their corners to 1/8 inch hardware cloth (wire screen); we then cut "freezer paper" (available in grocery stores in the U.S.; it has large size, is fairly thick, white and durable) to size, spray one side with "PAM" (a non-stick spray for frying pans), and then staple it to the wooden strips on the side opposite the hardware cloth. These can be assembled in a few minutes at a cost of \$2 U.S. or less. Many bee supply houses provide them at a fairly expensive price.

Timing of Treatments:

We have found that colonies, heavily infested with varroa mites in August, September and October, probably can not be saved. The treatments with grease patties and tracking strips will kill mites, but it is too late in the season to allow rearing of new, uninfested brood which would enhance survival of the colony. But, more importantly, even if all varroa mites are killed after heavy infestation, the bees may still die from the acute bee paralysis virus, Kashmir virus or other viruses transmitted earlier by the varroa mites.

However, strong, healthy colonies--free of mites in early September, but later overwhelmed by an influx of lost bees and mites from dying feral colonies or from untreated neighboring colonies--can be protected and saved by using the grease patties + essential oils throughout the

fall and winter. Since little or no brood will be available as shelters, the contact of the grease and essential oils will kill virtually all of the mites--before they are able to transmit the viruses. We recommend: 1) That grease patties + essential oils (and other medicaments as desired) be kept on the hives throughout the winter. 2) That grease patties and essential oils be kept on hives throughout the rest of the year (be sure no other medicaments are used). 3) When temperatures are warm enough to allow flight, that colonies be treated with syrups containing essential oils delivered to the brood area (e.g., boardman feeders with 1/2 gallon jars). 4) When temperatures are warm enough to allow flight, that tracking strips be used to administer essential oils to the bees, especially during honey flows.

Recent Findings:

In <u>colonies treated with tracking strips</u> at the entrance and grease patties over the brood chambers, the varroa mites were able to escape treatment. Displaced nurse bees, which gather under the inner cover, were used as a hiding place by the mites. We treated this group of bees and mites by putting a piece of paper towel, treated with canola oil and winter-green, just under the inner cover. Very few mites were found on these bees after treatment.

The <u>tracking strips</u> and <u>grease patties</u> are <u>lethal to exposed mites</u> (those on adult bees or walking over frames), but the mites can escape these treatments by going into brood cells about to be capped. There were very few mites on adults in treated colonies, but the mites were reproducing at normal levels in drone cells and worker brood cells (especially in August and September). The few remaining drone cells had lots of mites in them (13 Sep 96). We concluded that the essential oils in tracking strips and grease patties do not enter the food chain sufficiently to impair mite development.

The colonies that were fed syrup with essential oils, in addition to using tracking strips and grease patties, were virtually free of varroa mites, without using Apistan.

Robbing can be greatly reduced by making an entrance cleat that surrounds the boardman feeder and provides an entrance at the other end of the cleat:

bee entry	feeder	

Diagram of a feeder cleat made to fit in the entrance of a hive to prevent robbing-dimensions will vary.

Diagram of a wooden cleat made to fit in the entrance of a hive--dimensions will vary. Large colonies with many supers and lots of space made mite control more difficult: the treatments were too diluted by the volume of space and number of dispersed bees. Therefore, we recommend that beekeepers remove honey supers as soon as possible and reduce colonies to 1 or 2 brood chambers, at most.

In some of our colonies, grease patties containing spearmint oil were propolized; but not those patties made with wintergreen, tea tree or patchouli oils.

We believe that during the late fall and early winter the varroa mite is very susceptible to control by essential oils due to the formation of a cluster and to the lack of brood. By treating hives with the grease patties (and tracking strips and syrups if weather is warm enough) containing wintergreen, tea tree and/or patchouli oils, and making sure the treatments are near the cluster, then the varroa mites will have no place to hide and all can be killed. (No brood cells will be available as shelters.)

Queens: A potential problem may be that queens on mating flights may have their pheromone masked or may become somewhat disoriented by essential oils. We recommend that the essential oil treatments be removed from the hives when queen rearing and mating is taking place. Care must be taken to avoid getting canola with essential oil directly onto the queen. Drones: We found that large numbers of drone cells provide protection to the varroa mites and are definitely the source of most breeding varroa mites. Consequently, we recommend that the amount of available drone cells be kept to a minimum; be sure to remove and replace old comb containing lots of irregular drone cells. (See the literature for techniques using periodic drone removal to reduce varroa mites).

Comments on Varroatosis:

There are two forms of varroatosis (infestation of bee colonies by the varroa mite):

- 1) **Epidemic Varroatosis**: This form of varroatosis occurs when the disease spreads uncontrolled through feral colonies, and/or through untreated apiaries in a large area. In this form of the disease, "rapid development" of varroa mites is seen accompanied by the complex of ailments known as PMS (parasitic mite syndrome) or bee PMS (see papers by Shimanuki and Hung), followed by sudden collapse and death of colonies. What is happening? Dying feral colonies, or dying apiaries, send forth lost bees that are carrying large numbers of varroa mites. [Or, robber bees may be picking up large numbers of mites from dying feral colonies.] Thus a "queenright" colony is suddenly invaded by large numbers of phoretic, parasitic mites. Under "normal" conditions, the mites in a colony do not produce huge numbers in just one or two months; sudden increases in mite numbers are the result of mass influx of bees and mites deserting dying colonies. At the same time, the mites, coming from many diverse colonies, have acquired many different pathogens: agents of sac brood, chalk brood, acute bee paralysis virus, kashmir virus, Egypt (deformed wing) virus, chronic bee paralysis, etc. The sudden appearance and spread of these pathogens in a colony produces the syndrome called PMS. The colony becomes very weak and unable to fend off common pests such as wax moth, yellow jackets, etc., and the colony rapidly dwindles and then dies. Epidemic varroatosis was spread into various centers throughout the U.S. by migratory beekeepers, who were not aware of the degree of mite infestations nor of the various pathogens being transmitted. This epidemic varroatosis has thus spread throughout the U.S. in a series of expanding circles in nearly every state; the disease reached Bob Noel's colonies in summer 1995, when he began his research with essential oils. The most dramatic results of effective research will be evident when applied to colonies suffering from epidemic varroatosis.
- 2) **Chronic Varroatosis**: Once the epidemic has passed (as shown by the loss of all feral colonies and the loss of all colonies in apiaries that were not treated), the varroatosis will take its "normal" chronic form: much slower rate of development of mite populations in hives, which if left untreated, will eventually succumb in 3 or 4 seasons. Also, very few of the other virus diseases will be seen, which produced the PMS symptoms described above. Treatments using Apistan, formic acid, etc., will prevent the mites from overwhelming colonies. Thus, here in the U.S., beekeepers will enter the phase that the Europeans have been experiencing for the past several years. This milder form of varroatosis causes some beekeepers to erroneously believe that varroatosis is not such a severe pest of honey bees, and that the terrible scourge that decimated bee colonies must have been due to other causes. Despite its less severe expression, chronic varroatosis is an insidious disease capable of the eventual demise of all European Honey Bee Colonies.

Comments on Natural Resistance to Varroatosis:

There are many papers in the literature that remark on "bee resistance" to varroa mites, or on regions and countries where the varroa mite, although present, is not a problem. We believe that in many of these cases that essential oils in nectars (and/or pollens?) collected in and around the colonies had a suppressive effect on varroa mite reproduction. None of the papers considered the possibility that a component of the bees' diet (from nectar or pollen) or from propolis may have adversely affected the development of the varroa mites. A series of appropriate experiments in Brazil, et al. locations where this was observed, would be able to determine if this indeed is what causes the large numbers of "sterile" or non-reproducing varroa mites found in these colonies.

A recent report of varroa mites on *Apis cerana* in Papua New Guinea mentions that none have been found in nearby colonies of *Apis mellifera*, suggesting that these colonies may have resistance to the varroa mites. However, we believe that the "jump" made by *Varroa jacobsoni* from *Apis cerana* (its original, natural host) to *Apis mellifera* may be a comparatively rare event. Maybe such a jump occurs only once or twice per decade: perhaps our current varroa mite problem comes to us from a jump that occurred in Japan last century. This possibility could be experimentally investigated by performing a series of transfer experiments--especially one where varroa mites from varroa-infested *Apis mellifera* are transferred to these supposedly resistant colonies in an experimental setting (e.g., on some small Island distant from Papua New Guinea). Similar experiments should be conducted in Japan and in China where *A. cerana* and *A. mellifera* are artificially sympatric.

The possibility of European Honey Bees in the U.S. developing hygienic behavior to remove mites has been mentioned in several papers. We believe that the evidence suggests there apparently are no populations that exhibit this behavior. Our conclusion is based on the fact that virtually all feral bees have been destroyed by the varroa mite. If there had been hygienic behavioral types present, they would be evident by now. We also believe that the Eastern U. S. had considerable diversity among the formerly immense feral bee populations. For decades beekeepers bought different kinds of bees originally derived from many parts of Europe and Central Asia (eg., Italians, Dark Germans, Carniolans, Caucasian, and many types of hybrids such as Starlines, Midnights, Buckfast, et al.)--and from these colonies, swarms have issued all through the eastern U.S. and produced the feral colonies that were abundant until varroa mite arrived. We believe that the enormous diversity of these feral colonies throughout the U.S. would have contained the genes for hygienic behavior, if such genes were available. But since virtually all feral bees are now gone, it is apparent that even this extensive diversity was not sufficient to provide any meaningful resistance to the varroa mite. However, we need to be alert for the possibility that somewhere, in some corner of the formerly vast feral populations, one such resistant hygienic colony or population managed to survive.

EPA Exemption of Essential Oils:

We learned recently that EPA has exempted certain active ingredients from the requirements of FIFRA (Federal Insecticide, Fungicide and Rodenticide Act) including some essential oils; this information can be found in the Federal Register, Vol. 61, No. 45:8876-8879, Weds., Mar. 6, 1996 ("Exemption of certain pesticide substances"); materials listed include cinnamon oil, citronella oil, lemongrass oil, mint and mint oil [eg., Patchouli oil], peppermint oil, etc. EPA further stated that the inert substances in Fed. Reg. Vol. 61, No. 45 can be added to the active ingredients listed in Fed. Reg. Vol. 59, No. 187:49400-49401, Weds., Sep 28, 1994 ("Inert ingredients in Pesticide Products"), including cloves, mineral oil, parafin wax, wintergreen oil,

etc. The label for exempted products must list the name and percentage (by weight) of each active ingredient and the name of each inert ingredient, and must not contain any false or misleading statements (40 CFR Art. 156.10, 1 July 1994). Many states require some form of state labeling such as "Attention: this product is a pesticide which is exempt from federal safety testing requirements. State registration does not imply safety or efficacy. User assumes full liability of use." Contact your state pesticide office for their requirements.

Notes about tracheal mites:

We checked Bob's colonies for tracheal mites by collecting older foragers: those that have frayed wings and a bald thorax. We removed their heads and first pair of legs and examined the tracheal trunks that are visible inside the resulting cavity (you may need to peel off the "collar" with forceps on some specimens); infested tracheae could be seen with a hand lens and were dark spotted or uniformly dark. Results: in some control colonies (no treatments) 30% had tracheal mites; in treated colonies, 10% or less had tracheal mites. Since bees are able to drift considerable distances, we suspect that some of those in our treated colonies may have come in from other, declining feral colonies in the neighborhood (a few feral colonies still remain near Cumberland, but these will probably all disappear by 1997). The older bees give a more reliable test for the presence and extent of infestation by tracheal mite. We learned that British beekeepers, up until about 1950, used a bottle of wintergreen oil (methyl salicylate) with a wick through the top and placed in the bottom of their hives to control acarine disease (tracheal mites) (courtesy of David Eyre, Ontario, Canada). We also learned that Dr. Rennie, around 1919-1925, recommended oil of wintergreen over the original Frow treatment (safrolnitrobenzene-petrol) as a treatment for Isle of Wight Disease (acarine disease or tracheal mites) (courtesy of Mr. Joe Hemmens, UK).

On 23-25 December 1996, four colonies at WVU were examined for tracheal mite. Exams for tracheal mites were negative in July, August, and September. The colonies were treated with grease patties and syrups containing wintergreen and tea tree oil in summer and fall. Eight of 36 bees on 23 December had tracheal mite (22.2%); eggs, larvae, males and females were present indicating active development of the tracheal mites. The sample consisted of live and dead bees from the front of colonies. Each hive was sampled again on 24 December: Hive 1: 0/19 (0 %). Hive 2: 3/17 (17.6%). Hive 3: 3/23(13.04%). Hive 4: 6/21 (28.6%). Overall, the tracheal mite infestation was 20/116 (17.2%) total (20/97 or20.6% for the three infested hives). Three colonies (1,2,4) were treated by shaking warm sugar syrup containing dissolved menthol (1 tsp stock menthol solution per pint) and canola oil (1 tsp/pint) onto the bees on 30 Dec. 1996: 1/2 pint per cluster (ambient temperature = 61 F.) [provide access here to the file "tm.wpd"] The hives will be examined in January and February to determine tracheal mite infestation.

Experimental Treatment: Menthol + Oil for Tracheal Mite (Acarapis woodi) Control

Useful Equivalents and Measures for Mixing Essential Oils

Essential oils used to control mites in honey bees

General Safety Considerations for Using Essential Oils

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