Mechanisms of Varroa Control by Thymol and Other Essential Oils

Project No.: **07-POLL3-Huang**

Project Leader: **Zachary Huang**
243 Natural Science
Michigan State University
East Lansing, MI 48824
(517) 353-8136
bees@msu.edu

Project Cooperators: Gorden Wardell
S.A.F.E. R&D. LLC
2526 E. Blacklidge Dr.
Tucson, AZ 85716-1802

Objectives:

In this study, my objectives are to:

1. Determine whether essential oils fed to bee larvae in a laboratory setting reduce varroa mite reproduction
2. Compare efficacy, and quantify the optimal dose, of essential oils for suppressing mite reproduction
3. Determine whether essential oils fed to bees in a colony reduce varroa mite reproduction

Interpretive Summary:

Rational
Many studies have shown that essential oils can reduce varroa mite populations at efficacies up to 90% (Ali et al. 2003, Ariana et al. 2002, Calderone and Spivak 1995, Fassbinder et al. 2002, Imdorf et al. 1999 and 1995, Kraus and Berg 1994, Mattila and Otis 1999, 2000, Melathopoulos et al. 2000 and 2003, Noel et al. 2002, Rice et al. 2002, Ruffinengo et al. 2002, Sammataro et al. 1998, Shenk et al. 2001, Whittington et al. 2000). Recently ApiGuard and ApiLifeVar have been registered in the US (one federally and one as section 18 by individual States). In both medications, the main ingredient is thymol, the chemical from thyme oil. Despite these studies and the registrations, the mechanism of how mites are reduced by essential oils is not well understood. Undoubtedly essential oils can kill adult varroa mites by direct contact as well as by vapor, as evidenced by laboratory tests (e.g. Lindberg et al. 2000, Melathopoulos et al. 2000). In addition, it is possible that essential oils can also kill adult mites on brood, or reduce their reproduction. This is done either through ingestion of oil from larval food, or through oil vapor across

wax capping into sealed brood cells. To my knowledge, these potential effects of essential oils on varroa mites have not been studied. There is preliminary evidence that micro-encapsulated or emulsified essential oils (developed by S.A.F.E. Research & Development, LLC, Tucson AZ) applied to the colony or fed to the larvae also reduced mite populations (unpublished study, Gorden Wardell). This suggests that the oils are being fed to the larvae by nurse bees. These essential oils might circulate in the larval hemolymph. They can either deter mite feeding, or are picked up by the feeding mites and then reduce their reproduction. In this study I will determine whether unprocessed or "derivatized" (micro-encapsulated and/or emulsified) essential oils can cause mortality or reduce mite reproduction in honey bee brood.

Research Plans

Experiment 1. Laboratory Study. **Determine whether essential oils fed to bee larvae in a laboratory setting reduce varroa mite reproduction; and compare efficacy and quantify the optimal dose of essential oils for suppressing mite reproduction**. We will use the above-mentioned in vitro rearing method to assess the effects of several essential oils. The whole oil and derivatives of **cineole, clove, origanum, lemon, lemon-grass, and thymol**, will be the main oils to be tested. They are selected because of their higher efficacy in killing adult mites in other studies (Lensky et al. 1996, Calderone et al. 1997, Imdorf et al. 1999, G. Wardell, unpublished data). Combinations of these oils will also be evaluated. Of these that shows the highest efficacy, we will also assess the optimal dose of essential oils for causing mite mortality and suppressing mite reproduction. LC50 for honey bee larvae will also be assessed to make sure the effective dosage does not harm honey bees.

Experiment 2: ***Colony Level Study***. **Determine whether essential oils fed to bees in a colony reduce varroa mite reproduction: artificial feeding to patches of brood.** A patch of four-day-old larvae (n = 500) will be selected, and 100 per group will be randomly selected to receive one type of essential oils or a control (vegetable oil). The time of brood capping will be precisely determined, and those capped during the most recent six hours will receive a mite harvested from a mite source colony (n = 50 mites for each group). The mite-introduced brood will be kept in an incubator (35°C, RH 75%). Ten days later, the cells with introduced mites will be uncapped and the contents carefully examined. We will record whether the mother mite is alive, how many offspring she has (eggs, nymph and adults of male or female mites), and the location of mite defecation. These data will help understand the mode of action of essential oils in reducing mite population. The experiment will be repeated for either straight, or encapsulated or emulsified of each oil, and repeated across 3-4 different source colonies.

Experiment 3: ***Colony Level Study***. **Determine whether essential oils fed to bees in a colony reduce varroa mite reproduction: whole colony treatment.** We will use the most effective oil and derivatization combination based on results from previous studies. Honey bee colonies (n = 10 colonies) with high mite

infestations will be treated with essential oil (mix provided by S.A.F.E. Research & Development, LLC, Tucson AZ) or without any chemicals (control group, n = 10 colonies). After 30-40 days of treatment, both worker and drone brood that are about one day before emergence will be used to assess mite reproduction in the laboratory. These frames were previously caged with the queen to precisely determine the age of brood. To assess mite reproduction, the cell capping will be carefully removed and the pupae (dark-eyed stage) extracted and observed for mites. The number, the status (alive or dead), and the stage of mites (egg, protonymph, deutonymph, and adult) will be recorded for each mother mite. From this data, the *mortality of mites, rate of reproduction, and the average number of offspring* can be calculated in the two groups of colonies. Oils can affect mite mortality and reproduction in two different pathways. Nurse bees can feed or "track" the oil to larval food, or oil vapor can transmit across the wax capping into brood cells. To separate the two, newly sealed brood (within eight hours) will be exchanged between the two groups of colonies. If mite reproduction is still depressed in brood cells sealed in a control colony but transferred to a treated colony, this suggests that oil vapor can penetrate wax capping to reduce mite reproduction. Conversely, if mite reproduction is still reduced in the reverse transfer (mites sealed in oil treated colonies but transferred to control colonies after cell is capped), then transfer of oil from nurse bee to larvae is more important. Newly sealed brood is obtained by mapping partially capped brood cells at one time period, and comparing the capping status with the map six hours later. Any previously open cells capped at the second time must be capped within the last six hours. Mapping will be done on a piece of transparency paper. This method has been used successfully when I was studying mite reproduction in China (Zhou et al, 2001). We will also monitor the number of mites in the entire colony to determine if reduction in mite reproduction in laboratory studies translates to less mite population in the colonies.

References cited:

Ali, M. A., P.A. Macedo, J. Wu, M.D. Ellis. 2003. Evaluation of three concentrations of tebufenpyrad for the control of *Varroa* destructor (Acari: Varroidae). J. Econ. Entomol. 96: 259-263.

Ariana, A., R. Ebadi, G. Tahmasebi. 2002. Laboratory evaluation of some plant essences to control *Varroa* destructor (Acari: Varroidae). Exp. Appl. Acarol. 27: 319-327.

Br∅dsgaard, H.F.; C.J. Br∅dsgaard, H. Hansen, G.L. Lövei. 2003. Environmental risk assessment of transgene products using honey bee (*Apis mellifera*) larvae. Apidologie 34: 139-145.

Calderone, N. W., and M. Spivak. 1995. Plant extracts for control of parasitic mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the western honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 88: 1211-1215.

Calderone, N. W., W.T. Wilson, M. Spivak. 1997. Plant extracts used for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol. 90: 1080–1086.

Chen, Y.W., C.H. Wang, K.K. Ho. 2002. Effects of oxytetracycline of larval honey bee, Apis mellifera, reared in vitro. Formosan-Entomologist 22: 53-64.

Chen, Y., J.S. Pettis, J.D. Evans, M. Kramer, M.F. Feldlaufer. 2004. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. Apidologie 35: 441–448.

De Jong, D., R. A. Morse, G.C. Eickwort. 1982. Mite pests of honey bees. Ann. Rev. Entomol. 27: 229-252.

Downey, D.L., M.L. Winston. 2001. Honey bee colony mortality and productivity with single and dual infestation of parasitic mite species. Apidologie 32: 567-575.

Fassbinder, C., J. Grodnitzky, J. Coats. 2002. Monterpenoids as possible control agents for *Varroa destructor.* J. Apic. Res. 41(3-4): 83-88.

Imdorf, A., J.-D. Charriere, C. Maquelin, V. Kilchenmann, B. Bachofen. 1995. Alternative Varroa Control. Fed. Dairy Res. Inst. Switzerland. 11 pp.

Imdorf, A., S. Bogedanov, R. I. Ochoa, N. Calderone. 1999. Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie 30: 209-228.

Kraus, B., S. Berg. 1994. Effect of lactic acid treatment during winter in temperate climate upon *Varroa jacobsoni* Oud. and the bee (*Apis mellifera* L.) colony. Exp. Appl. Acarol. 18: 459-468.

Lensky, Y., Y. Slabezki, H. Gal, U. Gerson, U. Dechmani, U. Jirasavetakul. 1996. Integrated control of Varroa mite (*Varroa jacobsoni* Oud.) in *Apis mellifera* colonies in Thailand. Final report. German Israel Agricultural Research Agreement for Developing Countries. Rehovot, Israel. 96 pp.

Lindberg, C.M., A.P. Melathopoulos, M. L. Winston. 2000. Laboratory evaluation of miticides to control *Varroa jacobsoni* (Acari: Varroidae), a honey bee (Hymenoptera: Apidae) parasite. J. Econ. Entomol. 93: 189–198.

Malone, L.A., E.L. Tregidga, J.H. Todd, E.P.J. Burgess, B.A. Philip, N.P. Markwick, J. Poulton, J.T. Christeller, M.T. Lester, H.S. Gatehouse. 2002. Effects of ingestion of a biotin-binding protein on adult and larval honey bees. Apidologie 33: 447 –458.

Martin, S., A. Hogarth, J. Breda, J. Perrett. 1998. A scientific note on *Varroa jacobsoni* Oudemans and the collapse of *Apis mellifera* L. colonies in the United Kingdom. Apidologie 29: 369-370.

Mattila, H.R., G. W. Otis. 1999. Trials of Apiguard, a thymol-base miticide. Part 1. Efficacy for control of parasitic mites and residues in honey. Am. Bee J. 139: 947-952.

Mattila, H.R., G.W. Otis. 2000. The efficacy of Apiguard against *Varroa* and tracheal mites, its effect on honey production: 1999 trial. Am. Bee J. 140: 969-973.

Melathopoulos, A.P., J. Gates. 2003. Comparison of thymol-based acaricides, API LIFE VAR ® and Apiguard ™, for the control of Varroa mites. Am. Bee J. 143: 489-493.

Melathopoulos, A.P., M L. Winston, R. Whittington, H.A. Higo, M. LeDoux. 2000. Field evaluation of neem and canola oil for the selective control of the honey bee (Hymenoptera: Apidae) mite parasites *Varroa jacobsoni* (acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae). J. Econ. Entomol. 93: 559-567.

Nazzi, F., Milani, N. 1996. The presence of inhibitors of the reproduction of *Varroa jacobsoni* Oud. (Gamasida: Varroidae) in infested cells. *Exp. Appl. Acarol.* 20: 617–623.

Nazzi, F., N. Milani, G.D. Vedova. 2002. Z-8-Heptadecene from infested cells reduces the reproduction of *Varroa destructor* under laboratory conditions. J. Chem. Ecol. 28: 2181-2190.

Noel, B., J. Amrine., A. Kovacs. 2002. Organic treatment IPM for honey bee mites. Am. Bee J. 142: 359-361.

Nordström, S. 2003. Distribution of deformed wing virus within honey bee (*Apis mellifera*) brood cells infested with the ectoparasitic mite *Varroa destructor*. *Exp. Appl. Acarol. 29*: 293–302.

Peng, Y.-S.C., E. Mussen, A. Fong, M.A. Montague, T. Tyler. 1992. Effects of chlortetracycline of honey bee worker larvae reared in vitro, Journal of Invertebrate Pathology 60: 127–133.

Rice, N.D., M.L. Winston, R. Whittington, H.A. Higo. 2002. Comparison of release mechanisms for the botanical oils to control *Varroa constructor* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 95: 221-226.

Ritter, W. 1981. *Varroa* disease of the honeybee *Apis mellifera*. Bee World 62: 141-153.

Ruffinengo, S.R., M.J. Eguaras, D. Cora, E. Rodriguez, E. Bedascarrasbure, P. N. Bailac, M. I. Ponzi. 2002. Biological activity of *Heterotheca latifolia* essential oil against *Varroa jacobsoni*. J. Ess. Oil Res. 14: 462-464.

Sammataro, D., G. Degrandi-Hoffman, G. Needham, G. Wardell. 1998. Some volatile plant oils as potential control agents for *Varroa* mites (Acari: Varroidae) in honey bee colonies (Hymenoptera: Apidae). Am. Bee J. 138: 681-685.

Shenk, P., A. Imdorf, P. Fluri. 2001. Effect of neem oil on *Varroa* mites and bees. Am. Bee J. 141: 878-879.

Vandenberg, J.D., H. Shimanuki. 1987. Technique for rearing worker honeybees in the laboratory. Journal of Apicultural Research 26: 90-97.

Whittington, R., M.L. Winston, A.P. Melathopoulos, H.A. Higo. 2000. Evaluation of the botanical oils neem, thymol, and canola sprayed to control *Varroa jacobsoni* Oud. (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of honey bees (*Apis mellifer*a L., Hymenoptera: Apidae). Am. Bee J. 140: 567-572.

Zhou, T., J. Yao, S.X. Huang, Z.Y. Huang. 2001. Larger cell size reduces varroa mite reproduction. Am. Bee J. 141: 895-896.