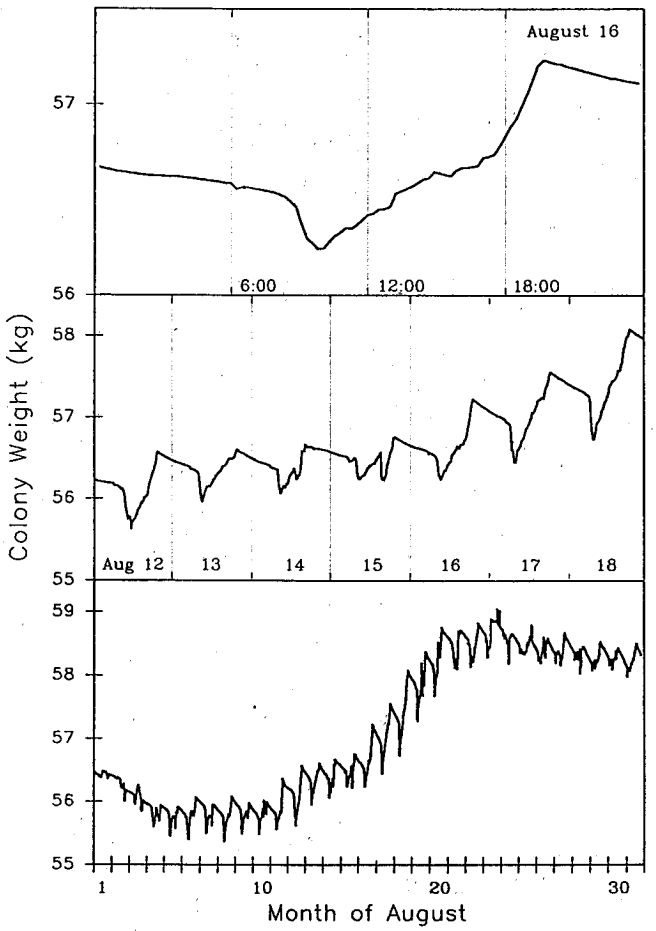
Special Feature

Proceedings of the American Bee Research Conference

The 1990 American Bee Research Conference was held on October 1 and 2 in Tucson, Arizona. Meetings were in the Meats Auditorium at the Campus Agricultural Center of the University of Arizona. The sixth American Bee Research Conference will be held in Tucson on October 7 and 8, 1991. The following are abstracts from the 1990 conference.

1. Buchmann, S. L.[a] – ELECTRONIC SCALE COLONY INTERFACED WITH A DATALOGGER[qq] – Routinely weighing colonies on mechanical scales has been used for decades to monitor honey flows. Knowing colony weights on a weekly or daily basis is a powerful research tool for studying population dynamics, transient pulses of harvested nectar and pollen, swarming behavior, general health, and a management device for honey extraction. An automated method is now presented which interfaces an industrial scale (Sartorius model F330S industrial balance) to a modern data logger (Polycorder from Omnidata, Logan, Utah). A custom Polycode program was written that prompts the electronic balance then stores the downloaded data at any user-defined time interval. Several months of data can be stored in tabular format inside the Polycorder memory until it is downloaded to a floppy or hard disk on a host PC. A sampling interval every 15 minutes around the clock is advantageous and data on behavioral phenomena such as nocturnal weight loss due to honey ripening or the number of foragers are easily obtained. This sensitivity is achieved since the scale can accommodate hives up to 300 kilograms with a resolution of 1 gram (the weight of 8-10 bees). Data for two colonies at different Sonoran desert sites has been collected for two years and provides an invaluable database for understanding colony population and foraging dynamics. The accompanying figure is a plot of the raw data for a large colony during the month of August, 1989 and illustrates daily patterns of nectar collection.

The Sartorius industrial balance is a rugged unit intended for dusty or wet warehouse applications for counting parts or in quality control during assembly lines. As such it has a rugged stainless steel base and sealed electronic components, including a built-in calibration weight and tare function, and is ideal for outside biological weighing applications when an AC power source is available. Our scale has now operated for a two-year period on a cement slab and under a shade screen during rainy and hot conditions (often exceeding 45 degrees Centigrade) without malfunctioning. Such a device is ideal for studying swarming and colony foraging ergonomics in honey bees and other central place foraging insects. Other temperate and tropical eusocial bees, such as *Bombus*, *Melipona* and *Trigona* spp. would be ideal candidates for long-term dynamical research using smaller Sartorius models. Perhaps the best feature of the electronic scale/datalogger interface is the ability to acquire vast amounts of error-free data, and the ability to estimate the number of bees out foraging at any time.



2. Buchmann, S. L.,[a] M. K. O'Rourke[b] and C. W. Shipman[a] – POLLEN PREFERENCES AND DIETARY BREADTH OF MANAGED AND FERAL SONORAN HONEY BEES[qq] – Since September 1980 an OAC pollen trap apiary of three colonies has been maintained near Pima

Canyon, north of Tucson. Trapped pollen was collected weekly and its weight recorded. A 100 g subsample was frozen until year's end when the annual diet was reconstituted by proportionally mixing portions of the 52 samples according to the total mean weight harvested weekly. Pollen samples were rehydrated, homogenized, acetolyzed and prepared for microscopic identification and counts according to methods given elsewhere (see O'Rourke and Buchmann, *J. Environ. Entomol.* in press). Palynological results indicate that from September 1980 to December 1989 the colonies collected pollen from 55 species of angiosperms in 40 genera and 25 families. Numerically, the dominant pollen types present during this 10-year period were: cf. *Aster* (22.7%), *Prosopis* (17.1%), Liliaceae (9.5%), *Eucalyptus* (7.0%), *Larrea* (6.6%), *Simmondsia* (6.6%), *Ambrosia* (6.4%), *Phoradendron* (4.8%), *Rhus* (3.1%), and Cruciferae (1.6%) with thse taxa accounting for 85.4% of the annual dietary pollen representation. Colonies harvested pollen from a total of 35-55 flowering plant species annually which represents about 18-28% of the species within their flight range.

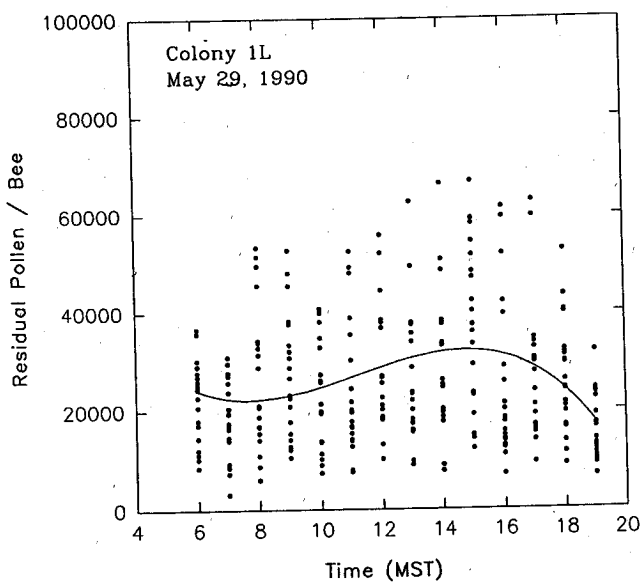
With their stored honey energy reserves *Apis* colonies are able to exploit pollen from anemophilous sources (eg. *Ambrosia, Ephedra, Rhus, Simmondsia*) early in the year when brood rearing is near maximum. During the period from 1981-1989 from 5 to 28% of the contribution to the annual pollen diet came from wind-pollinated plants. Unexpectedly, dominant plants in terms of vegetative or floral biomass (eg. *Cereus giganteus* and *Cercidium* spp.) represented only 0.8 ± 0.4 (SD) and 1.1 ± 0.7 (SD) of the mean annual diet. Although pollen is usually harvested proportionally to its community floral biomass, *A. mellifera* has distinctive preferences for certain plants, year after year. One example of a plant with minute flowers blooming in earliest Spring (Jan/February) and highly attractive to bee colonies is the parasitic mistletoe, *Phoradendron*, which occurs abundantly on host trees of mesquite (*Prosopis velutina*) and palo verde (*Cercidium microphyllum*). *Phoradendron* pollen was highly preferred (grand mean of 4.8% during the decade) by the bee colonies.

Although the site contains a largely undisturbed upper bajada Sonoran desert plant community, there are houses with ornamental plantings within flight range. From 1981-1989, pollen from exotic cultivated plants (eg. *Citrus, Eucalyptus, Morus, Olea, Rhus,* Tamarisk) constituted from 9-22% of the total pollen taxa harvested. Elsewhere (see Buchmann and O'Rourke, Grana, in press) we present data and caveats for quantitative assessment and understanding the importance of individual taxa (as numerical counts and by pollen volume) in pollen dietary assemblages from honey bees and other bee species.

Pollen diets can be quantified by processing dark *Apis* brood combs since these contain vast quantities of pollen harvested by the colony and consumed by larvae. As combs age, every 21 day brood cycle adds another layer of dark pollen-rich larval feces beneath silk cocoons. Little of this fecal pollen leaves via housecleaning bees. Solvent extraction methods for recovering and quantifying these comb samples from managed and feral colonies will be given elsewhere. Feral colonies living in Sonoran desert protected dry sites, produce unique debris middens rich in pollen. These can be exploited to determine foraging activity and pollen preferences over multiyear time spans. Such hardened, black, asphalt-like agglomerates of dead bees, wax, feces, dropped pollen and other materials are sometimes found below very old feral colonies in Sonoran rock outcrops. These bee middens can be processed using modified palynological techniques to yield a unique quantitative assessment of past pollen meals. One example of a multiyear (@5-15 yrs old) pollen diet from a feral colony located at Picacho Peak, Arizona is given below. Pollen from 37 species in 36 genera and 25 families were found. The dominant pollen types included; *Ambrosia* (25.7%), *Cercidium* (12.2%), Cheno-Am (8.1%), cf. *Aster* (7.9%), *Prosopis* (7,7%), *Larrea* (5.9%), *Trixis* (4.3%). Gramineae (4.1%), *Celtis* (3.2%), *Artemisia* (2.9%). Much older *Apis* middens were recently discovered in Organ Pipe Cactus National Monument and are being analyzed for their pollen content. Together, these data from managed and feral colonies constitute an invaluable database for bee diets prior to impending Africanization, and a means to assess intraspecific competition for floral resources.

3. Buchmann, S. L.,[a] C. W. Shipman[a] and H. M. Hansen[c] — POLLEN RESIDING IN SAFE SITES ON HONEY BEE FORAGERS — Pollen that increases male fitness by donation to stigmas, therefore pollination, represents a small fraction of the total pollen harvested by colonies. Honey bees are not sympathetic to plant reproductive needs, but are efficient floral herbivores of pollen and nectar for their own nutritional needs. *Apis mellifera* carry two corbicular payloads from 8.0 to 23.4 mg containing about 250,000 to 4,000,000 pollen grains, depending upon grain size. As a by-product of their sloppy harvest and grooming behavior on flowers and in flight, much pollen is lost to the bees and from pollination. Because of their plumose setae, large surface area and biophysical factors such as electrostatic charge and pollen characteristics (exine ornamentation, pollenkitt lipids) some pollen sticks to bees. Often, less than 0.007% of the pollen harvested by a forager gets lodged in "safe sites" where it cannot easily be groomed off for ingestion or transport. Safe sites for pollen grains occur in body regions including the occiput, thoracic notum, proboscideal fossa, between coxae and hind tibial pollen combs. For plants, some ungroomed residual pollen is donated to stigmas between floral visits. This diffuse coevolution between bees and flowers has directed the evolution of anther and stigmatic placement (*i.e.* noto- and sternotriby). Half-lives for residual pollen are unknown, but much is **not** donated to flowers or other bees. Many grains die, and often remain on bees for their entire lifetimes (Buchmann, in prep.).

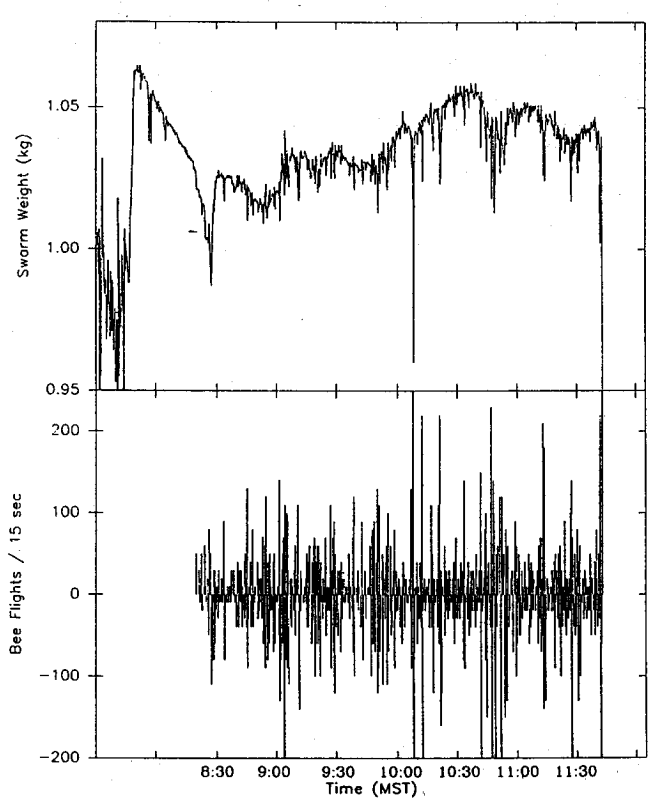
Experiments conducted in Tucson on full-sized colonies of Italian bees quantified residual pollen on outbound workers, drones and incoming nectar and pollen foragers. Additionally, newly emerged workers were marked and released into colonies to assess if any in-hive pollen transfer had taken place. Outbound foragers carried an average of 26,393 ± 3,952 (SD) pollen grains. Returning pollen foragers carried the most safe site pollen. The figure illustrates variable residual pollen on exiting foragers, but demonstrates a weak temporal pattern in pollen amount. Newly emerged bees acquired 6,780 ± 4,146 (SD) grains after 65 hours from nestmates, cell contacts, and feeding on bee bread. One sur-



prising result for newly emerged bees was the finding that bees could acquire up to 4,778 dead grains one hour post emergence from feeding on bee bread. If not ingested, this pollen was rapidly packed by young bees into the inner tibial pollen combs where it is not likely to be transferred to other nestmates. We conclude that in-hive pollen transfer from bee-to-bee is statistically unlikely for pollination compared with the predominant flower-to-bee-to-flower mode before grooming occurs.

4. Buchmann, S. L.[a] and S. C. Thoenes[a] – SCOUTING AND FORAGING BEHAVIOR BY BEES IN NATURAL SWARMS – From April 13 to May 15, 1990 a total of nine natural swarms were captured in Tucson, AZ and brought to the research center to study the scouting and foraging dynamics of unrestrained free-flying swarms. At capture these sugar-engorged swarm bees weighed on average 126.9 ± 18.9 mg. Within one hour of their collection, each swarm was installed on a screen cone suspended from a metal stand resting upon a Sartorius electronic balance linked to a Polycorder datalogger. The scale rested upon a concrete platform and the colony was shaded on three sides with dark green shadecloth which also provided protection from wind gusts. The support was tared out of the system and swarm mass was recorded every 15 seconds from installation until the swarms took flight to their new nest site usually on the first or second morning following collection. Since the swarm bees averaged 127 mg each, and the Sartorius balance is accurate to ± 1 gram, we have a weight resolution of ± 7.9 scouting/foraging bees for each time interval measured. No swarm traps or other artificial nest cavities were provided for the bees. Scouts were allowed to fly freely, come to a natural consensus, then move to unknown nest sites. Observations were also conducted on the number of waggle and DVAV dancers during certain days for some swarms. In addition to nocturnal measurements from the scale, swarm workers were randomly collected when found and at departure along with their weight and crop contents (volume and % TDS) to estimate the amount of nectar harvested during the scouting phase of nest establishment.

The figure illustrates the dynamic changes in weight for Swarm No. 8, May 3, 1990 plus estimated number of flights in lower panel. First bee flights began at 0615 MST and reached their peak at 1000 hrs. During maximum flight activity there were 400 to 1,000 bees away scouting or foraging. Pollen foragers were observed at a very low level (<0.01%) and no combs were constructed. During its entire stay prior to departure this swarm harvested 57 g of nectar, and made an estimated 15,000 scouting flights, or about 6 trips each by 2,798 bees. This is the first time that scouting/foraging dynamics from natural swarms has been studied. Our results indicate that about 80% of the bees are scouts, while only 20% are foragers. We estimate that even without foragers a swarm of this size (16.787 bees) could remain in a scouting mode for up to 5 days existing entirely on carbohydrate reserves in their honey crops.



5. Clark, K. J.[d] – 1990 FIELD TRIALS COMPARING VEGETABLE OIL AND MENTHOL AS A CONTROL FOR TRACHEAL MITES – Honey bee colonies infested with tracheal mites were treated with: 1) 250 g of a 1:3.5 canola oil:sugar mix applied as a patty in the brood nest, 2) 2 packets, each containing 25 g of menthol pellets, or 3) 2 plastic foam strips, each containing 10 g of re-solidified menthol, wrapped in perforated polyethylene.

Weather during the treatment period (July 6-21) was close to the warmest of the year for the region, with mean daily temperatures about 17°C and maximum daily temperatures generally above 25°C, occasionally reaching above 30°C. The mean amount of menthol vaporized in treatment 2 was 35 g per colony, and in treatment 3 was 12 g per colony. Menthol remaining at the end of the 15 days was removed. The oil patties were left on the colonies, and lasted 4 to 7 weeks.

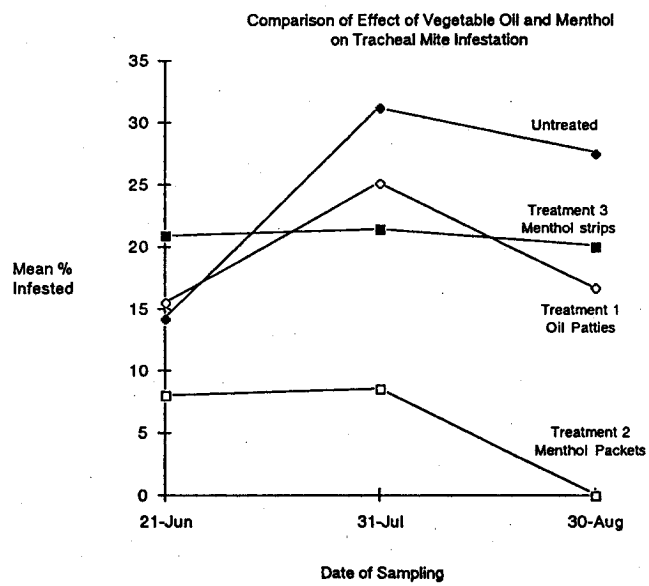
The mean tracheal mite infestation in untreated colonies increased from about 14% to about 30% during the 2 month trial (figure).

Eight weeks after the vegetable oil patties were installed, the mean mite infestation in that group of colonies was 40% below ($p < .05$) the infestation in the untreated colonies.

No change in infestation was indicated 25 days after installation of the menthol, although the thoracic disc examination method used, did not distinguish between live and dead mites.

Eight weeks after the menthol had been applied, fewer than 1% of bees in samples from treatment 2 had mites, a reduction of over 99% from the untreated group.

Perforations in the polyethylene wrappers around the menthol strips in treatment 3, became propolized enough to



reduce vaporization in that formulation. Samples indicated that the mite population in treatment 3 did not change over the period of the trial, but since the infestation in the untreated colonies had increased, the treatment 3 infestation at week 8 was lower than that in the untreated colonies.

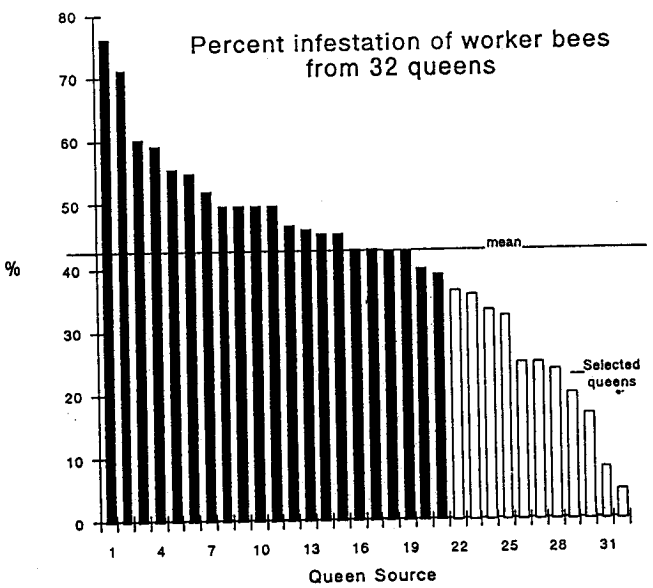
6. Clark, K. J.,[d] E. Huxter,[e] N. J. Gates[f] and T. I. Szabo[g] – SCREENING BREEDER HONEY BEE STOCK FOR RESISTANCE TO TRACHEAL MITES – Worker honey bees of 35 breeder queens from 11 commercial queen rearing operations in British Columbia were assessed to determine their relative tendency to become infested by tracheal mites in infested bee colonies. Workers from an additional 12 queens representing families of Alberta bee stock were assessed simultaneously. Results for the latter group are still being processed.

Newly emerged bees from each queen were marked with a unique 2 color combination. For each of 9 replicates of the assessment, 25 marked bees from each of the queens were introduced within 20 hours of emergence, into an infested colony. The bees were retrieved 2 weeks after introduction, sorted by color mark, and dissected (thoracic disc method) to determine the presence of tracheal mites. Three infested colonies were used on each of 3 dates, 1 week apart, providing 9 replicates with a maximum total of 225 marked bees per queen.

About 36% of the nearly 5,000 marked bees were retrieved with their marks intact. In 5 of the 9 replicates, less than 10% of the marked bees became infested, owing to low mite populations in the host colonies. Data from these 5 replicates were not included in the analysis. Marked bees from the other 4 replicates had a mean infestation of 43% (range 40-49%) resulting from host bee colonies with a mean infestation of 63% (range 53-83%).

The infestation of groups of bees from individual queens ranged from 4.8% (0.11 of the mean of all the marked bees) to 76% (1.8 of the mean) (figure). From the 35 breeder queens, 11 whose retrieved workers had a mean infestation of 23% (0.55 of the mean) were selected for production of a second generation. Ten daughters were grafted from each of 9 of the selected queens. The resulting queens were mated in an isolated location with drones from the same 9 queens. The project currently has about 70 queens which will be reassessed in the spring of 1991.

We intend further selection within the lines, inclusion of other apparently resistant stock, exchange with other projects, and distribution of stock to project cooperators.



7. DeGrandi-Hoffman, G.[a] and J. Bromenshenk[h] – AN UPDATE ON THE BEEPOP HONEY BEE COLONY POPULATION DYNAMICS MODEL – Two years ago a group of scientists from the Carl Hayden Bee Research Center (CHBRC) created a computer simulation model of honey bee colony population dynamics (BEEPOP) (DeGrandi-Hoffman *et al. Ecol. Modelling* 45:133-150). BEEPOP uses parameters known to influence population dynamics including: weather conditions, daylength, queen age, egg laying potential, the amount of sperm in the queen's spermatheca, and the size of the adult worker population.

BEEPOP is an interactive, menu driven, mainframe computer program. Default values obtained from the literature are provided for all parameters. By entering climate along with colony parameters, users can simulate population growth for very specific sets of conditions, times of year, and geographic locations. Users can test parameters for their effects on population size and identify those with the greatest influence. From simulations conducted under U.S. southwestern desert and midwestern weather conditions, we determined that the queen's egg laying potential is the most important parameter influencing population growth, because it limits the colony's ability to replace adult workers even when all other factors are optimized.

We constructed BEEPOP on a mainframe computer to take advantage of the virtually unlimited memory and rapid mathematical computations. However, many people do not have access to a mainframe computer and program portability is limited.

The problem of BEEPOP program portability was resolved at the University of Montana. At U.M. we developed PC-BEEPOP, a software program for IBM-compatible personal computers (Bromenshenk *et al. Environ. Tox. & Chem.* in press). Conceptually, PC-BEEPOP is similar to BEEPOP and retains all the options present in the original model. To facilitate use of the model, we added pull down menus, extensive on-line help, and graphics. We were interested in simulating colony responses to stress, including disease and exposure to toxic chemicals such as pesticides and hazardous wastes. Consequently new features were added to PC-BEEPOP. These include the ability to: vary development time, longevity, and mortality rates due to pesticides, predation, or diseases for specific lifestages and periods of time; randomize or manually initialize the population's age structure, control ratios of adults to brood, induce swarming, limit space for brood rearing and food storage, account for winter losses, and profile food gathering and food consumption rates, as well as entering the percentage of foragers that collect water, nectar, pollen, resin, or act as scouts.

PC-BEEPOP was designed for use in applied research and to assess bee population responses to environmental variables and exposures to toxic chemicals. We believe that PC-BEEPOP can be particularly useful as a teaching tool for courses in apiculture and social insects, population ecology, and general biology. Extension agents can use the program in beekeeping courses to illustrate the repercussions of various management practices. Beekeepers also may be interested in using PC-BEEPOP to identify the best management strategies for their particular geographic region. Regulatory agencies could better educate applicators about the effects of various pesticides using PC-BEEPOP.

PC-BEEPOP requires an IBM-compatible machine with a hard disk, 520K of memory, and a graphics adapter card. A 286 or 386 processor and a color monitor are preferred for ease of use and optimal performance.

8. Eischen, F. A.,[i] E. D. Akre,[j] and R. L. Hellmich[k] – AGONISTIC INTERACTIONS BETWEEN ANTS AND HONEY BEES IN THE TROPICS – Colonies of African-

ized and European honey bees were presented with living and freshly killed ants [*Camponotus sericeiventris* (Guerin)] at their entrances. Africanized colonies responded more quickly and with greater numbers of defending workers than did European colonies.

We speculate that because of their prevalence and incessant predatory behavior, ants have played an important role in the evolution of honey bees in the tropics. We suggest that ant predation prevented extensive utilization of the neotropics by European bees, but that effective defenses by Africanized honey bees allowed population growth and migration.

9. Ferrari, T. E.[l] – "ENPOLLINATION" OF HONEY BEES WITH PRECOLLECTED POLLEN IMPROVES POLLINATION OF ALMOND FLOWERS – Many crops have strongly self incompatible flowers and require cross pollination for fruit, nut or seed set. Poor environmental conditions, however, often interfere with optimal dispersion of pollen. Procedures that improve transfer of compatible pollen to flowers by bees are useful when undesirable situations threaten to limit crop production.

Dispersal of hand- or machine-collected pollen onto bees is termed enpollination. The procedure is fast, safe and easily implemented for many crop pollination situations. Enpollination of bees at the hive entrance improved pollination of cherry and apple flowers (Mayer & Johansen, *Goodfruit Grower* 38:32-33).

Tests with almonds support two components of pollen dissemination (near vs far) in orchards depending on kinetics of in-hive, bee-to-bee pollen transfer and orientation of tree rows. Three hours after almond pollen was applied to colonies, the number of grains on exiting bees peaked. At that time, transfer of viable pollens to flower pistils by bee bodies was improved 1.6- to 2.9-fold. Thus, an additional nut set would manifest itself where bees are foraging in an orchard when the pulse of increased pollination efficiency occurred.

Enpollinated colonies – placed so that bee flight was perpendicular to tree rows – produced significantly more nuts (table) within 300 feet of hives (near). When bee flight was parallel to rows, more nuts were produced beginning about 500 feet (far) from hives. No such changes were measured in orchards which did not receive supplemental pollen (-P). Results confirm known preference of bees to forage down, rather than across tree rows.

Table – Improved almond yields after enpollination of colonies with precollected pollen – based on two independent foraging patterns.

Orchard +/-Pollen	Nuts/Tree Diff	Chance	Student T-Test
Perpendicular	Near-Far	%	Level
1 + P	2415	+56.3	0.01
2 - P	-225	- 4.4	NS#
3 + P	1867	+44.4	0.01
4 - P	-291	- 3.2	NS
Parallel	Far-Near		
5 + P	1168	+25.8	0.05
6 - P	-556	- 11.4	NS
7 + P	1640	+17.8	0.1
8 - P	9	+ 0.1	NS

#: NS = not significant at 0.2 confidence level

10. Garza-Q.,[m] C., J. H. Dustmann,[m] W. T. Wilson[n] and R. Rivera[n] – CONTROL OF THE HONEY BEE TRACHEAL MITE *(ACARAPIS WOODI)* WITH FORMIC ACID IN MEXICO[pp] – One of the most important parasites affecting honey bees, *Apis mellifera*, is the tracheal mite, *Acarapis woodi*. Since first being reported in Mexico in 1980 (Wilson & Nunamaker, *Am. Bee J.* 122:503-505, 508), the parasite has spread rapidly and is now endemic in bee colonies in areas such as northeastern Mexico (Eischen, *et al., J. Kan. Entomol. Soc.* 63:56-73). Eischen *et al. (Apidologie* 20:1-8) reported that heavy mite infestations in Mexico caused serious economic damage to colonies and Garza-Q. (unpublished) found that the level of infestation increased during winter. Although non-chemical and management methods exist in Europe for mite control (Dustmann, *Allgemeine Deutsche Imkerzeitung* 21:2-8), chemical control is currently the method of choice in Mexico. Although several acaricides have been used by Mexican beekeepers, most chemicals have not been successful for reasons such as difficulty in dispensing, toxicity to bees or excessive cost.

Since formic acid is a naturally occurring compound and has been used effectively as an acaricide in Europe, it was decided to test formic acid fumes in 27 mite-infested honey bee colonies near Linares (N. L.) Mexico in 1988 for the control of *A. woodi*. Each of 9 colonies was given 4 applications of formic acid at intervals of 4 days. The chemical was dispensed on a cardboard sheet in the top of each colony (1 brood chamber & 1 super per colony). There were 3 colonies in each of 3 groups: I) 5 ml per colony per application (total 20 ml), II) 10 ml per colony per application (total 40 ml), III) 20 ml per colony per application (total 80 ml). Adult worker bees were collected from the colonies and dissected to determine the number of dead adult mites per bee on days 0, 4, 8, 12 & 16. The mite infestation level in the bee population was 30% or more at the beginning of the study. The entire test series was repeated three times. Therefore, the summary data is an average of the 3 replications.

Formic acid fumes gave good control of the tracheal mite in autumn field tests in northeastern Mexico. The 5 ml and 10 ml treatments killed about 40% of the adult mites after the 1st treatment and 61 and 76% of the mites, respectively, following the 4th treatment with the final mite counts made on day 16. The 20 ml treatment produced excellent control with 56% of the mites dead after the 1st, 71% after the 2nd, 90% after the 3rd and 96% after the 4th treatment (in 4 colonies 100% mite control was achieved). The normal adult mite mortality in the tracheal tubes prior to chemical treatment was approximately 6%.

The data showed that 4 treatments were necessary and that a 4-day period between treatments was suitable to achieve effective mite control. Although the bees did a lot of fanning when the treatments were applied, no bee mortality or abnormal bee behavior was observed in any of the treated colonies. Consequently, formic acid was not only efficacious in mite control, but also it was not damaging to the bees. The use of formic acid fumes shows much promise in Mexico as a cost-effective control for *A. woodi*.

11. Gilliam, M.[a] and S. Taber III[o] – DISEASES, PESTS, AND NORMAL MICROFLORA OF HONEY BEES, *APIS MELLIFERA*, FROM FERAL COLONIES – The opinion that feral colonies of honey bees are a menace to beekeeping because they harbor and spread diseases and pests is not expressed as frequently now as it was in the past. A review of the limited available literature revealed that the incidence of diseases in feral colonies has been found to be lower than that in managed colonies.

Data on wax moths in feral colonies are lacking. Since abandoned combs are often destroyed by wax moth larvae, these insects have been thought to play a role in slowing or preventing the spread of pathogens, particularly *Bacillus larvae*. However, an often overlooked, early study (Phillips, USDA Bull. 75) concluded that wax moths do not eat scales formed from honey bee larvae that have died from American foulbrood.

Recent interest in feral honey bee colonies, limited information available on surveys of feral colonies for diseases and pests, and lack of data on microflora of feral colonies prompted this work which was conducted before the mites, *Acarapis woodi* and *Varroa jacobsoni*, were reported in the United States. Our purposes were to survey feral colonies for disease and pests, to determine intestinal microflora of worker bees from feral colonies, and to compare the intestinal microflora of bees from feral colonies to that of bees from managed colonies.

Examinations and collections were made from 7 colonies located in rock caves, overhangs, or holes south of Cottonwood in central Arizona. Access to both colonies and brood combs within the colonies prevented examination of additional feral colonies in the area. Colonies were inspected and tested for diseases, pests, and parasites according to Shimanuki and Cantwell (USDA ARS-NE-87). Microflora was determined by isolating intestinal bacteria, yeasts, and molds from 34 foraging worker bees from 5 colonies.

Nosema apis, the only pathogen found, was observed in the intestinal tracts of 9 of 21 bees which were unable to fly and were crawling on the ground below one of the colonies. Only 1-5 spores of *N. apis* per bee were found after thoroughly searching numerous slides prepared from each intestinal tract.

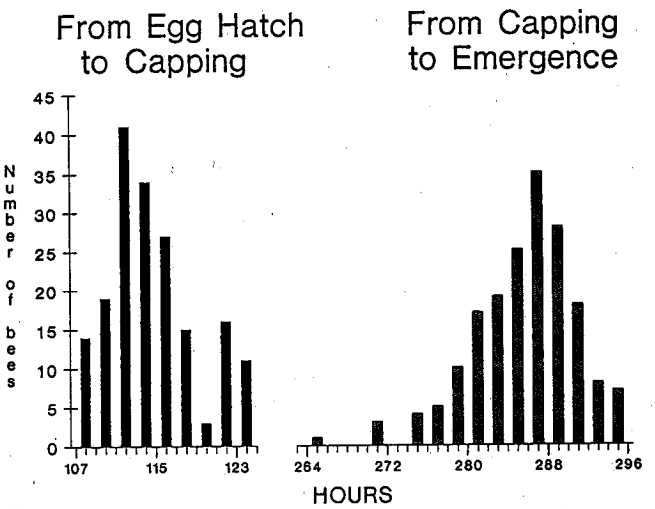
Frass and larvae of the greater wax moth, *Galleria mellonella*, were present in 2 colonies. One colony, below which the crawling workers with *N. apis* were found, had combs that were disintegrated as a result of attack by wax moth larvae. The other had no queen; but egg-laying workers, honey, and pollen were present.

Intestinal microorganisms were isolated from 79% of the foraging worker bees. Gram-variable pleomorphic bacteria were the most frequent isolates. Species of *Bacillus* were the second most common organisms; only *B. alvei* and *B. megaterium* were found. Some bees from all colonies contained Gram-variable pleomorphic bacteria and *B. megaterium*. Actinomycetes, Gram-negative bacterial rods, Gram-positive cocci, molds and yeasts were present in less than 10% of the bees. Thus, the honey bees from feral colonies in central Arizona contained the same kinds of intestinal microorganisms as honey bees from managed colonies in southern Arizona.

Ten samples of wax moth frass from each of the two colonies were stained and examined microscopically for bee pathogens and were also plated to test for *B. larvae*. Results were negative. The microflora of the frass was determined and found to be comprised of Gram-variable pleomorphic bacteria, *Bacillus* spp. including *B. alvei* and *B. megaterium*, Gram-positive cocci, actinomycetes, and molds. Thus, there are similarities in the kinds of microorganisms in bees and in wax moth frass from feral colonies.

12. Harbo, J. R.[k] — BREEDING FOR A SHORT CAPPED PERIOD IN WORKER BROOD — A capped period of about 240 hours is required for a female mite, *Varroa jacobsoni*, to produce 1 mature female in the cell of a worker bee, and additional females are produced at 30 h intervals (Rehm & Rittner, *Apidologie* 20:339-343). Moritz (*J. Hered.* 76:267-270) found that the capped period of worker brood differed by more than 48 h between European and African races of bees, and he reported a high heritability (0.8) for this trait. Therefore, it should be possible to acquire resistance to *Varroa* by selecting bees for a short capped period.

I surveyed bees in Baton Rouge to measure variability and heritability of development time. Newly hatched larvae were obtained from 26 different colonies by placing brood combs in an incubator without adult workers. Larvae from eggs that had hatched in the incubator were identified by their not having brood food in their cells. These larvae (0-3 h old) were then transferred to a small patch of worker cells



Development times for 180 worker bees from 26 different colonies. The mean ± SD was 114.5 ± 4.3 hours for the uncapped period and 285 ± 5.1 h for the capped period. Combined times ranged from 379 — 417 hours, so by adding 71 hours for the egg stage (Harbo & Bolten, *Ann. Entomol. Soc. Am.* 74:504-506), total development times ranged from 450 — 488 h (18.8 — 20.3 days) with a mean of 19.6 days.

and put into a strong colony to be reared. This equalized the ages of the larvae and placed larvae from different stocks into a uniform environment for comparison. Development time was established for each worker by checking the comb at 2 hour intervals during the capping and emergence periods (see figure). Heritability was 0.56 for the capped and 0.40 for the uncapped period. Stocks with rapidly developing workers did not always produce rapidly developing queens (worker-queen phenotypic correlation = 0.4), so one must evaluate workers rather than queens. With standard breeding methods, it should be possible to select within th. Baton Rouge population to produce a stock with a capped period <270 h (probably some resistance to *Varroa*), but a higher level of resistance (<240 h) may be difficult to achieve.

13. Houck, M. A.[c] — BEE MORPHOMETRICS AND THE INFLUENCE OF SIZE ON THE DISCRIMINATION OF AFRICANIZED AND NON-AFRICANIZED BEES — In any multivariate analysis, change in morphological characters must be evaluated within the context of some allometric model. Variation (due to such factors as ontogeny, nutrition, and phenotypic plasticity) that effects general size can contribute significantly to the variance structure of discriminant function analyses. Such influences must be considered as separate from conclusions concerning evolutionary or populational differences. In the past, discriminant function analyses on *Apis* have been accomplished without consideration of the allometric effect of size on discriminatory characters.

I present the first of a series of analyses that will examine the discrimination of Africanized bees from non-Africanized bees in an allometric context. This work represents an extended collaboration with R. Strauss, E. Dyreson, H. Daly, and E. Erickson. It investigates discrimination due to forewing and hindwing characters of worker bees as related to 4 measures of head and thorax size. Later reports will focus on similar analyses applied to the stinger, leg III, mouth parts, and antennae of workers.

Bees were collected from 14 geographic localities representing 4 groups: African bees, Africanized bees, non-Africanized North and Central American bees, and European bees. Data were entered into the discriminant function analysis with geographic location as the categorical variable. The condition of Africanization was not a categorical variable.

When size was retained in the 14-locality analysis, meaningful discrimination by degree of Africanization was not evident in the structure of the data. When size was partitioned from the data set, using the method of residual analysis, a clear two-group separation occurred that discriminated African/Africanized bees from European/Europeanized bees. A regional subset of 7 localities (feral bees from Arizona, managed bees from Arizona, feral Californian bees, and 3 Africanized localities: Brazil, Costa Rica and Colombia) resulted in the discrimination of 3 groups: Africanized bees, CA/European bees, and AZ feral/AZ managed bees.

Results from these analyses are encouraging, and size-independant discriminant procedures may be very helpful in distinguishing Africanized from non-Africanized bees, especially on a regional level where within-group variances are low as compared to among-locality variation.

14. Kitto, G. B.,[p] F. Davidson,[p] E. Broussard,[p] J. Lemburg,[p] and W. Rubink[n] — DEVELOPMENT OF BIOCHEMICAL DETECTION METHODS FOR AFRICANIZED BEES — Africanized Bees are now approaching the southern border of the United States, and it is imperative that rapid and reliable means of detecting and monitoring the spread of these bees be available so that effective control measures can be implemented. Electrophoretic and immunological techniques offer great potential for identifying Africanized Honey Bees (AHB). We have identified three proteins (termed A-1, A-2, and B-1) which are unique to AHB (Davidson *et al, Am. Bee J.* 129:813) using narrow range non-denaturing isoelectric-focusing (IEF). Such AHB specific proteins have subsequently been confirmed (Hung, *Am. Bee Jour.* 130:49), also using such IEF procedures.

Africanized honey bees from 16 different sources in Mexico, Honduras, Costa Rica, Venezuela, and Brazil, have been screened by IEF (more than 800 individuals). Only 7% of these samples lack the AHB-specific proteins. Tests using the three AHB-specific proteins should make it possible to have a 93% accuracy of AHB detection. Trials using USDA Mexican trapline samples have been consistent with this figure, and correlate with other methods of AHB detection.

AHB-specific proteins have been purified by a combination of IEF and SDS-gel electrophoresis for structural studies and antibody preparation. Rabbit polyclonal antibodies directed against the A-1 and A-2 proteins cross react not only with these proteins in AHB samples, but also with the other AHB-specific protein, B-1, and, to a lesser extent, with a protein termed B-2 which is found in all European bee populations studies and in approximately 50% of AHB populations. This cross-reactivity is indicative of a degree of structural relatedness of the 4 proteins. Preliminary trials indicate that absorption of these antisera and an EHB extract provides antibodies that are AHB specific. The availability of such antibodies allows for the development of ELISA assays for AHB detection and the development of dipstick-type immunoassays for rapid and economical detection of AHB. The ELISA approach should provide a means for quantitative estimation, in a laboratory setting, of the degree of Africanization of large scale samples from swarms and traplines. The ready availability of automated equipment for this type of assay makes its use attractive for tests at centralized facilities. The dipstick, or test-strip, type of assay has proven very reliable in clinical analysis and this approach is well suited for rapid, low cost analyses in the field. Potential uses include hive sampling and rapid field identification of Africanized swarms.

Analysis of malate dehydrogenases (MDH) of honey bees has shown the presence of 3 alleles for the cytoplasmic form of this enzyme. Previous studies (Nunamaker, PhD Dissertation, Univ. Wyoming) on *Apis mellifera scutellata* from African populations showed that the "fast" or "5" allele was present in 100% of these colonies, but was only present in low frequencies in other *Apis mellifera* subspecies. These findings suggested that the MDH "5" allele might be a useful marker for assaying the degree of Africanization of bee colonies in North and South America. In order to assess the usefulness of this approach, we have determined the MDH allele frequencies of a number of Africanized bee samples from Central and South America, and have carried out a brood survey of European Bees (EHB) across the United States. The AHB samples from Central and South America have high MDH-5 allele frequencies, but certainly not 100%. More importantly, allele frequencies of EHB samples show a very broad range of variation (3%-65% MDH-5). Even different colonies of European Bees from the same apiary can have very different MDH allele frequencies (13%-65% MDH-5). We conclude that MDH allele frequency analysis is an inappropriate tool for assessing the degree of Africanization of honey bees in the U.S.

These studies were supported in part by a grant from The Texas Advanced Technology Program.

15. Loper, G. M.[a] and M. M. Fierro[q] — USE OF DRONE TRAPPING AND DRONE RELEASES TO INFLUENCE MATINGS OF EUROPEAN QUEENS IN AN AFRICANIZED HONEY BEE AREA; TAPACHULA, CHIAPAS, MEXICO — This study was very similar to that reported by Hellmich, *et al. (J. Econ. Entomol.* 81:796-799) which had been conducted in Venezuela. However, instead of supplying European drone source colonies located in the central queen nuc apiary, the drone colonies were placed 1 km away from the queen nucs in each of 4 cardinal directions (Taylor and Rowell In *Africanized Bees and Bee Mites*, Chap. 21, pp. 173-192. 1988). Additionally, modified Taylor aerial net traps (*J. Apic. Res.* 23:18-20) were used to eliminate many of the Africanized drones before the release of the second batch of sister Cordovan virgin queens. The drone colonies supplied about 6,400 yellow or cordovan colored drones whereas the drone trapping eliminated about 6,400 "feral" Africanized drones. Some of the drones may have come from some managed colonies, but over 80% of the trapped drones were black (Table) and highly Africanized and only 2% were yellow or Cordovan.

The progeny of 12 queens from the pre-drone control mating and 8 queens from the post-drone control mating were rated in terms of the number of yellow bands on their abdomens. There seemed to be a "break point" at 1½ yellow bands; *ie.* 52.6% of the progeny from the first mating (with the feral drone population) had 1½ or fewer yellow bands whereas only 6.4% of the progeny from the controlled mating had 1½ or fewer yellow bands.

It is obvious that drone populations, and therefore mating results, can be influenced with current technologies. But only in very isolated situations would total drone control be possible. Some introgression of African genes may be desirable in producing genetic lines for the future (Hellmich and Waller, *Am. Bee J.*, 130:537-542.)

Table — Drone trapping, color sorting results. Ejido Joaquin M. Gutierrez, Chiapas, Mexico, 1989.

Date	Color Sort				TOTAL
	% Black		% Yellow		
Nov. 17	85.7		14.3		63
	% Banded Black	% Black	% Semi-Yellow	% Yellow + Cordovan	
Nov. 30-Dec. 7	64.5 ± 7.8*	15.8 ± 6.0	18.5 ± 8.2	1.2 ± 0.8	6,036
	% Black		% Semi-Yellow	% Yellow + Cordovan	
Dec. 8	39.9		9.3	50.8	801
Dec. 9, 14, 19	66.7 ± 11.7		28.0 ± 10.1	5.2 ± 4.4	803

* ± Standard deviation

16. Loper, G. M.[a] W. W. Wolf[r] and O. R. Taylor, Jr.[s] – SUMMARY OF STUDIES ATTEMPTING TO INFLUENCE HONEY BEE DRONE FLIGHT DIRECTION – RADAR OBSERVATIONS – The use of a portable X-band radar has documented that drones orient to tree-lines and other structural features and form broad (80-100 m wide) flyways to the side (60-100 m) of such features. For example, in a flat desert area west of Tucson, AZ, drones from an apiary formed flyways on either side of a line of short mesquite trees growing in a dry wash. The drone flyway curved with the tree line and extended in excess of 4 km along the wash. At various places, the flyway branched and congregation areas formed apparently in relation to other features encountered along the way. Beginning in 1989, some efforts were made to attempt to influence drone flight direction by providing new lines of visual cues. In 1989, the studies were conducted west of Tucson on an old air field where there were only a few small (<3 m) trees and low (<1 m) shrubs, but where drone flyways were known to exist (*i.e.* "natural" conditions). In 1990 the artificial lines of cues were established on the flat, barren bed of a large 15 x 15 km (roughly heart-shaped) dry lake – the Wilcox Playa near Willcox, AZ (*i.e.* "unnatural" conditions). The reason for going to the Playa in 1990 was that even the low shrubs on the airfield interfered with the radar viewing. In both areas a line of "artificial trees" up to 1,200 m long was created. A "tree" consisted of a 2 m steel fence post (placed at 3-5 m intervals) to which a triangular piece of plywood was wired (cut from 4' x 8' plywood sheets, 2/sheet). The first tree line (1989) was essentially straight with a slight curve "dog leg" at the end. Another was a straight line and another formed a semi-circular curve intersecting a known flyway. The one straight line also had a width of mosquito netting (Army green, 28 cm wide) which was weighed down with fence posts. In 1990, we used 1 straight line of the "artificial trees" (1,200 m) and another line (curved near one end) of the netting (850 m long). Additionally, on the lake bed, any vehicle track was also an obvious cue and care was taken to establish long straight sets of tracks in a rectangular pattern near the central apiary.

In 1989, the radar viewing was interfered with – at the lower levels (10-15 m above ground) by the shrubs, but occasionally, drones appeared to be diverted to new directions by the artificial cues. The curved tree line that crossed a known flyway was installed in one morning and as the drones began to fly, they "piled up" in the curve. However, later in the day they were crossing it reforming their normal flyway. (This was not documented by radar, but from the sound of drones flying (or not flying) on either side of the tree line).

On the Playa, there were very few other insects and most of the workers were diverted to a source of sugar-water. However, both workers and drones were flying low (½-5 m) in the experimental area and the radar could not tell worker from drone targets. Only by comparing pictures before and during drone flight time and by using queen pheromone could some deductions of drone flight be surmised. Since the bees flew so close to the ground visual observations were made to complement the radar.

Unfortunately, the "tree line" was a strong reflector of radar energy and since the bees flew so low, bee activity near it was obliterated by radar "clutter." However, bee activity near the netting and tracks was obvious. In addition to the scatter of bees in the general radar viewing area (800 m radius, 360°) the radar pictures show insects flying over and alongside all straight line and curved features in greater numbers than in the rest of the viewing area (except to the sugar-water). Only during drone flight time, a DCA-like feature developed at the end of a set of tracks: drones flew away from the apiary along these tracks for approximately 800 m until they reached the end of the road. About 50 m beyond this point, a separate group of targets formed from which they dispersed in all directions, but this grouping continued for many minutes.

We feel that we have sufficiently documented that drones use (even seek out) visual orientation cues and form flyways along them using them in both outward and returning flight.

17. Loper, G. M.[a] and E. A. Sugden[n] – USE OF AVERMECTIN-TYPE INSECTICIDE APPLIED TO DRONE HONEY BEES TO CONTROL FERAL COLONY POPULATIONS[pp] – A large population of feral colonies exists in the United States especially in locales where the African honey bee (AHB) is expected to become endemic. In most cases, these feral populations are sufficiently isolated such that they pose no threat to humans and they provide a beneficial source of pollinators for wild plants. However, in some locales, urban, recreational and queen mating areas, especially, feral AHB may become a socioeconomic problem. In these areas, periodic elimination of feral colonies may become necessary. Most feral colonies, especially AHB colonies, produce a large number of drones, and when flying they can be attracted in large numbers (using queen pheromones) and caught in aerial net (Taylor) traps.

The Avermectins are a class of compounds derived from *Streptomyces avermitilis* produced by fermentation. Commercial preparations of these compounds: Avermectin ("Avid") and Ivermectin ("Zimectrin") have been formulated to use against the "fire ant" and numerous internal parasities in animals, respectively. Drones escaping from the top of a modified Taylor drone trap were forced to contact either Ivermectin or a mixture of Avermectin and Ivermectin (A + I). This resulted in the death of the drones in less than 24 h, but their nest mates survived (Loper, unpubl.). A procedure of manually dosing drones with 5 mg of A + I (≈ 70 μg active ingredient) was developed and this paper describes the experiments and results of tests conducted in 1989 and 1990.

In Arizona (1989) 60 drones returning to a feral colony were caught at the nest entrance and treated with 41 μg/drone of A + I and released 100 m from the nest. Treated drones were observed (but not counted) returning to the nest. Within 13 h, the colony was severely affected (many dead drones and bees at the entrance) but it took approximately 10 weeks for it to die. Apparently, the sealed brood (worker and drones) survived after emergence, but with no queen the colony was doomed.

In Texas (1990) a series of domesticated colonies were similarly treated (58-81 μg/drone). The number of treated drones allowed to return to a colony were: 25, 35, 45 and 55. All colonies were severely affected; colonies treated with 45-55 drones died quickly. In the sub-lethal treatments, each treated drone resulted in the death of approximately 250 nest mates. Some drones flew at least 2 km after treatment to return to their colony. Feral drones were caught during the experiment, but no feral colony was located. On one afternoon, 128 feral drones were dosed and no more drones could be caught at that location on subsequent days. However, virgin Cordovan queen successfully mated with wild-type drones after this treatment. These drones could have come from: (1) newly emerged and matured drones from the treated feral colony(s); (2) drones from untreated (or under-treated) feral colonies within flight range of the queen.

Samples of dead bees, honey and wax from treated colonies were analyzed by Paul Rivera for residues of A and I, but no detectable levels (less than 0.1 μg/bee) of residues were found, even with samples of 30 dead bees.

18. Macdonald, J.[t] and K. J. Clark[d] – 1989 FIELD TRIALS OF MENTHOL AS A CONTROL FOR TRACHEAL MITES – Honey bee colonies in an apiary infested with tracheal mites were divided into 4 groups:

1) Infested colonies to be treated with menthol,