# Proceedings of the American Bee Research Conference

***The 2004 American Bee Research Conference was held in conjunction with the annual meeting of the American Honey Producers Association in San Antonio, Texas on January 8 and 9. The 2005 meeting will be held with the American Beekeeping Federation at John Ascuaga's Nugget Casino in Reno, Nevada on January 14 and 15. Abstracts from the 2004 conference follow.***

**1. Aliano, N.P.[a] & M.D. Ellis [a] – STRATEGIES FOR USING POWDERED SUGAR TO REMOVE VARROA MITES FROM ADULT HONEY BEES** – Varroa mite populations on adult bees can be reduced by applying powdered sugar to a small population of adult bees in a screened jar (Macedo *et al.*, 2002 *J. Apic. Res.* 41: 3-7). Macedo *et al.*'s findings suggest that powdered sugar could be used to remove 90 % of Varroa mites from large populations of adult bees. To test this hypothesis, fourteen colonies were shaken, frame-by-frame, into a deep hive body with screened top, bottom and sides. The bees were dusted with 225 grams of powdered sugar, confined for five minutes and then released. Honey bees treated in this manner dropped 32 ± 5.61 % of their mites (see figure).

Our lower than expected result led us to conduct a separate experiment to identify factors contributing to mite fall. Three factors were considered, each with two levels: (1) box design (solid *vs.* screened bottom), (2) agitation (agitation *vs.* no agitation), and (3) bee density (high *vs.* low). Only the density-by-box design factor significantly affected the percentage of mites dropped from adult bees when dusted. Given high density, solid bottom design yielded 33.7 ± 7.84 % more mites than screened bottom. Agitation had no effect on mite fall ($P$ = 0.1049). We conclude that dusting adult bees with powdered sugar is most effective in a solid bottom container and increased crowding of bees results in greater mite fall.

We modified our bee-boxes based on the above study by adding solid bottoms and sides. In addition, boxes were designed to attach to the front of a honey bee colony, forming a bee-tight seal with the hive entrance. We isolated the adult populations of 28 colonies (from 4 apiaries) in our modified bee-boxes by applying a bee repellent (Bee Go®) to each colony. The repellent forced the adult bees into the detachable box where they were subsequently dusted with 225 grams of powdered sugar. We recovered 76.7 ± 3.63 % of the mites on adult bees in our modified box design (see figure).

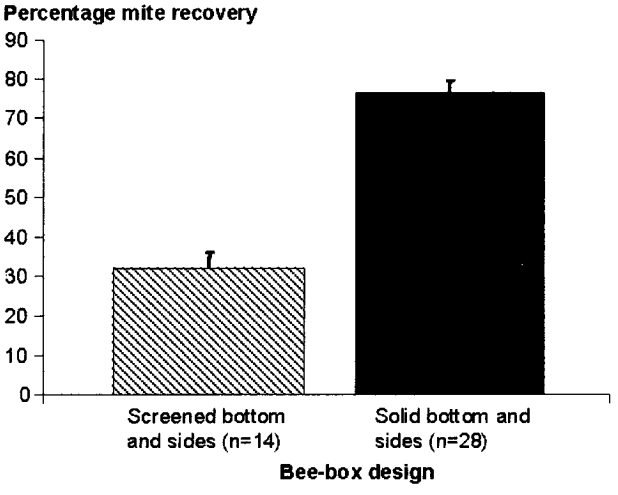



**Figure. Percentage mite recovery for two box designs used to treat adult honey bee populations for Varroa mites with powdered sugar.**

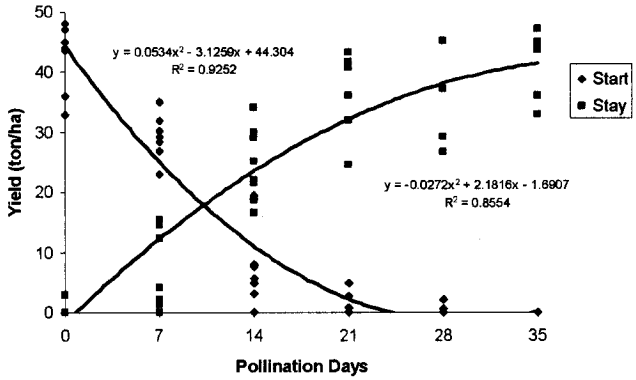
**2. Callaway, C. [a] & M.D. Ellis [a] — DRONE CONGREGATION AREAS IN URBAN SETTINGS** — We investigated the formation of drone congregation areas (DCAs) in an urban setting (Lincoln, Nebraska). Our main purpose was to locate DCAs on the University of Nebraska campus for use in teaching. We also compared the location of DCAs in our urban study with those reported in the literature for rural areas.

We were able to find five distinct DCAs within a one mile radius of the University's East Campus. We did not find DCAs in open areas, in areas with randomly distributed vegetation, around buildings or near prominent man-made structures such as smoke stacks and transmitting towers. We did find DCAs at the intersection of tree lines, along a creek, in the vicinity of high voltage power lines and at the intersection of a gravel road and a row of apple trees. DCAs were not found in any of the landscapes that are unique to urban settings. All DCAs that we located were found near landmarks that have been reported to trigger DCA formation in rural settings (Loper *et al.* 1992, *J. Kansas Entomol. Soc.* 65: 223-230). Our findings demonstrated the existence of DCAs in urban settings. They also suggest that drones orient to the same landmarks in urban and rural settings and that man made structures, abundant in urban areas, are not a factor in DCA formation. Green corridors may be important for DCA formation in urban settings.

**3. Cano-Ríos, P.[b], J. L. Reyes-Carrillo[c] & U. Nava-Camberos[b] — EFFECT OF POLLINATION PERIODS ON THE QUALITY AND YIELD OF MUSKMELON** — Muskmelon is one of the main vegetable crops in Mexico. It is grown in about 38 thousands hectares with an average yield of 15.2 tons/ha. In La Comarca Lagunera region, muskmelon is the main fruit crop and is cultivated in about 4 thousands hectares with an average yield of 25.6 tons/ha. In this region, muskmelon production has several problems, but the main problems are pests, poor irrigation management, and inadequate use of bees for pollination.

We wanted to how much yield is lost because of a late introduction of beehives for pollination and when the beehives could be removed from the muskmelon fields. Therefore, we carried out two experiments in 2001 and 2002 at La Laguna Experimental Station using the muskmelon hybrids Gold Rush (2001) and Cruiser (2002). Planting dates were April 18 and April 26 for 2001 and 2002, respectively. Four beehives/ha were used for pollination. In 2001 nine treatments were studied: In treatments 1, 2, 3, 4, and 5 pollination began at 1$^{st}$, 2$^{nd}$, 3$^{rd}$, 4$^{th}$ or 5$^{th}$ week of blooming, respectively ("start" group in fig.). For treatments 6-9 ("stay" group in fig.), pollination began on the 1$^{st}$ week of blooming, continued for 1, 2, 3, or 4 weeks, and were then covered with Agribon® the rest of the blooming period (*e.g.* tmt 6 = pollination the 1$^{st}$ week and then covered, tmt 9 had pollination in weeks 1-4 and was then covered). In 2002, a 10$^{th}$ treatment covering all the blooming period was added.

A significant quadratic relationship was found between the first five treatments and commercial yield with a determination coefficient of 92.5%. This quadratic model showed that when pollination began at the beginning of blooming the yield may be about 44.3 ton/ha. A significant quadratic relationship was also found between the last treatments (6-10) and commercial yields with a determination coefficient of 85.5%. This data indicates that beehives should remain in the muskmelon field no more than 28 days. That can be observed in the following figure.




**4. Danka, R. G. – AN OBSERVATION OF HIGH LEVELS OF COTTON POLLEN COLLECTED BY ITALIAN AND RUSSIAN HONEY BEES IN LOUISIANA** - Honey bees typically reject pollen of upland cotton, *Gossypium hirsutum*, as a resource. Stimulating bees for enhanced pollen collection may help overcome this rejection.

Sixteen equal-sized colonies of each of two commercial stocks of bees (Italian and Russian) were placed adjacent to 70 ha. of cotton fields at Rosedale, LA, in late July 2002. Half of the colonies of each bee type were given high stimulus to collect pollen and half were given low stimulus. Differential stimuli were achieved by exchanging combs having relatively large amounts of brood with combs having pollen between colonies of the two treatment groups (*i.e.*, high stimulus colonies received brood and donated pollen; low stimulus colonies had the converse). Stimulus manipulations resulted in significantly more general pollen collection, but not cotton pollen collection, in the high stimulus group on days 1 and 6 after treatment. Foraging responses of the treatment groups returned to being equal by day 11 (7 August). Italian colonies had greater total foraging activity and pollen collection effort on day 1 after treatment, but the bee types foraged similarly on days 6 and 11. There were no interactions of the effects of stimulus treatment and bee type. Collection of cotton pollen was minimal during this period and was not affected by stimulus treatment. After the treatment effects dissipated, however, collection of cotton pollen increased substantially. Approximately one-fourth of all foragers and 80% of pollen collectors carried cotton pollen pellets during a 2-week period in mid August (table). This appears to be the highest rate of upland cotton pollen collection recorded for honey bees. The reason for the dramatic shift in pollen gathering is undetermined, but may have been related to increased cotton flower availability in conjunction with the high humidity of the area. The phenomenon warrants further investigation because of the potential importance for cotton pollination by honey bees.

**Table. Collection of pollen from upland cotton (mean ± sd) by honey bee colonies during and after a test of effects of pollen collection stimuli (stimulus treatments were applied from 26 July to 7 August 2002). Data are from observations of all returning foragers during two, 4-min counts on all days except on 7 and 26 August when 60-80 returning foragers were captured and examined. Asterisks indicate that Italian colonies had a greater rate of cotton pollen collection than Russian colonies on the specified date ($P < 0.01$). Stimulus treatment had no effect on collection of cotton pollen.**

| | Date | | | | | |
|---|---|---|---|---|---|---|
| | 1 Aug. | 7 Aug. | 11 Aug. | 16 Aug. | 21 Aug.[1] | 26 Aug. |
| % of all foragers with cotton pollen | <1 | 2±3 | 25±14 | 24±11 | -- | 21±12 |
| Italian | <1 | 3±4 | 31±15* | 26±9 | 31±8 | 27±12* |
| Russian | <1 | 1±2 | 19±2 | 21±14 | -- | 14±9 |
| | | | | | | |
| % of pollen foragers with cotton pollen | 2±4 | 12±19 | 82±22 | 81±23 | -- | 67±25 |
| Italian | 3±6 | 21±23 | 89±21 | 91±14* | 86±10 | 78±19* |
| Russian | <1 | 3±8 | 76±22 | 72±26 | -- | 56±27 |

**[1]Data are incomplete for 21 August because of the onset of rain after eight Italian colonies were sampled.**

**5. Danka, R. G. & J.D. Villa - CONTEMPORARY SOYBEANS SHOW NO EVIDENCE OF YIELD INCREASES ASSOCIATED WITH PROXIMITY TO HONEY BEE COLONIES** – Research largely conducted during 1970-1985 on conventionally bred soybeans showed that the crop is generally self-fertile and often self-pollinating, that foraging activity by honey bees is highly variable both within fields and between fields, but that soybean yields in some circumstances may be increased by 10-15% following honey bee pollination (see summary by Erickson, 1984, *Am. Bee J.* 124: 775-779).

We assessed the potential for honey bee foraging to increase yields in recently bred soybean cultivars (some conventional, some transgenic). In measurements of seven commercial fields, we found no relationship between proximity to an apiary and soybean yield (see figure). Yields in these fields ranged from ca. 20 to 63 bushels per acre. The experimental situations represented a range of cultural practices, cultivars, bee-colony diversities, weather conditions during bloom, etc.

Densities of honey bee foragers relative to apiary locations were measured in 2001 in two fields in Louisiana by counting bees and flowers in 25-m sections of rows during mid day on multiple days during bloom (late June to early August). Forager densities varied greatly between the two fields. In Concordia Parish ('Deltapine 3478' soybeans), average densities (bees per 1000 flowers) ranged from 0.80 at 100 m to 0.21 at 600 m. In St. John Parish ('Deltapine 5915 RR'), average densities were 0.05 at 75 m, 0.01 at 200 m and 0.01 at 700 m. Yield data from each distance were not obtained from the Concordia Parish field, but the overall yield was 38 bushels per acre. Despite a much lower density of honey bee foragers, the field in St. John Parish yielded 53 bushels per acre (see figure).

Overall, we found no evidence that yields of contemporary soybean cultivars rise with increased foraging activity by honey bees during bloom.

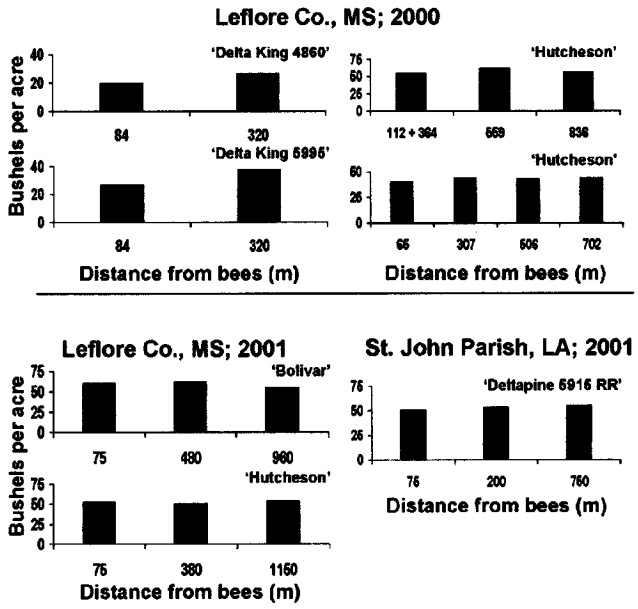



**Figure. Relationship of soybean yield and distance from honey bee colonies. Each graph shows data from an individual field. Yield typically was measured in a one-acre plot at each indicated distance from a group of honey bee colonies placed on one edge of each field. Colonies were isolated from other known bees by at least one mile. Harvested plots were located 50 feet from field edges and field roads to avoid confounding distance with edge effects.**

**6. Dedej, S. [e] & K.S. Delaplane [e] – ENERGETIC ADVANTAGE DRIVES HONEY BEES TO ROB RABBITEYE BLUEBERRY FLOWERS, *VACCINIUM ASHEI* READE** – Carpenter bees (*Xylocopa virginica* L.) invariably rob flowers of rabbiteye blueberry *Vaccinium ashei* Reade by perforating the corolla laterally to reach basal nectaries. Honey bees subsequently learn to switch from legitimate flower visits at corolla apertures to illegitimate visits at the lateral perforations, thus becoming secondary nectar thieves (Dedej & Delaplane 2004, *Environ. Entomol.* 33: 100-106). We hypothesized that honey bees switch to illegitimate visits because in doing so they realize a net energetic advantage. We tested this

hypothesis in a study conducted at a permanent blueberry orchard in Oconee County, Georgia USA during blooming season of 2003.

Based on the measurements of nectar standing crop in unvisited flowers ($n$=299) and in flowers after one visit by a honey bee ($n$ = 275) (both perforated and intact flowers) on six days, the quantity of energy (J) ingested per bee visit from one flower was calculated. The energy spent per bee visit (illegitimate or legitimate) for one flower was calculated based on volumes of oxygen consumption as referenced by Wolf et al. (1989 *Funct. Ecol.* 4: 417 – 424), using the coefficient of 21.3 J per one 1 ml oxygen consumption (Harrison *et. al.*, 2001 *J. Exp. Biol.* 204: 805-814). The monitored and timed (with a hand-held stopwatch) foraging activity of 102 honey bees on *V. ashei* flowers performing either robbing or legitimate visits was used in these calculations.

Honey bees ingested approximately the same quantity (mg) of sugar per visit per flower performing either legitimate (0.5 ± 0.1, $n$ = 6) or robbing (0.4 ± 0.1, $n$ = 6) behavior, and consequently gained non different quantities of net energy per visit per flower, but robbers visited more flowers per unit time and showed superior nectar ingestion rates and net energy gain per s handling time per flower visited. Nectar ingestion rate and net energy gain per s handling time was either not different or (on 3 of 6 sampling days) significantly higher for illegitimate flower visitors (table).

The result of this study suggest that honey bee robbers visiting perforated flowers perform higher rates of visitation and ingest more nectar and energy per unit time, thus confirming in the affirmative the hypothesis stated above.

| Julian day | Number flowers visited per min | | | | Net energy gain (J) per sec handling time | | | | Nectar ingestion rate µl/s | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leg | | Illeg | | Leg | | Illeg | | Leg | | Illeg | |
| 87 | 2.6 | 0.3b (5) | 6.3 | 0.9a (7) | 0.8 | 0.05a (5) | 1.2 | 0.1a (7) | 0.1 | 0.01b (5) | 0.3 | 0.02a (7) |
| 88 | 2.5 | 0.2b (10) | 6.8 | 0.6a (10) | 0.8 | 0.2a (10) | 0.8 | 0.1a (10) | 0.2 | 0.04a (10) | 0.2 | 0.02a (10) |
| 91 | 3.3 | 0.5a (5) | 4.2 | 0.4a (6) | 1.1 | 0.3a (5) | 1.0 | 0.2a (6) | 0.1 | 0.03a (5) | 0.1 | 0.02a (6) |
| 92 | 4.0 | 0.3a (10) | 4.2 | 0.5a (10) | 0.5 | 0.08b (10) | 0.9 | 0.1a (10) | 0.1 | 0.01a (10) | 0.1 | 0.02a (10) |

**Table. Number flowers visited per min, net energy gain per sec handling time, and nectar ingestion rate by one honey bee during legitimate (Leg) or illegitimate (Illeg) rabbiteye blueberry flower visits for all days of measurements. Data were collected only for individual bees successively expressing one behavior type. Values are mean ± standard error, with *n* in parentheses. Means within a row for each variable followed by the same letter are not significantly different at the α = 0.05 level.**

**7. Delaplane, K.S. [e] — IPM PRACTICES DELAY ECONOMIC THRESHOLD FOR VARROA** — An economic threshold forms the conceptual and practical foundation for an effective IPM program against Varroa mites. This measure is defined as the mite level at which a colony should be treated in order to prevent the mite population from reaching damaging levels. By treating only when economic threshold is triggered, beekeepers eliminate unnecessary chemical treatments, reduce overall chemical use, protect product purity, reduce sublethal effects of chemicals on bees, and delay the evolution of chemical resistance in mites. Delaplane & Hood (1999 *Apidologie* 30: 383) derived an economic threshold of 60-190 mites on an overnight sticky sheet (unassisted by acaricide).

If beekeepers can chronologically delay chemical treatments, it not only reduces overall chemical use, but enables mites through genetic recombination and reproduction over time to conserve their chemical susceptible genes (see Metcalf, 1982. *In* Introduction to insect pest management, 2d. ed, John Wiley). Numerous IPM practices against Varroa have been developed, but in a collaborative project between the Univ. Georgia, Univ. Tennessee, and Clemson Univ. my colleagues and I have focused on three: apiary isolation (Sakofski *et al.*, 1990 *Apidologie* 21: 547), heritable suppressed mite reproduction in bees (SMR) (Harbo and Harris, 1999 *J. Econ. Entomol.* 92(2): 261-265), and screen hive bottoms (Pettis & Shimanuki, 1999 *Am. Bee J.* 139: 471). Our goal was to determine if these IPM practices reduce colony mite levels and delay onset of economic threshold.

The experiment was set up in March 2002 with 40 full-sized overwintered colonies in Oconee County, Georgia. Each colony was randomly assigned to an isolated apiary (no closer than 2 km to a known managed apiary) or a non-isolated apiary (i.e., within an existing managed apiary). Within each apiary situation each colony randomly received one of the following treatments (1) SMR queen + solid bottom, (2) non-selected + solid, (3) SMR + screen bottom, and (4) non-selected + screen. Colonies were monitored *ca.* every three weeks (except over winter 2002-2003) until December 2003 with 3-day sticky sheets to appraise colony mite levels and the onset of economic threshold. Three-day mite counts were converted to daily averages to correspond them to the economic threshold of Delaplane and Hood (1999).

Results were favorable or neutral for the practices examined. Over 87 weeks of sampling, colonies headed by SMR queens had significantly lower daily mite drop (7.8 – 1.1, $n$ = 317) than non-selected queens (9.5 – 1.5, $n$ = 236). Similarly, colonies with screen bottoms had significantly lower daily mite drop (6.7 – 1.0, $n$ = 275) than those with solid bottoms (10.4 – 1.5, $n$ = 278). On average it took 12.5 weeks longer for colonies headed by SMR queens to achieve economic threshold than those with non-selected queens (71.7 – 3.9 weeks, $n$=14 versus 59.2 – 4.4, $n$=13, $P$ = 0.012). The trend, although not significant, was the same in favor of isolated apiaries (66.3 – 3.0 weeks, $n$=17 versus 64.6 – 6.9, $n$ = 10). Screen bottoms did not delay economic threshold (65.9 – 4.8 weeks, $n$ = 17 for screens versus 65.5 – 4.0, $n$ = 10 for solid).

**8. Eischen, F.A.[f], R.H. Graham [f], & R. Cox [f] — REGIONAL DIFFERENCES IN THE PREVALENCE OF TETRACYCLINE-RESISTANT AMERICAN FOULBROOD IN HONEY BEE COLONIES OF WESTERN UNITED STATES** — We randomly sampled 567 honey bee colonies pollinating almonds in California during February, 2003 and examined them for the presence of *Paenibacillus larvae* spores. Positive identification was found in adult worker bees from 33% of the colonies. Colonies originating from the Rocky Mountain region and California produced significantly higher numbers of bacterial colony forming units (mean = 400 per 30 adult bees, $P < 0.05$), than colonies from the upper-Midwestern region (mean = 1.3). Colonies from the West, Northwest, Central and Southwest had intermediate bacterial colony forming unit (CFU) levels. Percentages of colonies with high CFUs (≥400 per 30 bees) differed significantly, with those from the Rocky Mountain region having 9.4% compared with 0% found in the upper-Midwest.

The significance of CFU levels in the California survey was evaluated by inoculating healthy colonies with diseased immatures. Random samples of 30 bees were taken from the broodnest at 3, 6, 12, 24, 48, 72, and 168 hours after inoculation. *P. larvae* spores were detected in 0%, 25%, 57%, 90%, and 82% of all samples drawn from colonies that received 0, 1, 10, 48.2, and 141.0 (n = 5) diseased immatures, respectively. The number of CFUs detected per diseased immature was conservatively calculated to be about 399 CFUs per 30 adult bees. We define this to be one Disease Equivalent number of *P. larvae* CFUs (this level of spores may arise from different sources, e.g. current AFB disease, robbing diseased colonies, past AFB disease or scale, etc.). Based on this, 3.86% of colonies in our California survey had one or more Disease Equivalent number of *P. larvae* CFUs. Our culturing techniques were accurate 80-90% of the time in detecting 48+ cells of active American foulbrood. When a 15% error factor is included, we project that of the 1.4 million colonies in the almond orchards, about 34,485 colonies had at least one Disease Equivalent number of *P. larvae* CFUs.

Resistance to tetracycline was found in 39.7% of the AFB-positive colonies or 10.4% of the total colonies sampled. The Inhibition Zone Diameter (IZD) of *P. larvae*-infected colonies from the Pacific Northwest were on average 25.9 ± 14.1mm, which was significantly smaller than those from the West and Southwest which averaged 35.8 ± 16.5mm ($P <$ 0.05). Upper Midwestern, Central and Rocky Mountain states had intermediate averages that were not significantly different. All *P. larvae* isolates were scored either resistant (IZD ≤ 20mm) or susceptible (IZD ≥ 40mm), i.e., no isolate was observed to be moderately resistant to OTC (IZD 21-39mm). The prevalence of OTC-resistance tended to be inversely related

to the population density of *P. larvae* spores, i.e., areas with higher spore counts tended to have lower levels of resistance to tetracycline.

It is not clear what the cause(s) of regional differences are. Aggressive control of AFB may be a factor. In any case, operations with high *P. larvae* spore levels in their colonies will likely observe American foulbrood if prophylaxis is not practiced diligently.

**9. Ferrari, T.E. [g] - ENPOLLINATION OF HONEY BEES DURING BLOOM IMPROVES CROP PRODUCTION IN ALMONDS: CASE HISTORIES** — Honey bees are used to cross pollinate almonds, but poor weather often limits foraging activity, and non synchronous bloom conditions between cultivars often limits availability of cross compatible pollen. Such problems encourage farm managers to improve forager efficiency by applying precollected pollen directly onto bees (enpollination) using a dispenser located at the beehive entrance, a cultural practice termed *supplemental pollination*. Recent advances in pollen application strategies (Ferrari, 2003 *Hort. Sci.* 38:740) were evaluated for 8 orchard varieties during the 2002 and 2003 bloom periods.

Pollen was collected from 100% compatible pollinizers by mechanically shaking trees during bloom, shredding the flowers, sifting unripe anthers from other flower parts, and ripening cleaned anthers 12 to 16 hours on paper-covered racks at 29-31 C. Fluorescence microscopy was used to determine viable pollens per gram and also to measure pollen performance *in situ* using live flower pistils on cuttings maintained at 21 C. The pollen dose applied was 150 million viable pollens per acre and pollen preparations produced more than 50 germ tube penetrations per pistil. Pollen applications of 50 and 100 million VPPG, respectively, were made at 15% and 30% blooms. Enpollination of honey bees was performed after foraging flight had begun. Hive densities were from 2 to 3 per pollen-treated acre.

Varieties exposed during bloom to enpollinated bees ranged from 15 to 150 acres and orchards contained from 2 to 4 different varieties (Nonpareil, Butte, Sonora, Carmel, and Peerless) which ranged from 8 to 23 years in age. Nuts were harvested by growers and production information was provided by hullers after processing. Yield histories ranged from 3 to 9 years. When pollen was dispersed by enpollinated bees, either (A) only one cultivar was in bloom; (B) two were in bloom, but only one was compatible with applied pollen; or (C) one variety was past peak bloom and flowers were no longer receptive, whereas other varieties were at an early stage of bloom and receptive. Historical yield ratios between two varieties, when no extra pollen was applied, were determined. The *average* historical yield ratio (before) was then compared with the ratio for the year when pollen was applied (after) to a treated variety: in each orchard, a non treated variety acted as the *reference*. The average historical ratio with the least variation (std. dev./average) was used to evaluate yield changes. A 1-tailed Z-score was the test statistic employed to analyze data.

All 8 varieties exposed to enpollinated honey bees resulted in an increase in the yield ratio and, consequently, yield. Evidence indicates highly significant ($P < 0.01$) improvements in crop production were achieved in the year pollen was applied for 4 of 8 varieties exposed to extra pollen. Supplemental pollen accounted for from 7% to 53% (avg. = 32%) of total nut production for the 8 case histories provided. The maximum increase in yield due to supplemental pollen was 1,154 lbs. (median increase = 632 lbs.) and accounted for 48% of nut production. *The consistent and sometimes drastic improvement in nut production for all varieties exposed to enpollinated honey bees is evidence that foragers are inherently inefficient at cross pollination.*

**10. Gregory, P.G. [d], T.E. Rinderer [d], & J.R. Harbo [d] – PATERNITY OF OFFSPRING FROM HONEY BEE QUEENS RE-INSEMINATED AFTER PRODUCING WORKER BROOD** – Being able to re-inseminate honey bee queens could be a valuable breeding tool. It may increase the length of a queen's reproductive period and maintain a queen's fecundity. It could be used to increase genetic variation after inbreeding or produce an inbred line by re-inseminating a queen with her own drones.

Honey bee queens from various sources were inseminated with one drone and then re-inseminated 3-8 months later with a second drone. Before the second insemination, we determined the genotype of each queen and 9 of her worker progeny for one polymorphic microsatellite locus A14 (Estoup *et al.*,1994 *Proc. R. Soc. Lond., B* 258:1) to infer the genotype of the first drone. Queens were removed from their colonies, caged, and stored together in a colony for 1 or 2 weeks prior to re-insemination. For the second insemination, about 50 drones were caught, marked with identification numbers on their thorax, and then genotyped. Based on genotype, a second drone was chosen for each queen so that progeny could be differentiated from that of the first drone. Genotypes were obtained nondestructively from queens and drones by extracting DNA using the Chelex boiling method from wing clippings (Walsh *et al.*,1991 *Biotechniques* 10:506; Gregory & Rinderer, *Entomol. Exp. Appl.*, in press). Workers were collected from brood cells and genotyped using leg segments of a pupa or wing clippings of an imago.

Queens were re-inseminated after producing worker brood for several months, and those queens subsequently produced offspring sired by both the first and second inseminations (Fig). There was, however, a time component in the use of sperm from the second insemination. For eggs fertilized about 1 week after the second insemination, all were progeny of the first insemination. By week 3, 5% of the offspring were fathered by the second drone, and by week 12, the percentage had increased to 38%. Physiological studies are needed to understand the initial absence and the gradual appearance of progeny from the second insemination. Re-inseminating queens shows promise as a breeding tool and may reveal information about sperm utilization by queen bees.

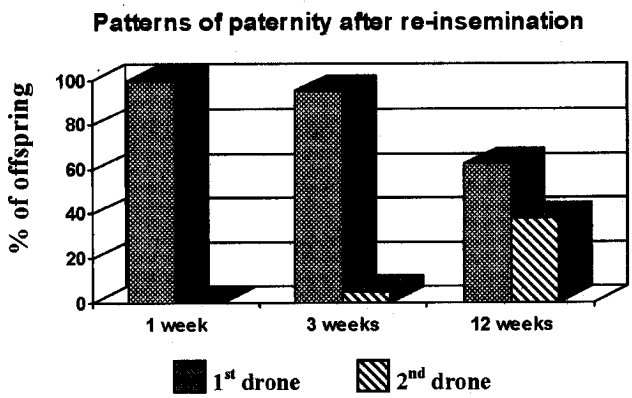



**Figure. Columns represent 451 workers from 12 queens, 168 workers from 10 queens and 80 workers from 4 queens, respectively. Eggs that developed into the workers described in the figure were laid (and fertilized) 1, 3, or 12 weeks after the second insemination.**

**11. Harbo, J.R. [d] & J.W. Harris [d] — EFFECT OF SCREEN OR WOOD BOTTOM BOARDS ON POPULATIONS OF HONEY BEES AND *VARROA* MITES** — Screen instead of wood on the floor of a bee hive has been tested over the years for decreasing moisture in honey, increasing ventilation in a colony, and monitoring mite populations. Most recently, screen bottom boards have been associated with the control of varroa populations (Pettis & Shimanuki, 1999 *Am. Bee J.* 139: 471-473; Ostiguy *et al.*, 2000, *Am. Bee J.* 140: 906-907; Ellis *et al.*, 2001, *Am. Bee J.* 141: 813-816). Our objectives were to determine if screen bottom boards affect bees and mites during a 9-week field test. We measured the effect of screen bottom boards on the growth of bee populations, the production of brood, and the growth of mite populations.

Two experiments were conducted in Baton Rouge, Louisiana, one in winter (beginning 23 Jan., 19 colonies) and one in summer (beginning 26 June, 14 colonies). In both tests, we established uniform colonies of honey bees by subdividing 30 kg of mite-infested bees that had been collected into a large cage. Each colony was established with about 11,000 bees, no brood, a free-mated queen that was not selected for resistance to varroa, and 127 mites (480 mites per colony in the summer experiment). Hives had either a traditional wood bottom board or an open-screen bottom board that had 20 cm of outside air space between the screen and the ground. All screen consisted of 1/8 inch (3.2 mm) mesh.

Analyses combined winter and summer data. After nine weeks, colonies with the open-screen treatment had significantly fewer mites (23% less in winter, 39% less in summer; df = 1, 28; $P = 0.03$), more cells of capped brood (17% more in winter, 10% more in summer; df = 1, 26; $P = 0.04$), and a lower percentage of their mite population in brood cells (21% less in winter and 13% less in summer; df = 1, 26; $P = 0.004$) than colonies with traditional wood bottom boards. A honey bee colony with open-screen bottom boards may slow the rate of growth of its mite population by decreasing the rate at which mites invade brood cells.

The summer test also included a third treatment (8 colonies) where a wooden tray (5 cm deep) created a closed space beneath a formerly open-screen bottom board. Results with a closed-screen bottom board were similar to results with an open-screen when measuring brood production and final mite population, but more similar to results with a wood bottom board when measuring the rate of mite invasion into brood cells. This suggests that open and closed screeens may affect mites in different ways.

**12. Harris, J.W.[d] & J.R. Harbo[d] – Selective breeding for honey bees with a low percentage of varroa mites in capped brood** – We selected colonies of bees for extremes in the percentage of varroa mites in capped brood (P-MIB). P-MIB provides a measure of the duration of the mite reproductive cycle (Otten, 1991 *Apidologie* 22: 466). Time that mites spend on adult bees increases as P-MIB decreases. Therefore, a lower P-MIB should result in diminished growth of mite populations (Fig).

Selection for extremes in P-MIB occurred in two field trials (22 colonies in each) that began on 20 May and 12 June 2003. Bees for each experiment were taken from a uniform mixture of bees and mites that had been collected into a large cage. Each colony began with 2.5 lbs (1.1 kg) of bees, no brood, 253 mites (921 in second test), and a test queen that was inseminated with semen from one drone. P-MIB was measured in each colony 9 weeks later (Harbo & Harris, 1999, *Apidologie* 30: 183-196). We selected three colonies with the lowest P-MIB and three with the highest P-MIB from each apiary as breeding stock. Because amounts of brood and bees influence P-MIB (Boot *et al.*, 1994, *Bull. Entomol. Res.* 84: 3-10), colonies were selected only if they had ≥ 150 square inches of capped brood and ≥ 1.0 kg of adult bees at the end of the test. The means of P-MIB (mean ± SD) for the low groups were 42 ± 8% (apiary A) and 30 ± 16% (apiary B), and those for the high groups were 85 ± 10% (apiary A) and 66 ± 2% (apiary B). As breeding continues, our goals are: (1) to produce lines with high and low P-MIB, (2) to determine the lowest limits for P-MIB, and (3) to show that low P-MIB can control mite populations in the absence of other varroa resistance traits.

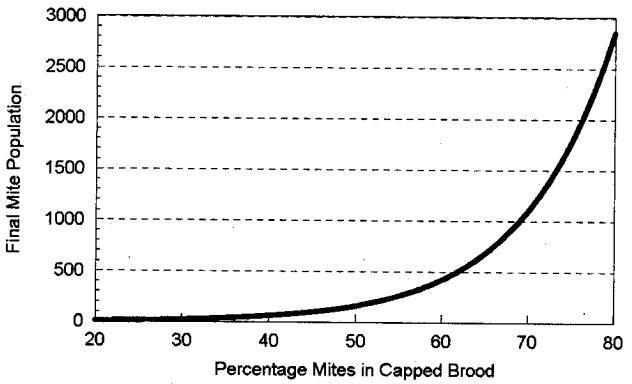



**Figure. Predicted effect of P-MIB on growth of a varroa mite population after 100 days. Model assumes no drone brood, an initial population of 100 mites, fertility rate of 75%, 1.45 female offspring per fertile mite per cycle, and a mortality rate of 0.019 mites per day. With this model, zero population growth of mites occurs when P-MIB = 47%.**

**13. Hood, W.M.[h] - EVALUATION OF AN UPPER HIVE ENTRANCE FOR CONTROL OF SMALL HIVE BEETLES, *AETHINA TUMIDA* MURRAY** - Small hive beetles (SHB) have now spread throughout most regions of the eastern USA and will likely spread to the western regions of the country in the next few years. SHB have caused major problems only in the coastal areas of the southeastern region of the country, but the pest has become a problem in other areas when conditions were favorable for SHB population buildup. Chemical products Check Mite +® for in-hive treatment and Gard Star® for soil treatment are now available for SHB control in many states in the USA. Other non-chemical alternatives are needed to provide safe and efficient control of this new hive pest. One alternative that has been investigated is the use of an upper hive entrance (Ellis et al., 2003 *Am. Bee J.* 142(2): 288-290; Ellis *et al.*, 2003 *J. Econ. Entomol.* in press). They tested polyvinyl chloride (PVC) pipe hive upper entrances (1.9 cm ID [inner diameter] and 3.8 cm ID) which gave mixed results for beetle control and a decrease in brood production for upper hive entrance colonies. Brood losses were partly mitigated by use of screened bottom boards.

I conducted further research testing the PVC pipe hive upper entrance (3.8-cm ID) over a full season from April-November. Nuc honey bee colonies (2# package bees and queen) were established in April in two apiaries located in two coastal South Carolina counties where SHB populations were well established and had been present for a minimum of 3 years. Each apiary contained eight colonies with four colonies having a PVC pipe hive upper entrance and the other four had a normal lower hive entrance as controls. Colonies were surveyed for SHB populations at 3-4 week intervals and capped bee brood was measured at 6-8 week intervals. The number of SHB counted in the PVC pipe hive entrance colonies and the control colonies were not significantly different (Figure). But, the amount of capped brood in the upper hive entrance colonies was significantly less. The use of a PVC pipe hive upper entrance as tested here is not recommended, especially in areas where SHB are well established. I conducted these tests using solid wood bottom boards. If I had used screened bottom boards, brood loss may have been partly mitigated.

| Hive Entrance | Adult Beetles | Sealed Bee Brood ($25cm^2$) |
|---|---|---|
| Lower | 41.31 ± 5.89 a | 28.99 ± 3.83 a |
| Upper | 29.64 ± 5.77 a | 16.88 ± 3.85 b |

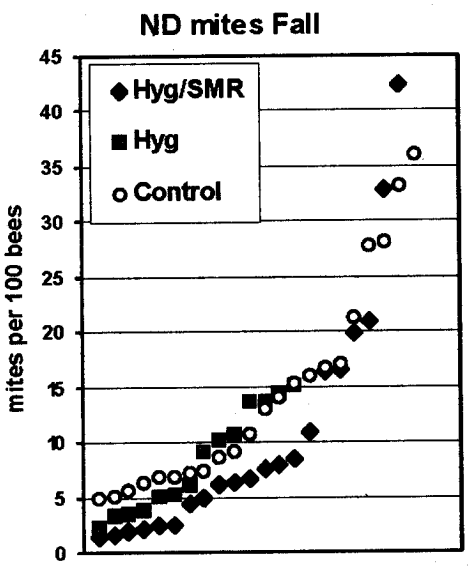
**Table. Data (least square means ± SE) are small hive beetles counts and honey bee capped brood measurements in nuc colonies having an upper PVC pipe (3.8 cm ID) hive entrance versus a standard lower hive entrance. Means within rows followed by different letters are different at the $P \leq 0.05$ level.**

**14. Hunt, G.J.,[i] E. Guzmán-Novoa [j], & R.E. Page [k] - PATERNAL EFFECTS INFLUENCE THE DEFENSIVE BEHAVIOR OF HYBRIDS BETWEEN AFRICANIZED AND EUROPEAN HONEY BEES** - We recently reported that the highly defensive behavior of Africanized honey bees (AHB) is genetically dominant to the gentle behavior of European honey bees (EHB) as a colony level trait. We also reported that in mixed-genotype colonies AHB efficiently recruit EHB to sting and that genotype by environment interactions occur that influence guarding behavior. Further analyses of over 30 hybrid colonies from 4 different sets of crosses showed that reciprocal crosses resulted in colonies that were not equal in their stinging tendencies. In each set of crosses, F1 colonies fathered by Africanized drones always gave more stings on average than those fathered by European drones. The paternal effect explains most of the dominance effect previously observed. This paternal effect is important for beekeepers to be aware of because queen breeders have good control over the queen source, but it is more difficult to control the drone source. Given this fact and the interactions that occur in recruitment, a queen that mates with a small number of Africanized drones may result in an unacceptably defensive hive.

**15. Ibrahim, A. [m], G.S. Reuter [m] & M. Spivak [m] – PROGRESS IN BREEDING HONEY BEES FOR RESISTANCE TO *VARROA DESTRUCTOR*** - Since 1994, we have been breeding honey bees for hygienic behavior (HYG), winter survivorship, honey production and gentleness. Field trials (e.g., Spivak & Reuter, 2001 *J. Econ. Entomol.* 94:326-331) demonstrated that HYG colonies have good disease resistance, but only partial resistance against mites. To increase mite resistance, we began incorporating the trait, Suppression of Mite Reproduction (SMR; Harbo & Harris, 1999 *Apidologie* 30: 183-196) by crossing SMR and HYG queens and drones through instrumental insemination. In 2003, queens that were 75%SMR:25% HYG mated naturally with HYG drones in a commercial apiary in eastern TX to produce workers that were on average 63% HYG:37% SMR. These HYG/SMR colonies were compared to HYG colonies and control (unselected) colonies in commercial apiaries in Minnesota and North Dakota (24 colonies from each line were transported to each state in May). Of the colony parameters measured (hygienic behav-

ior, honey production, brood viability), we report only mite loads on adult bees and in worker brood.

In both states, the average number of mites per 100 adult bees in spring was the same, but by fall, the HYG/SMR colonies in MN had significantly fewer mites (0.9 - 0.2 s.e.) than the HYG (2.7 - 0.8) and control colonies (3.9 - 1.1 ) ($P = 0.01$). In ND by fall, mites on adult bees for individual colonies were lower in the HYG/SMR than in HYG and control colonies (Figure); exceptions have been eliminated from breeding program. In ND, the average percent infestation of mites in worker brood (mites / 200 sealed cells) in the HYG/SMR and HYG colonies was 25.9% - 4.4 and 27.1% - 4.9, respectively, both significantly lower than in the control colonies, 53.9% - 4.3 ($P < 0.001$; data from MN still being analyzed). Partial data on reproductive success of mites in worker brood (at least one deutonymph on tan-colored pupae) indicate mites in HYG/SMR colonies had less reproductive success (32.8% - 26.2) than mites in Hyg (45.9% - 19.9) and controls (50.4% - 27.4). Tentative conclusions suggest that the incorporation of the SMR trait into the hygienic line can significantly increase mite resistance.




**16. Ibrahim, A. [m] & M. Spivak [m] - THE RELATIONSHIP BETWEEN SUPPRESSION OF MITE REPRODUCTION (SMR) AND HYGIENIC BEHAVIOR** - In 2002, we tested colonies with inseminated queens bred for Suppression of Mite Reproduction (SMR) (Harbo & Hoopingarner, 1997 *J. Econ. Entomol* 90:893 -898) for hygienic behavior, using the freeze-killed brood test (Spivak & Reuter, 1998 *Am. Bee J.* 283:186). The SMR colonies (n = 7) removed 98.6% - 0.9 of the freeze-killed brood compared to 97.5% - 1.9 by hygienic colonies (n=12). These surprising results led us to test the following questions: (1) do bees bred for SMR detect and remove brood infested with *Varroa destructor*? (2) If so, do SMR bees preferentially remove pupae infested with reproductive mites (mites with offspring) leaving pupae with non-reproductive mites? And, (3) When SMR bees are not allowed to remove infested brood, how well do mites in SMR colonies reproduce on SMR brood vs. non-SMR brood? Here we report preliminary data from ongoing experiments.

We first introduced mites from one non-SMR colony into recently sealed worker brood cells within 5 SMR, 2 hygienic and 2 non-hygienic colonies (following Spivak, 1996 *Apidologie* 27:245 -250). The percent of mite-infested pupae removed after 10 days was 91.8% (of 110 infested cells), 89.1% (64 cells), and 50.0% (20 cells), respectively.

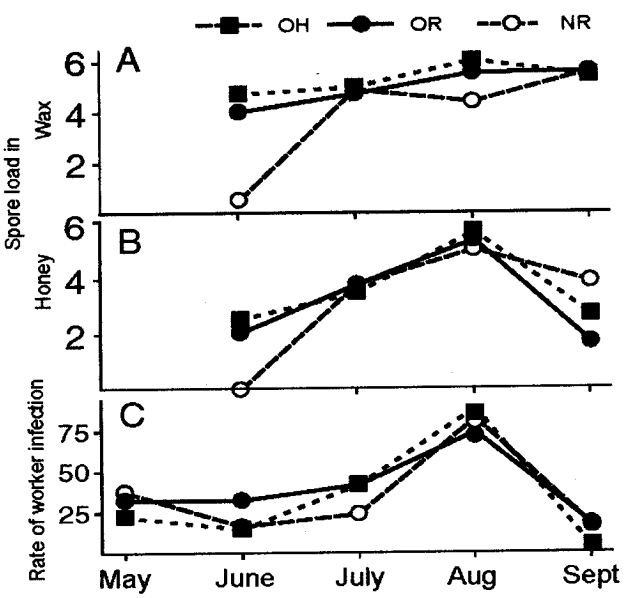
For question 2, we recorded mite reproductive success on naturally infested worker brood. Comb sections containing 4th instar larvae from SMR and non-SMR colonies were introduced into different, infested SMR and non-SMR colonies. Each infested colony received brood from both lines, and after the worker cells were sealed, the combs were replaced in their original colonies. Three days before the pupae eclosed, we recorded the reproductive success of the mites, defined by the presence of at least one deutonymph on a pupa with a yellow thorax (Martin, 1994, *Exp & Appl Acarol*, 18:78 -100). No mites from SMR infested colonies were found in SMR worker brood; however, 47.1% (8 of 17 remaining infested cells) of the mites from SMR colonies reproduced successfully on non-SMR brood. In contrast, successful reproduction of mites from non-SMR colonies on SMR brood was 71.4% (5 of 7 remaining infested cells) and on non-SMR brood was 72.7% (56 of 77).

For question 3, we placed cages over sections of worker brood that were artificially or naturally infested with mites. Of pupae artificially infested with mites from SMR colonies, 42.1% of mites (8 mites of 19 infested cells) reproduced successfully on SMR brood compared to 73.7% (14 of 19 cells) on non-SMR brood. Of the naturally infested pupae, 12.5% (1 of 8) of the mites from SMR colonies reproduced successfully on SMR brood, compared to 48% (24 of 50) on non-SMR brood. In contrast, 79.3% (23 of 29) of the mites from non-SMR colonies reproduced successfully on SMR brood compared to 90% (43 of 48) in non-SMR brood.

Although we are repeating these experiments, our preliminary data suggest that bees bred for SMR are also hygienic and remove a high proportion of mite-infested worker brood, which may contribute to their high degree of mite resistance. The mites on the remaining pupae appear to be reproductive; however, mites in SMR colonies have less reproductive success than mites in non-SMR colonies. The low reproductive success of SMR mites does not appear to be a permanent condition and increases if they infest non-SMR brood.

**17. Qin, Y. [n, o] & Z.Y. Huang [o] - DYNAMICS OF *NOSEMA* SPORES IN HONEY, BEESWAX AND THEIR RELATIONSHIP TO WORKER INFECTION** - Nosema disease (*Nosema apis*) can cause serious damage to honey bee colonies, resulting in queen loss, winter mortality and reduced honey yield. Although Nosema disease has been studied for over 50 years, there are still major gaps in our understanding of its epidemiology.

We determined dynamics of *Nosema* spore loads in honey and beeswax, and examined rates of worker infection in three treatments: colonies with new equipment and regular queens (NR, N=6), old equipment and regular queens (OR, N=4), and old equipment and hygienic queens (OH, N=5). We sampled two honey and two wax samples from each colony monthly. Honey samples were collected near the broodnest and wax was collected from 10 wax caps from brood cells. For worker infection rate, we introduced 100 newly emerged (0 day old) workers into their natal colonies and recovered 30-50 workers when they were 10 days old. Nosema spores were extracted and counted with a hemocytometer under 400x magnification. Fig. 1 shows that (A). Nosema spore levels remained very constant inside beeswax. The NR group showed much lower spore loads in June, but it quickly became the same as the other groups. (B). Nosema spores in honey increased steadily from June till Aug, but declined in Sept, again the spore load was low (zero) in the NR group in June, but quickly became the same as the others (C). When old workers from packages were sampled (May), they remained infected with Nosema despite being treated with Fumidil. The rate of infection in newly emerged bees peaked in Aug and dropped in Sept, similar to the dynamics of spore loads in honey. The correlation between the two variables was significant (n=12, r=0.77, P <0.01).

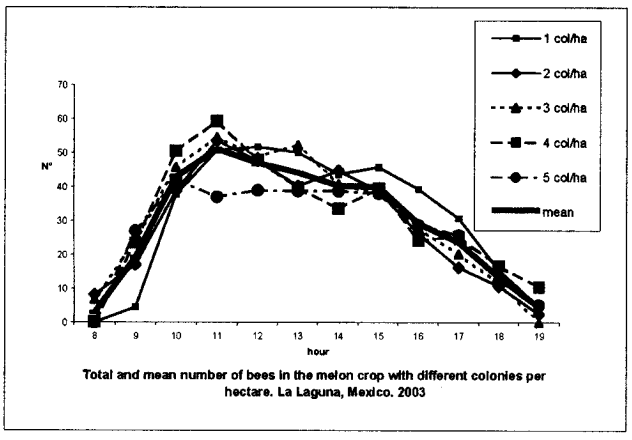


The correlation between rates of worker infection and spore loads in beeswax was not significant (n=12, r=0.25, P>0.05).

We conclude from this preliminary study that worker infection rates are more correlated with spore levels in honey. Spore loads remained high in beeswax in Sept, perhaps serving as the main source of re-infection for next year. When colonies were stocked with infected bees, new equipment did not help. Hygienic queens (artificially inseminated) did not show a significant impact on spore loads in this study. We thank Michigan Department of Agriculture for funding this study.

**18. Reyes-Carrillo, J.L [p] ,P. Cano-Ríos [b], F. Eischen [f] & E. Gaona-González [p] - BEE DISTRIBUTION IN THE CANTALOUPE CROP (*CUCUMIS MELO* L) WITH DIFFERENT NUMBER OF COLONIES** - The cantaloupe crop is the cucurbit with most hectares in Mexico. In 2002 La Laguna area (Coahuila and Durango states) had 3943 ha of muskmelon with an average yield of 25.6 ton/ha, but with the use of beehives this can be increased by at least 20 ton/ha, however, the appropriate number of pollinating colonies per hectare is not known. Therefore, the objective of the present work was to determine the number of beehives per hectare on the basis of the bee distribution on the cantaloupe field.

The research was carried out in a commercial melon crop planted with the Cruiser hybrid. During the second blooming week Italian beehives (Jumbo size) were increasingly placed from one up to five colonies per hectare transferring them one day before every observation. Weeds were mechanically controlled, so they did not affect pollination. Colonies were uniformly distributed adjacent to the field. In 5 randomly-selected rows of 105 m, we marked 10 m transects at 25, 50,75 and 100 m distances from the apiary. The foraging bees were observed in the transects simultaneously every hour from 8:00 am until 8.00 pm. Significant differences in the number of bees were found among the distances evaluated. 50m had the most bees (7.2a), 25 and 75m had an intermediate number (6.6ab) and (6.7ab), respectively, 100m had the fewest (5.3c)($p<.05$). The total number of bees present in the melon field varied with time of day($P<0.05$), reaching the highest between 11 AM and 3 PM (figure). Three colonies per hectare were adequate to maintain consistent populations of foraging bees in the melon field.




Total and mean number of bees in the melon crop with different colonies per hectare. La Laguna, Mexico. 2003

**19. Rivera, R., [f] F.A. Eischen,[f] H.R. Graham[f] & G.M. Acuña[f] - FUNGICIDE RESIDUES IN HONEY BEES, POLLEN, LARVAE, BROOD FOOD AND NECTAR DURING ALMOND POLLINATION** - About 1.2 million honey bee colonies are transported into the Central Valley of California for almond pollination. Weather conditions during almond bloom in this region of the Central Valley of California are often moist and cool. Fungal diseases such as almond blossom rot are actively controlled by spraying one or more fungicides during early and peak bloom using Rovral, Rally, Vangard or Ziram sprays. Captan is typically used prophylactically during late bloom and petal fall, but occasionally it is sprayed during peak bloom. Honey bees from colonies placed in the almond orchards for pollination are exposed to fungicide sprays during application. Atkins (1977) estimated the $LD_{50}$ for Captan to be 5.39ug for 3-day old larvae and indicated that, based on the level of mortality observed in his laboratory assay, that 30-40% of brood could be killed by the sprays applied to the trees. Mussen (pers. comm. 2003) reported that in a laboratory study, feeding brood directly with food spiked with Captan (parts per billion), killed 100% of larvae.

Samples of honey bees and their corbicular pollen loads were collected immediately after spray, using a sweep net. Hive samples were collected at 24, 48 and 72 hours after spraying, which included honey bees, stored pollen, nectar, brood food and larvae. We determined the levels of three commonly used fungicides, Captan, Rally (myclobutanil), and Rovral (iprodione) in these samples using gas chromatography. Based on these results, we fed a mixture of pollen, sugar, water and 0, 50, 500, and 2000 ppm of Captan, Rovral, Rally, Vanguard (cyprodinil), and Ziram in laboratory cages to newly emerged adult bees. Fifty bees in the cage were allowed water and 50/50 sugar water ad libitum and were kept in an incubator at 35°C. These same fungicides were topically applied to each one-day-old honey bee at 0, 10, 50, 100, 500 µg per bee and were kept in laboratory cages under the conditions described previously.

The highest level of Captan was found in corbicular pollen (avg = 97ppm). The highest level of Rally detected in corbicular pollen (avg = 20ppm), but none was detected in brood food. The highest levels of Rovral was again found in corbicular pollen (avg = 10.3ppm). Fungicide levels dissipated with time.

Lethal dose for caged honey bees consuming Captan ranged from 3,321 ppm to 184,000 ppm and ziram was 10,030 ppm. For honey bees consuming Rally, Rovral and Vanguard the lethal dose was higher than 200,000 ppm. These doses are all higher than concentrations used in field spray applications. $LD_{50}$ of topical application of Captan ranged from 34.4 µg/bee to 4158 µg/bee, Vanguard 3170 µg/bee and Ziram 1068 µg/bee. These doses are also higher than concentrations found in field spray applications.

**20. Sammataro, D.,[r] J. Finley,[r] & F.D. Guerreo[s] - ESTERASE ACTIVITY OF VARROA MITES THAT ARE RESISTANT TO ACARICIDES IN HONEY BEE COLONIES** - *Varroa destructor* mites that infest honey bee colonies are currently controlled using chemical pesticides. Two of the products commonly used to treat bee colonies in the U.S. are Apistan®, containing the synthetic pyrethroid (P) tau-fluvalinate, and CheckMite®, containing the organophosphate (OP) coumaphos. Recent reports indicate that pesticide resistance to fluvalinate has been found in mite populations in Europe, the UK and in the U.S.

We found mites from Florida that showed resistance to both pesticides (P and OP) and wanted to determine the mechanism of the cross-resistance. Up to now, esterase-mediated resistance has only been reported for Varroa in Israel. Strains of the cattle tick *Boophilus microplus*, which exhibited cross-resistance to both pyrethroids and OP's, also possess high levels of metabolic esterase activity. Live mites were collected from frames of capped drone brood sent to AZ by overnight post, and tested in a vial assay (Elzen *et al.* 1998 *Am. Bee J.* 138:674-676) for resistance/susceptibility to fluvalinate, amitraz and coumaphos. We ran an esterase activity gel (an electrophoretic analysis of proteins with esterase hydrolytic activity), comparing several strains of *B. microplus* with varying responses to pesticide with two samples of *V. destructor*. Since this analysis is performed under native conditions, molecular weights of visualized proteins cannot be determined. The most obvious difference between the suscepti-

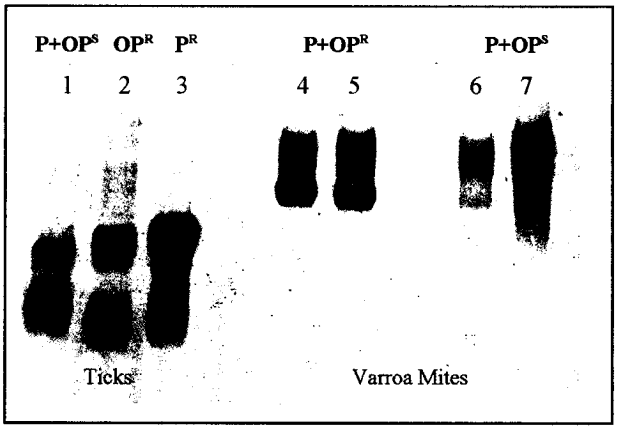



**Figure. Native Esterase Activity Gel. Lane 1: *B. microplus* Gonzalez strain susceptible to both Pyrethroid and OP; Lane 2: OP resistant Tuxpan strain of *B. microplus*; Lane 3: Coatzoacoalcos pyrethroid resistant strain of *B. microplus*; Lane 4 & 5: Pyrethroid and OP resistant *Varroa* (P+OP$^R$); Lane 6 & 7: *Varroa* susceptible to both OP and pyrethroid, (P+OP$^S$).**

ble and resistant mites was an intense band of esterase activity present in the OP-resistant mites, which is barely detectable in the susceptible mites. A similar pattern is seen in resistant ticks. The esterase profile for both the susceptible and resistant mites was not affected by eserine sulfate or triphenyl phosphate, indicating the esterases were probably not acetylcholinesterases or carboxylesterases. We are completing a detailed toxicological survey of resistant Varroa in several locations around the U. S. to determine specific mechanisms operative in resistant mites.

**21. Skinner, J.A.,[t] J.P. Parkman,[t] M.D. Studer[t] & W.M. Hood[h] - A THREE STATE VARROA IPM PROJECT: THE TENNESSEE AND SOUTH CAROLINA COMPONENTS** - The *Varroa* mite, *Varroa destructor*, is the most damaging pest to honey bees in North America and most of the world. Populations of *Varroa destructor* have exhibited resistance to the only chemical miticides registered for use in the US. A second year of a tri-state (TN, SC and GA) evaluation of non-chemical management tactics for *Varroa* was completed in 2003. Bee colonies in two groups of apiaries (isolated [apiaries located at least 2 km from varroa infested apiaries]and non-isolated) were subjected to one of four treatment combinations: (1) resistant honey bee line [suppression of mite reproduction (SMR) trait ] + open bottom board (OBB), (2) resistant honey bee line + conventional (closed-floor) bottom board (CBB), (3) non-resistant honey bee line + open bottom board and (4) non-resistant honey bee line + conventional bottom board. Test colonies taken from mature, overwintered colonies, were established in April in South Carolina and May in Tennessee. Colonies were requeened with new open-mated queens from commercial queen producers.

In Tennessee and South Carolina, results for 2003 were not as promising as those for 2002. *Varroa* populations rose steadily in most colonies and there was no difference in mite populations among the treatment combinations. Possible reasons for lack of mite reduction include (1) initial mite load at beginning of study was greater in 2003, and (2) using open-mated queens where the paternity of workers was unknown, possibly diluting the resistant trait. Selected colonies were tested for being hygienic or expressing the suppression of mite reproduction (SMR) trait. There was no difference in resistance between resistant and susceptible colonies.

In 2002, queens were instrumentally inseminated with resistant sperm. Therefore, workers were 100% resistant. Open-mated queens, in 2003, failed to maintain *Varroa* below damaging levels indicating improvements are needed to ensure queens mate with resistant drones.

There were differences among treatments for some indicators of colony health. At 68 d after requeening, isolated colonies had more bees (9.3 *vs.* 6.9 frames [frs]), uncapped brood (2.5 vs. 1.5 frs) and pollen (2.0 vs. 1.0 frs) than non-isolated colonies (bees: $F$=5.34; $P$<0.0279; df=1, 30; brood: $F$ = 9.79; $P$ <0.0039; df = 1, 30; pollen: $F$ = 4.67; $P$ <0.0389; df = 1, 30). At 107 d, isolated colonies had more bees (5.8 *vs.* 2.6 frs) and uncapped brood (2.3 *vs.* 1.1 frs) than non-isolated colonies (bees: $F$ = 11.30; $P$ <0.0021; df = 1, 30; brood: $F$ = 8.67; $P$ <0.0062; df=1, 30).

In South Carolina, at 108 d after requeening, isolated colonies had more bees (8.6 frs) than non-isolated colonies (6.4 frs) ($F$ = 6.66; $P$ <0.0138; df = 1, 38). At 150 d, colonies with CBBs had more uncapped brood (1.3 frs) than those with OBBs (0.8 frame) ($F$ = 5.06; $P$ <0.0306; df = 1, 37); isolated colonies had more pollen (0.8 frame vs. 0.5 frame for non-isolated colonies) ($F$ = 6.78; $P$ <0.0160; df = 1, 37).

Although 2003 results were disappointing, we believe the quality of commercially available resistant queens will improve and using a combination of non-chemical tactics is a viable management option for *Varroa*.

**22. Webster, T.C. [q], F.E. Vorisek [q] & E.M. Thacker [q] - SCREENED BOTTOM BOARDS SLOW THE DEVELOPMENT OF ACARICIDE RESISTANCE IN VARROA MITES** – Screened bottom boards prevent live *Varroa destructor* from returning to the bee colony after they have fallen from the bees in the hive. These bottom boards slow the development of acaricide resistance in the mites in two ways.

First, by allowing less frequent acaricide treatment the beekeeper will be exerting less selection pressure for acaricide resistance in the mites. A recent study in Kentucky (in preparation) showed a 57% reduction in mite infestation due to the use of screened bottom boards over a 15-month period.

Second, we found that screened bottom boards can eliminate many resistant mites when used in conjunction with the acaricide treatment. This possibility was suggested by a study (Webster *et al.*, 2000 *J. Econ. Entomol.* 93:1596-1601) showing that the number live mites falling to hive bottom boards increased when fluvalinate (Apistan®) was installed. We hypothesized that this increase in live, fallen mites consisted of mites that were stunned by the fluvalinate. Such mites would be more resistant to fluvalinate than the overall population of mites in the hive. To test the hypothesis we collected live, fallen mites from standard hive bottom boards daily before the hives were treated and then during fluvalinate treatment. We then evaluated the mites for fluvalinate resistance by placing them inside fluvalinate-coated glass vials and observing their subsequent survival (see Elzen *et al.*, 1998 *Am. Bee J.* 138:674-676). The mites collected during fluvalinate treatment were more resistant than those collected before the treatment. This finding supports our hypothesis. Possibly, similar results would be found for other acaricides used for varroa mite control.


**Addresses of authors**

a. Dept. of Entomology, Univ. of Nebraska, Lincoln, NE 68583
b. INIFAP-CeLaLa, Matamoros, México
c. Univ. Auton. Agrar.A.Narro, U.L. Torreón, Coahuila, México
d. ARS, USDA, Honey Bee Breeding Laboratory, Baton Rouge, LA 70820
e. Dept. of Entomology, Univ. of Georgia, Athens, GA 30602
f. Honey Bee Unit, USDA-ARS-SARC, 2413 E. Hwy 83, Weslaco, TX 78596
g. Pollen Bank, Box 6697, Bakersfield, CA 93386
h. 305 Long Hall, Clemson Univ., Clemson, SC 29634
i. Dept. of Entomology, 901 W. State St., Purdue Univ., West Lafayette, IN 47907
j. CENIFMA-INIFAP, Santa Crúz 29-B, Las Haciendas, Edo. de Méx., Mexico 52140
k. Dept. of Entomology, Univ. of California, Davis, CA 95616
m. Dept. of Entomology, 1980 Folwell Ave., Univ. of Minnesota, St. Paul, MN 55108
n. Dept. of Entomology, China Agricultural Univ., Beijing, China 100094.
o. Dept. of Entomology, Michigan State Univ., East Lansing, MI 48824
p. Autónoma Agraria Antonio Narro, U.L. carr. a Santa Fe y periférico, Torreón, Coahuila, México
q. Atwood Research Facility, Kentucky State Univ., Frankfort, KY 40601
r. Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ
s. USDA-ARS Knipling-Bushland, U. S. Livestock Insects Research Lab, 2700 Fredericksburg Rd., Kerrville, TX USA 78028
t. Dept. of Entomology & Plant Pathology, Univ. of Tennessee, Knoxville, TN