

# Proceedings of the American Bee Research Conference

The 2007 American Bee Research Conference was held January 8-13 at the Sheraton Crescent Hotel in Phoenix, Arizona. The twenty-first American Bee Research Conference will be held in conjunction with the American Honey Producers Association and the American Beekeeping Federation at the DoubleTree Hotel in Sacramento, California on January 9-12, 2008. The following are abstracts from the 2007 Conference.

**1. Baum, K. A.<sup>a</sup>, Tchakerian, M.<sup>b</sup>, Thoenes, S. C.<sup>c</sup> & R. N. Coulson<sup>b</sup> – AFRICANIZED HONEY BEES IN URBAN ENVIRONMENTS: A SPATIAL-TEMPORAL ANALYSIS –**

In the southwestern United States, including the greater Tucson metropolitan area, the feral honey bee population is predominantly Africanized. Feral colonies can be found in both natural and urban environments, and honey bee colonies will exploit urban sources of cavities, nectar, pollen and water. Africanized colonies will utilize smaller cavities than European colonies, expanding the range of suitable nest sites to include sites abundant in urban areas, such as flower pots, water meter boxes, tires, cement blocks, garbage cans and buildings. This broad range of nest sites increases the proximity of Africanized honey bees to humans, creating concerns over public health and safety. We obtained invoices with data on honey bee colony removals from 1994 to 2001 from BeeMasters Inc., a private company in Tucson, Arizona which specializes in the removal and control of Africanized honey bees. We used colony and swarm removal records to evaluate spatio-temporal patterns in the distribution of feral honey bees in the greater Tucson metropolitan area. We predicted that colony and swarm removals would show a strong spatio-temporal correlation with removed colonies and swarms located close together in space also occurring close together in time. We also conducted a cross-correlation analysis to identify any associations of colony and swarm removal numbers with rainfall and temperature across a range of lag times. Identifying patterns in the spatial and temporal distribution of Africanized honey bee colonies in urban areas is necessary to ascertain factors that contribute to Africanized honey bee use of urban habitats and to develop strategies to reduce contact between humans and honey bees in urban environments.

**2. Bourgeois, L.<sup>d</sup>, Sylvester, H. A.<sup>d</sup>, Danka, R. G.<sup>d</sup> & T. E. Rinderer<sup>d</sup> – PRELIMINARY ASSESSMENT OF GENETIC DIVERSITY OF ITALIAN HONEY BEES IN THE U.S.A. AND ITALY –**

Declining numbers of breeder queens and the concomitant loss of genetic diversity potentially could result in inbreeding and increased susceptibility to pests and disease in honey bees. Genetic diversity of commercial Italian bee colonies in the United States and Italy was assessed using microsatellite genetic markers. We sampled worker bees from colonies of major queen breeders in both countries. To date, 13 U.S. and 14 Italian suppliers were represented. DNA was extracted from the thoraces of 4 bees per colony. Data from four microsatellite loci are presented here, and six additional loci are being analyzed.

Overall allelic diversity (mean number of alleles per locus) did not differ between the two groups ( $P > 0.05$ ), although alleles were present that were unique to each group (Table). There were a total

of 10 unique alleles among U.S. bees and 5 among bees from Italy. The U.S. "Italian" bees likely are an admixture of subspecies and thus have alleles that may not be present in the bees of Italy. Estimates of genetic differentiation using the population genetic parameter  $F_{ST}$  showed that bees from the USA and Italy differ ( $P < 0.001$ ). Examination of the genetic structure within each group (USA and Italy), based on Cavalli-Sforza & Edwards genetic distance, indicated regional clustering for both groups. Samples from California queen breeders clustered together, as did all but one of the samples from the Bologna region of Italy. Higher resolution will be realized when more microsatellites and sample data are added.

**Table - Allele counts and  $F_{ST}$  estimates for Italian bees collected from commercial queen breeders in the United States and Italy.**

Locus	Repeat Unit	Country	Alleles	Unique Alleles	$F_{ST}$
L174	(GGA)10	USA	13	3	0.0393
		Italy	10	0	
L306	(GAA)20	USA	8	2	0.1597
		Italy	8	2	
L440	(TTTC)5	USA	3	0	0.2025
		Italy	6	3	
L504	(TC)12	USA	12	5	0.2625
		Italy	7	0	
Overall		USA	36		0.1633
		Italy	31		
Mean/locus		USA	9		
		Italy	7.75		

**3. Calderone, N. W.<sup>e</sup> - A THREE-YEAR STUDY OF SCREEN BOTTOM BOARDS IN THE NORTHEAST -**

Screen bottom boards were evaluated to determine their usefulness in managing the parasitic honey bee mite *Varroa destructor*. In 2001, this device was evaluated on colonies in four apiaries, each with 16 colonies. Half of the colonies in each apiary were managed with standard bottom boards and half with screen bottom boards. Mite-to-bee ratios (~250 bee samples) and cluster sizes were obtained in October and November, respectively, after reducing colonies to two deep hive bodies. Colony weight gain was measured from June through September. The average weight gains for colonies with standard bottoms ( $69.07 \pm 5.45$  kg) and screen bottoms ( $63.34 \pm 5.28$  kg) were not significantly different ( $F_{1,50} = 0.57$ ;  $P < 0.45$ ). Apiary effects were significant ( $F_{3,50} = 5.40$ ;  $P < 0.0027$ ), but Treatment\*Apiary effects ( $F_{3,50} = 0.50$ ;  $P < 0.69$ ) were not significant. The average October mite-to-bee ratios for colonies with

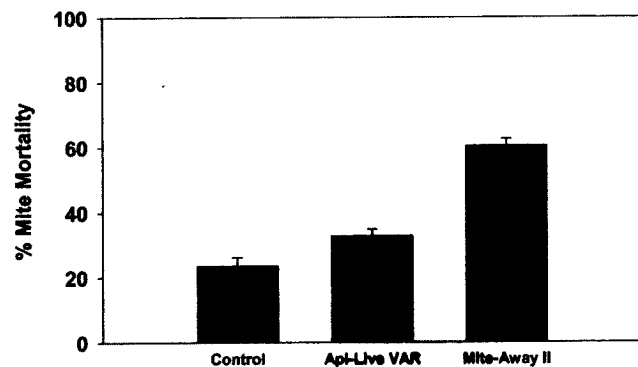
standard bottoms ( $0.09 \pm 0.02$ ) and screen bottoms ( $0.11 \pm 0.02$ ) were not significantly different ( $F_{1,49} = 0.91$ ;  $P < 0.35$ ). Apiary effects were significant ( $F_{3,49} = 20.72$ ;  $P < 0.0001$ ), but Treatment\*Apiary effects ( $F_{3,49} = 0.41$ ;  $P < 0.75$ ) were not significant. The average November cluster sizes for colonies with standard bottoms ( $9.26 \pm 0.55$  combs of bees) and screen bottoms ( $8.46 \pm 0.54$  combs of bees) were not significantly different ( $F_{1,49} = 1.10$ ;  $P < 0.30$ ). Apiary effects were significant ( $F_{3,49} = 13.54$ ;  $P < 0.0001$ ), but Treatment\*Apiary effects ( $F_{3,49} = 0.26$ ;  $P < 0.86$ ) were not significant.

Package bees were established in the spring of 2002 ( $n=24$ ) and 2003 ( $n=32$ ). Colonies were maintained in a single apiary each year, with half the colonies being managed with standard bottom boards and half with screen bottoms. The average weight gains for colonies with standard bottoms ( $49.04 \pm 4.82$  kg) and screen bottoms ( $55.76 \pm 4.82$  kg) were not significantly different ( $F_{1,52} = 0.97$ ;  $P < 0.33$ ). Year effects were significant ( $F_{1,52} = 17.96$ ;  $P < 0.0001$ ), but Treatment\*Year effects ( $F_{1,52} = 1.73$ ;  $P < 0.19$ ) were not significant. The average October mite-to-bee ratios for colonies with standard bottoms ( $0.12 \pm 0.02$ ) and screen bottoms ( $0.12 \pm 0.02$ ) were not significantly different ( $F_{1,52} = 0.00$ ;  $P < 0.97$ ). Year effects were significant ( $F_{1,52} = 29.56$ ;  $P < 0.0001$ ), but Treatment\*Year effects ( $F_{1,52} = 0.12$ ;  $P < 0.73$ ) were not significant. The average November cluster sizes for colonies with standard bottoms ( $7.21 \pm 0.51$  combs of bees) and screen bottoms ( $6.71 \pm 0.50$  combs of bees) were not significantly different ( $F_{1,51} = 0.49$ ;  $P < 0.49$ ). Year effects were significant ( $F_{1,51} = 6.43$ ;  $P < 0.01$ ), but Treatment\*Year effects ( $F_{1,51} = 0.04$ ;  $P < 0.83$ ) were not significant. Screen bottom boards are not currently recommended for control of *V. destructor* in the northeast.

**4. Calderone, N. W.<sup>c</sup> - EVALUATION OF API-LIFE VAR™ AND MITE-AWAY II™ IN THE NORTHEAST** - Two miticides were evaluated as fall treatments for control of the parasitic honey bee mite *Varroa destructor* in upstate New York. Colonies were reduced to two deep hive bodies in early October. In each of five apiaries, six colonies were treated with Mite-Away II™ (formic acid), six with Api-Life VAR™ (thymol), and four served as controls. Mites were collected on sticky boards for four weeks; and then, treatments were removed and remaining mites were collected for six weeks on new sticky boards after applying four strips of both Apistan and CheckMite+ to each colony. Formic acid pads and thymol tablets were weighed every 8-12 days to determine the amount of product delivered.

Average mite mortality (Figure) was  $32.66 \pm 0.02\%$  for colonies treated with Api-Life VAR and  $60.22\% \pm 0.02\%$  for those treated with Mite-Away II. Natural mite fall in the control colonies was  $23.66\% \pm 0.03\%$ . Treatment effects were significant ( $F_{2,63} = 68.06$ ;  $P < 0.0001$ ), and each mean was significantly different from the others (Tukey-Kramer;  $P < 0.01$  each test). Apiary ( $F_{4,63} = 1.06$ ;  $P < 0.38$ ) and Treatment\*Apiary ( $F_{8,63} = 0.26$ ;  $P < 0.98$ ) effects were not significant. The average temperature during the treatment period was  $47.87 \pm 1.29$  °F, lower than label recommendations. Overall, only  $53.60 \pm 2.14\%$  of the

**Figure - Percent mite mortality with Mite-Away II and Api-Life VAR used as fall treatments in upstate New York.**



formic acid and 12.90% of the thymol product was delivered. Neither product provided adequate levels of control under conditions typically encountered during October in upstate New York.

**5. de Guzman, L. I.<sup>d</sup> & A. M. Frake<sup>d</sup> - OBSERVATIONS ON THE LIFE HISTORY OF SMALL HIVE BEETLES** - The life history of small hive beetles (SHB) kept in an incubator (34°C) and at room temperature (24-28°C) was compared. Six slides of eggs were obtained using the glass slide technique, and each slide was placed in a rearing container kept either in an incubator ( $n=3$ ) or at room temperature ( $n=3$ ). Egg incubation period was based on the time when 100% of the eggs hatched, which was observed to be 51 h (ca. 2 days) in the incubator and 71 h (ca. 3 days) at room temperature.

Larvae were reared individually within Eppendorf vials (1.5 ml) closed with moistened cotton wads to prevent desiccation. All vials were placed in partitioned trays (50 vials/tray), and trays were kept either in the incubator ( $n=2$ ) or at room temperature ( $n=2$ ). Each larva was fed one honey bee pupa. When larvae stopped feeding, moist potting soil (1.2 g) was placed in each vial to supply a medium for pupation.

Our results showed that the duration of each developmental stage of SHB was affected by temperature (Table). Developmental time was accelerated when larvae were exposed to 34°C, while exposure to 24-28°C slowed their development. Beetles kept in the incubator took ca. 23 days to develop from egg to adult, which was shorter than the duration of 32 days reported by Schmolke (1974, Certificate in Field Ecology Project Report, 178 pp.) at 30°C. A total developmental period of ca. 39 days was observed at room temperature, which was similar to that observed by Mürrle and Neumann (2004 *J. Apic. Res.* 40: 111-112) at 8-25°C, and about half the highest duration (81 days) reported by Lundie (1940 *Science Bulletin* 220. 30 pp.). Typically, each beetle spent >75% of its developmental time in the soil. Additionally, higher temperature resulted in larger and heavier adult beetles.

Our results suggest that temperature may significantly influence the abundance and impact of SHB on honey bee colonies. High temperature accelerates reproductive ability and developmental rate of SHB, resulting in an increased population that may damage honey bee colonies.

**Table - Developmental time (days), weight and body size for *A. tumida* reared in an incubator and at room temperature.**

Stages	Incubator (34°C)	Room Temp. (24-28°C)	P
Egg*	2.0	3.0	
First instar	1.0 ± 0 <sup>b</sup>	2.6 ± 0.05 <sup>a</sup>	0.0001
Second instar	1.0 ± 0.02 <sup>b</sup>	1.8 ± 0.05 <sup>a</sup>	0.0001
Third instar (Feeding)	2.9 ± 0.03 <sup>b</sup>	3.1 ± 0.08 <sup>a</sup>	0.025
Third instar (Non-feeding or wandering phase, mobile + immobile)	5.3 ± 0.06 <sup>b</sup>	9.5 ± 0.08 <sup>a</sup>	0.0001
Pupa	5.4 ± 0.06 <sup>b</sup>	10.8 ± 0.07 <sup>a</sup>	0.0001
Teneral adult to emergence from soil	5.1 ± 0.08 <sup>b</sup>	8.5 ± 0.13 <sup>a</sup>	0.0001
First instar larva to adult emergence from soil	20.69 ± 0.08 <sup>b</sup>	36.31 ± 0.10 <sup>a</sup>	0.0001
Total development time (Egg* to adult emergence)	22.7	39.3	
Weight of newly emerged adult (mg)	12.23 ± 0.15 <sup>a</sup>	10.75 ± 0.16 <sup>b</sup>	0.0001
Length of newly emerged adult (mm)	6.15 ± 0.05 <sup>a</sup>	5.37 ± 0.03 <sup>b</sup>	0.0001
Width of newly emerged adult (mm)	3.46 ± 0.01 <sup>a</sup>	3.36 ± 0.02 <sup>b</sup>	0.0001

\* Estimates based on the time when 100% egg-hatching was attained.

Means in each row followed by different letters are significantly different ( $P < 0.05$ , Wilcoxon two-sample test).

6. Delaplane, K. S.<sup>f</sup>, Ellis, J. D.<sup>g</sup> & J. A. Berry<sup>h</sup> - **PROFITABILITY OF A VARROA IPM SYSTEM** - Our lab has been engaged for over ten years in the development of a comprehensive integrated control program for varroa mites. Phase one resulted in an economic threshold (ET) for the Southeast (1999 *Apidologie* 30: 383-395). Phase two was a demonstration that bottom screens and genetically mite-resistant queens reduce colony mite levels and delay economic threshold (2005 *J. Apic. Res.* 44(4): 157-162). The third and final phase, summarized here, examines the economic feasibility of IPM. Six beekeeper collaborators, ranging from sideline to commercial, each contributed 21-30 colonies in one apiary. Colonies were each assigned one of three treatments: (1) chemical (Feb and Aug treatments with acute miticide), (2) IPM (Russian queen plus screen hive floor), or (3) experimental check (no miticide, non-selected queen, conventional hive floor). IPM performed better than chemical treatment as measured by honey production, colony mortality, and queen supersedure (Table). IPM values for mite levels and percentage of colonies reaching ET were lower than the check group and comparable to the chemical group. Time inputs were highest for IPM colonies; a difference explained by the time spent counting mites on screens. When this factor was removed, time spent working IPM colonies was lower than chemical colonies. This study shows that IPM does not sacrifice profitability. Improved or abbreviated sampling will eliminate the only liability remaining to IPM.

7. Delaplane, K. S.<sup>f</sup> & A. M. Ellis<sup>g</sup> - **VARROA AND SHB IN CONTEXT OF PLANT POLLINATION** - If hive invaders like *Varroa destructor* and *Aethina tumida* impact crop pollination negatively, it may happen at two levels: (1) the bee population level where exotic invaders induce colony mortality or (2) the colony level where compromised foragers pollinate less efficiently. This preliminary report summarizes two years of field data acquired by manipulating bee colonies to achieve various levels of varroa mites or small hive beetles (SHB), then tenting them with one of two model plants in flower: canola and rabbiteye blueberry. Pod-set in canola was unaffected by different levels of honey bee nest invaders. Instead, a pod-set benefit was indicated by the presence of bees, regardless of the degree to which their colonies were infested. The fact that pod-set was lowest in the no-bee tent supports the belief that flower shaking, whether by wind or insects, is important to pollinate this crop (1988 *Apidologie* 19: 51-72). In blueberry, bee flower visitation rate was unaffected by different levels of honey bee nest invaders. Fruit-set, like pod-set in canola, depended more on the simple presence of bees rather than the degree to which their colonies were compromised by pests. Visitation and fruit-set were higher in plants tented with bees than in open plots, an artifact noted regularly in this system. Under our conditions we have failed to identify negative pollination impacts of nest invaders at the colony level.

**Table - Feasibility of integrated pest management (IPM) to control varroa mites as compared to regimented use of a miticide (Chem). Experimental controls (Check) were not treated with miticides, had standard bottom boards, and had bees derived from unselected queens.**

Treatment	Avg. mite counts	% of colonies reaching ET	2006 honey production (lb)	% of colonies dead after 2 yr	Queen supersedure rate	Time (h) spent working colonies	Time (h) working colonies, minus counting screens
Chem	13.1	69	1104	61	46	33.5	33.5
IPM	17.9	74	2053	39	39	40.4	31.5
Check	21.3	94	660	72	55	30.4	30.4

**Table - Comparison of different levels of two colony pests on the pollination success for canola and blueberry in a controlled environment. The presence of varroa mites or small hive beetles did not negatively impact the pollination of either plant. However, the presence of bees significantly increased the pollination for both plants.**

Canola		Blueberry	
Tent treatment	Pod-set (pods per flower), 2005	Tent treatment	Bee blueberry flower visits / 2 minutes, 2006 Blueberry fruit-set, 2006
Open	50.6a	Open	2.6b 36b
High Varroa	58.2a	Varroa	38.5a 55a
Low Varroa	53.5a	SHB	34.6a 58a
High SHB	62.6a	No pests	38.1a 44ab
Low SHB	55.6a	No bees	18c
No pests	62.5a		
No bees	36.1b		

**8. Eischen, F. A.<sup>1</sup>, Graham, R. H.<sup>1</sup>, Rivera, R.<sup>1</sup> & J. Traynor<sup>1</sup> - ALMOND POLLEN COLLECTION BY US OVERWINTERED COLONIES AND AUSTRALIAN PACKAGE COLONIES** - A possible honey bee colony shortage for almond pollination and high pollination fees (\$130-150 per colony) kindled interest in importing packages of adult bees from Australia. During the 2006 season 30,000+ packages were imported. We compared the performance of Australian (AUS) package colonies with overwintered US colonies.

Pollen collected by US 8-frame colonies was about 2.5 times that of AUS 4-lb package colonies established in late January 2006. Pollen collection by US 6-frame colonies was similar to the AUS 4-lb colonies and both of these were significantly higher than the AUS 3-lb colonies established in late January 2006. All groups collected significantly more pollen than the AUS 4-lb colonies established in mid-December 2005.

The weight of pollen collected per frame of bees (foraging rate) by AUS 4-lb colonies was about 65% as US 8-frame colonies. Over the 20-day collection period, AUS 4-lb colonies lost strength, but their pollen collection did not decline proportionately. Calculated on ending bee populations, pollen collection by Australian colonies was about 112% of US colonies. We assume that this was, in part, caused by an increased effort to meet the needs of an enlarged broodnest and an ageing adult population more likely to forage. However, based solely on pollen foraging behavior, an Australian 4-lb package colony was worth about half that of a standard overwintered US colony for pollinating almonds.

Almonds were pollinated throughout the day, but pollinator exclusion data indicates that a slightly higher percentage was set 10.30 - 13.00hrs. An analysis of foraging flights by test colonies and nut set data indicate that a disproportionate number were pollinated late in the afternoon. We speculate that diminished pollen per blossom caused by previous foraging caused bees to visit additional blossoms and this caused a higher rate of inter-cultivar visitation and nut set.

**9. Eischen, F. A.<sup>1</sup>, Graham, R. H.<sup>1</sup> & R. Rivera<sup>1</sup> - DEVELOPING HIVASTAN® (FENPYROXIMATE) FOR THE CONTROL OF VARROA DESTRUCTOR** - The efficacy of an experimental 0.3% fenpyroximate preparation (Hivastan®, Central Life Sciences-formerly Wellmark International) was tested against *Varroa destructor* known to be resistant to fluvalinate and coumaphos during 2004-2006. Fenpyroximate is a pyrazole acaricide and is lipid soluble. Its route of entry to the parasite is through contact. Using laboratory test cages, fenpyroximate demonstrated a LD<sub>50</sub> of about 118 µg/bee.

A series of preliminary field trials found that the most effective presentation was a grease patty composed of hydrogenated vegetable oil, powdered sugar, irradiated honey and 0.2 - 0.3% fenpyroximate. The honey was found useful in getting bees to interact with the patty to insure contact. The honey was irradiated to insure against viable American foulbrood spores and other disease organisms. Patties weighing 227g were applied above waxed paper to the top bars of brood frames. Evaluations of colony strength, broodnest size and mite load were assessed at the beginning and end of trials.

Several large field trials (20-30 colonies per treatment group) have been conducted in central and south Texas, as well as the central valley of California. Efficacy (ca. 95%) was equal to or better than Apiguard® [Vita (Europe) Limited]. Efficacy remained high under a wide range of temperatures (5 - 40°C) and various climatic conditions. High temperatures caused patties to be a little more difficult to handle, and we routinely held them in chilled ice chests for convenience.

Todd dead bee trap studies found that adult worker bee mortality was elevated above controls during the first 24hrs of treatment. Thereafter, no difference in adult mortality was observed. We do not know the cause for this. It is possible that many of dead bees in our traps had been previously weakened by varroa (many had twisted wings). We doubt that beekeepers will observe any signif-

icant colony impairment if patty material is removed after 42 days of treatment.

The Weslaco laboratory is committed to finding alternative control products for the parasitic mite *Varroa destructor*. Our goal is to assist in bringing to market an array of products for its control so that U.S. beekeepers can conduct an effective rotational scheme and reduce the impact of acaricide resistance. Our evidence shows that Hivastan® provides effective control.

**10. Ferrari, T. E.<sup>k</sup> & A. B. Cobb<sup>k</sup> - ONE COLONY WITH SUPPLEMENTAL POLLINATION IS BETTER THAN TWO WITHOUT EXTRA POLLEN: CASE HISTORIES** - Consistent improvement in almond production for flowers exposed to enpollinated honey bees is evidence foragers are inherently inefficient at cross pollination. Enpollination is the application of compatible pollen directly onto bees using a dispenser located at the hive entrance - a practice termed *supplemental pollination*.

Thirteen orchards were evaluated in which new pollen application strategies were used during the 2002 to 2006 bloom periods. Varieties exposed to enpollinated bees ranged from 12 to 150 acres; orchards contained 2 to 4 different varieties; and ages ranged from 8 to 23 years. Pollen doses ranged from 100 to 200 million *Viable Pollens /acre*, and pollen was 100% compatible with targeted flower pistils. Production histories were provided by growers and ranged from 3-9 years. In 7 cases, only 1 colony/acre was used when pollen was applied to a targeted variety; additional hives were introduced after enpollination was complete, as 2 to 3 colonies/acre is customary.

Historical yield ratios between 2 varieties when no pollen was applied (before) were compared with ratios for years when pollen was applied (after) to a treated variety (non-treated varieties acted as controls since they were either not in bloom or flowers were no longer receptive when pollen was dispersed). Changes in "before" and "after" ratios were used to calculate yields due to natural vs. supplemental pollination. A 1-tailed Z-score was the test statistic used to analyze probabilities that a yield ratio between a treated and non-treated cultivar was different than the historical average (no treatments).

All 13 orchards exposed to enpollinated foragers had increased yield ratios and, consequently, almond production (Table). Statistically significant (P < 0.05) changes in ratios were achieved in 9 cases. Supplemental pollination caused an average of 525 lbs/acre *EXTRA* for 7 cases when 1 hive/acre was used and 988 lbs/acre extra after pollen was applied to the stronger of 2 colonies when 2 hives/acre were used.

Evidence indicates it is feasible to improve almond yields while reducing colony numbers, which can mitigate demand and annual rental costs.

**Table - Almond production for varieties exposed to enpollinated honey bees**

(Supplemental Pollination = SP).

Case	Variety	Yield	Production	SP as percent
		without SP	due to SP	of total
		lbs/ac	lbs/ac	%
1.	Sonora	1352	1473	52.1 **
2.	Butte	1120	1125	50.1 **
3.	Sonora	1351	1154	46.1 *
4.	Nonpareil	946	599	38.7 **
5.	Nonpareil	2227	1134	33.7 **
6.	Butte+Padre	1964	773	28.2 *
7.	Sonora	2042	779	27.6 **
8.	Nonpareil	2237	826	26.9 **
9.	Sonora	1748	557	24.2 *
10.	Sonora	1876	492	20.8 *
11.	Sonora	1987	391	16.4 *
12.	Sonora	1939	176	8.3 *
13.	Nonpareil	1625	133	7.6 *

\* Indicates 1 hive/ac, \*\* 2 hives/ac.

**11. Harris, J. W.<sup>d</sup> – VARROA-SENSITIVE HYGIENE AND RECAPPED BROOD CELLS** – Honey bees bred for “suppression of mite reproduction” (SMR trait) resist the growth of *Varroa destructor* by removing mite-infested pupae from combs. This is varroa-sensitive hygiene (VSH), and the bees are called VSH bees. VSH is likely a multi-step process by several bees that involves detection of infested pupae, uncapping of brood cells, and removal of the pupae (Arathi *et al.*, 2000 *Ethology* 106: 365-379). Although VSH bees target mite-infested pupae, they and other bees expressing varroa-sensitive hygiene also uncapped some cells that contain uninfested pupae (Vandame *et al.*, 2000 *Can. J. Zool.* 78: 2037-2044). When a cell containing a mite-infested pupa is uncapped, the mite either escapes the cell, or she is removed when the bees eliminate the pupa (Aumeier & Rosenkranz, 2001 *Apidologie* 32: 253-263). However, cells with a mite-infested pupae may be uncapped by hygienic bees, only to be recapped by nestmates without removal of the pupae (Arathi *et al.*, 2006 *Anim. Behav.* 72: 431-438) and with or without escape of the mites. Caps of recapped cells have patches of wax that seal the holes made previously by hygienic bees (Boecking *et al.*, 2000 *J. Anim. Breed. Genet.* 117: 417-424). These patches lack the silk which was spun by the last instar larvae before pupation, which gives the wax a granular appearance.

The relationship between varroa-sensitive hygiene and the percentage of reproductive mites has not been firmly established. Harbo & Harris (2005 *J. Apic. Res.* 44: 21-23) suggested that VSH bees preferentially remove pupae with ovipositing mites and ignore those with non-ovipositing mites, and this bias produces the low percentage of reproductive mites that was the primary selection criterion in the SMR breeding program (Harbo & Harris, 1999 *Apidologie* 30: 183-196). However, the phenomenon of recapped cells was not investigated in that study, and it is unknown to what extent VSH bees recap cells. The purposes of this study were (1) to compare the incidence of recapped brood cells between combs that were exposed to either VSH or commercial Italian bees, and (2) to compare the frequencies of ovipositing and non-ovipositing mites in normally capped cells to those in recapped cells for combs exposed to the two types of bee.

A comb with only mite-infested prepupae was put into each VSH (n=11) and Italian (n=9) colony for 1 week. VSH bees removed 55% of the mite-infested pupae during the test, while Italians removed 1% (P<0.01). Combs from VSH colonies had significantly more recapped cells (1,226 of 2,117 sampled) than combs from Italians (421 of 1,771 sampled) (P<0.01). The percentage of non-ovipositing mites was not significantly different between normally capped and recapped cells (P=0.28) for combs given to Italian colonies; about 21% of mites in each type of cell had not laid eggs. Recapped cells had significantly more non-ovipositing mites (53%) than normally capped cells (22%) for combs from VSH colonies (P<0.01). Thus, a higher percentage of non-ovipositing mites was associated with cells that had been manipulated by hygienic bees. This suggests that the presence of some non-ovipositing mites in brood cells is directly related to hygienic uncapping and recapping of brood cells, which confounds the notion that VSH bees ignore brood cells that contain non-laying mites.

**12. Hood, W. M.<sup>1</sup> & M. Nolan<sup>1</sup> – A COMPARISON OF TWO SMALL HIVE BEETLE ATTRACTANTS INSIDE HONEY BEE COLONIES** – Trapping small hive beetles (SHB) is one alternative to controlling this hive pest. Although traps have been developed for controlling SHB, an economical and readily available material is needed for placement in traps to lure and kill the pest. We report here field tests of a comparison of two SHB attractants, cider vinegar and a USDA yeast-based product.

Investigations were conducted in 2006 to compare the attractiveness of the two materials when placed inside the Hood Beetle Trap. The Hood trap is a one-way beetle trap (Brushy Mountain Bee Farm, Moravian Falls, North Carolina, Ph. 800-233-7929, <www.brushymountainbeefarm.com>) that can be fastened by two screws to a hive frame bottom bar and placed in the top or bottom of a hive depending on season and SHB activity. The trap lid

is constructed in a manner which allows beetles to enter, but impedes their escape especially when the trap is partially filled with certain liquids such as food grade mineral oil, which disrupts beetle mobility. The Hood trap is constructed with three compartments which allows the beekeeper to apply various materials in the trap simultaneously.

Eight honey bee colonies were established on 1-2 April 2006 with .9 kg (2 lb) package bees in each of four apiaries located in Anderson, Bamberg, Barnwell, and Pickens Counties, South Carolina, where beetles were present at various levels. Colonies were allowed to become naturally infested with SHB from nearby colonies, and on 18-19 May, 100 SHB adults were released inside each test colony near the colony entrance, and traps were installed. Colonies were randomly selected to receive one of three treatments in the Hood beetle traps: USDA yeast-based attractant in the middle compartment and mineral oil in the two side compartments (n=11), cider vinegar in the middle compartment and mineral oil in the two side compartments (n=11), or trap only as a control (n=10). The trap middle compartment was 80% filled with attractant and the side compartments one-half filled with mineral oil. The traps were placed in hive body position number one or number ten. Colonies were serviced at 3-week intervals through 13-14 November to remove and replace traps with fresh attractant and mineral oil. At each service visit, colony strength was measured by counting number of 25cm<sup>2</sup> capped bee brood, and colony SHB population was surveyed by adding the number of beetles counted under the colony inner cover to the number of beetles counted on the three exposed vertical hive body surfaces and hive floor following removal of five frames.

The number of dead SHB adults counted in traps with the USDA yeast-based attractant was significantly greater (P = 0.02) on one (15 August) of the nine sampling dates. Although there was no significant difference in beetles sampled in colonies having the two attractants, the control colonies had significantly more (P < 0.04) beetles than colonies having either attractant on two sampling dates (24 July and 15 August) during the six months test period. The amount of capped bee brood did not vary by treatment.

When used in the Hood trap, the USDA yeast-based attractant proved to be a better SHB attractant in warmer months of the year, July and August. There was no difference found in the two attractants in the cooler months. The USDA yeast-based attractant proved to be a more stable material than cider vinegar, which had a tendency to evaporate quickly in hot weather. Test colonies having traps with no attractant (controls) had more small hive beetles than test colonies having either attractant in July and August, which indicates that trapping efforts resulted in maintaining a lower beetle population at a critical time of year.

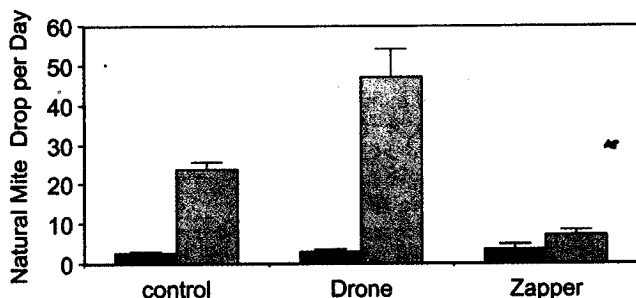
**13. Huang, Z. Y.<sup>m</sup>, Langenberger, M. W.<sup>m</sup> & J. S. Riddle<sup>m</sup> – EFFECT OF MITEZAPPER ON VARROA MITE POPULATION: A FIELD TEST** – Varroa mites are more attracted to drone brood than worker brood, and mites are also more sensitive to high temperature than bees. Based on these two principles, a device called “Mitezapper” was invented (Huang, 2001 *Am. Bee J.* 141: 730-732). The device consists of a heating element sandwiched between two plastic pieces of drone foundation. When the drone brood is capped, varroa mites and drone brood can be killed thermally by applying electricity from a 12 volt car battery.

In summer of 2006 we tested whether Mitezapper can be effectively used against varroa mites under field conditions. We tested the effectiveness of Mitezapper at three apiary locations. Each location had 5 colonies with one Mitezapper (Z group), 5 colonies with a frame of drone comb (D group) and 4 colonies with neither a Mitezapper nor a drone comb (C group). Natural mite drop was monitored every 8-14 days for each colony. The Z group was treated 3-4 times during the season. Treatment involved connecting a control box and a portable car battery to the Mitezapper, which has a temperature probe on its center. During each session, the control box maintained the temperature on the Mitezapper at 58 °C for 6 min.

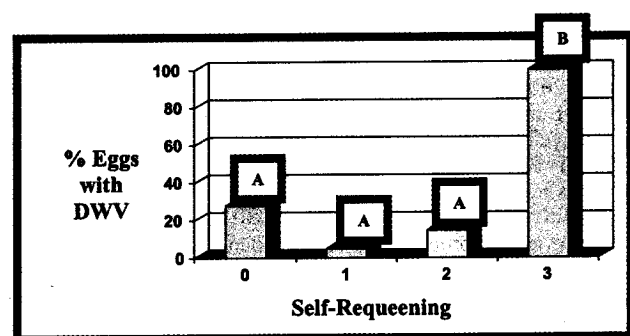
Results are shown in the Figure. The black bars represent data on June 8th, about one month into the experiment, and the hatched

bars represent data on August 24, near the end of the experiment. The percentages of colonies that showed >30 naturally dropping mites per day were 24.4%, 73.3%, and 0%, for C, D and Z groups, respectively. We used 30 mites as a "threshold" for treating mites in Michigan, considering our longer and harsher winter than Georgia, where ~60 mites per day is used (Delaplane & Hood, 1999 *Apidologie* 30: 383-395). Our study clearly indicates that the Mitezapper can be a powerful tool in reducing mite populations, resulting in 70% reduction of mite population when compared to the C group and 85% reduction when compared to the D group.

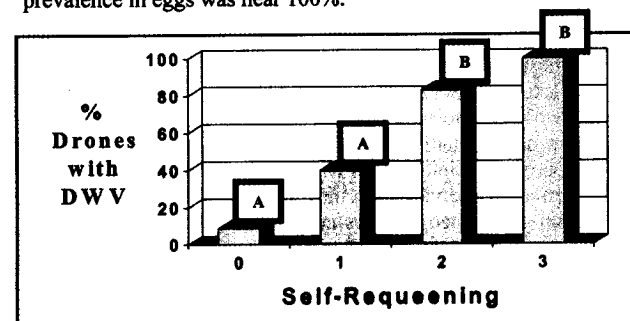
Figure - Mean mite drop per day for C, D and Z groups near the beginning (solid bars) and the end (hatched bars) of the experiment.



14. Ostiguy, N.<sup>n</sup>, Cox-Foster, D.<sup>n</sup>, Kalkstein, A.<sup>n</sup> & O. Thompson<sup>n</sup> - THE CONTINUING STORY OF HONEY BEE VIRUSES - In 2004, frequent colony self-replacement of queen was observed, anecdotally, in colonies with elevated prevalence of Deformed Wing Virus (DWV). In 2005 (one location) and 2006 (2 locations), colonies were established with packages and queens were marked. Once every two weeks in 2005 and once every week in 2006, colonies were inspected for disease and presence of queen, and samples of 20 eggs and 10 drones per colony were collected. Mite counts were obtained using the Penn State Sticky Boards<sup>®</sup> (Great Lakes IPM) and counting followed the procedure in Ostiguy and Sammartaro (2000 *Apidologie* 31: 707-716). No associations were observed between self-requeening and the number of mites, presence of eggs, or year. A significant correlation between colony self-replacement of queens and prevalence of



A - Queen replacement occurred more frequently when DWV prevalence in eggs was near 100%.



B - Infection in drones is more related to self-requeening than DWV prevalence in eggs.

DWV in drones was observed ( $R^2=0.21$ ;  $P=0.0001$ ). Prevalence of DWV in drones was a better indicator of colony self-replacement of queen (Figure A and B). When DWV prevalence in drones was high (over 80%), colonies replaced their queen two or more times ( $P<0.05$ ) (Fig. 1b). Colonies with high DWV prevalence had a shorter time to a first requeening event, 30 days earlier than colonies with low or no DWV ( $P<0.001$ ). Self-replacement of queens in August was observed only in colonies with the highest DWV prevalence.

15. Rogers, R. E. L.<sup>o</sup> & G. R. Williams<sup>p</sup> - HONEY BEE HEALTH IN CRISIS: WHAT IS CAUSING BEE MORTALITY? - Honey bee colony strength and health have been assessed by Wildwood Labs Inc. from hives in various locations in North America since 2001. Also, working visits to western Europe since then have provided a clearer understanding of the bee health situation there. The picture that is emerging is alarming - honey bee losses are severe in many countries.

When honey bee colonies die in large numbers, the cause is often considered mysterious, and this leads to various allegations and theories as to what caused the bee losses. Using a Colony Condition Assessment and Survival Prediction Analysis (CCA/SPA) approach, it has been possible to define the status of bee health in individual colonies and to predict the chances of surviving winter.

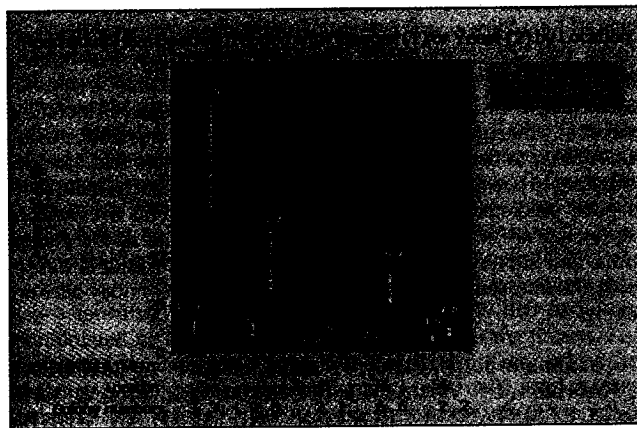
A CCA is a process that measures a wide range of factors that can affect bee health or can be indicators of a problem. This process must be performed in a living, managed colony of honey bees. Samples are also collected and processed in the laboratory to complement the in-hive assessment. This information combined with data related to equipment, apiary, environment, and management completes a data snapshot of colony condition at the time of assessment. Using provisional thresholds and interaction considerations, it is possible to do a SPA to realize the effects of multiple stressors. The system is objective, unbiased, accurate and comprehensive.

What has become clear from using CCA/SPA is that honey bees are suffering from what is being referred to as Multiple and Various Causative Agents Syndrome (MVCAS). This syndrome is caused by additive or synergistic combinations of more than one factor that affects bee health. The combinations may vary among hives, apiaries, regions and countries. The factors most frequently associated with MVCAS are parasitic mites, diseases (including viruses), management, and nutrition. It is possible, using CCA/SPA, to determine the specific various factors that are contributing to bee mortality. As well, the system has been found to be a good predictor of the chances of colony survival over winter. Assessing all of the factors that affect bee health at the correct time, and interpreting the results in relation to provisional thresholds and interactions, takes the mystery out of why honey bees are dying.

16. Sammartaro, D.<sup>q</sup>, Finley, J.<sup>q</sup> and J. Hooper<sup>r</sup> - TEMPORAL CHANGES IN VOLATILE COMPOUNDS FROM VARROA-RESISTANT EUROPEAN AND AFRICAN HONEY BEE WORKER BROOD AS DETECTED BY SPME - Solid Phase Microextraction (SPME) techniques (to sample short-range vapor-phase chemicals) were used to collect unique compounds from immature worker brood of European and Africanized honey bees to determine if there were changes during the development process of the honey bee. All immature stages of the bees were sampled every 24 hours throughout brood development, using 5 individuals for each stage. The GC-Mass Spec was used to identify some of the compounds and quantify the amounts of compounds produced (recorded as peak area counts). E- $\beta$ -Ocimene was present in workers and in drones. In general, E- $\beta$ -Ocimene levels increased sharply when the egg hatched and throughout the larval stage. Between Day 8 and Day 11 (from egg) a second spike in ocimene was recorded until mid-pupal stage, when it gradually declined. The drones had lower levels of E- $\beta$ -Ocimene than the workers. The ratio of signal output to body mass was highest during the egg and larval stages. This preliminary investigation will

direct future work in certain age groups of immature bees and in particular bee lines.

**17. Skinner, J. A.<sup>s</sup> & M. D. Studer<sup>s</sup> - COMPARING BAITS TO USE WITH THE "HOOD TRAP" FOR SMALL HIVE BEETLE IN TENNESSEE** – The small hive beetle (*Aethina tumida* Murray) has now spread throughout the United States and other parts of the world. (Hood, 2004 *Bee World* 85: 51-59). A study was initiated in October, 2006 in Knoxville, Tennessee using forty-two colonies of small hive beetle infested honey bees in five locations. Initially, a single plastic-box trap known commercially as the "Hood Small Hive Beetle Trap" (Brushy Mountain Bee Farm, Inc.) was attached to the bottom bar of an empty frame and inserted into a honey bee colony. Each trap had three compartments. Food grade mineral oil was added to the outside two compartments as a scentless killing agent, while either apple vinegar or pollen patty substitute (Global Patties) plus small hive beetle yeast (USDA, Torto) was added to the middle compartment as bait. A comparison of mean number of adult beetles trapped in the mineral oil over four dates (approximately a weekly period) indicated that significantly ( $P < 0.07$ ) more beetles were trapped in the traps with yeast plus pollen patty (YPP) compared to traps baited with apple cider vinegar (V) (See Figure). There was a significant difference among the sampling dates by treatment because more beetles were trapped with YPP bait on the first sampling date, one week after installing the traps. This indicates that beetles found the traps more rapidly when baited with YPP. After the second sampling date, two additional traps were added to each of ten colonies that started the trial with vinegar baited traps only. One trap contained pollen patty only; the other contained YPP. Although more beetles were collected in traps with YPP, suggesting that the yeast component of YPP added to attractiveness of beetles to pollen patty alone, this trial should be repeated with a balanced experimental design. Beetles oviposited in the YPP, and larvae were subsequently collected in the mineral oil in traps baited with YPP. It is possible that larval development could add an olfactory cue to this bait. Further study is needed to determine if this factor is important.



**18. Villa, J. D.<sup>d</sup> - INTERMEDIATE LEVELS OF RESISTANCE TO TRACHEAL MITES IN CROSSES BETWEEN RESISTANT AND SUSCEPTIBLE STRAINS** – Bioassays and sampling of field colonies were used to test the hypothesis that the resistance to tracheal mites in Russian honey bees is a dominant trait. Earlier studies with Buckfast bees as a resistant parent had suggested dominance or partial dominance in their crosses with either a Canadian susceptible line (Lin *et al.*, 1996 *Exp. Appl. Acarol.* 20: 87-101) or with U. S. sources (Danka & Villa, 2001 *J. Econ. Entomol.* 93: 1602-1605).

Starting in 2003, colonies of Russian origin ( $R \times R$ ) and from selected susceptible colonies in the U.S. ( $S \times S$ ) were propagated using instrumental insemination and further selected for diverging levels of tracheal mite resistance. Five groups of  $F_1$  colonies were produced by crossing queens and drones from these diverging parental colonies in spring or autumn (2003-2006). In a series of

10 bioassays, 20-50 young workers ( $< 12$  h from emergence) from surviving  $R \times R$ ,  $F_1$  and  $S \times S$  colonies were simultaneously introduced into infested colonies and retrieved 5-7 days later. On average, each bioassay tested 3.5  $R \times R$ , 4.7  $F_1$ , and 3.3  $S \times S$  colonies coming from a total of 21, 26, and 26 colonies, respectively. Average mite abundance (female mites per worker) was calculated for each colony in each bioassay and used as the variable for analyses. In July 2005, a group of 27  $R \times R$ , 17  $F_1$  and 28  $S \times S$  queens were randomly assigned to colony divisions and established in two apiaries near Baton Rouge, Louisiana. Initial colony infestation was highly variable, but evenly distributed between the three groups. Colonies were sampled and the queen status checked bimonthly until May 2006.

The average mite abundance of  $F_1$  colonies averaged over all bioassays was intermediate ( $0.96 \pm 0.15$ , mean  $\pm$  SE) and significantly different from that of both  $R \times R$  colonies ( $0.63 \pm 0.15$ ) and  $S \times S$  colonies ( $1.37 \pm 0.15$ ). However, when results for the ten bioassays are analyzed individually, the infestation of  $F_1$  colonies gave variable outcomes: in five of the ten bioassays, the  $F_1$  colonies were similar to the  $R \times R$  and significantly different from the  $S \times S$  colonies; in two bioassays, the  $F_1$  colonies were similar to the  $S \times S$  colonies; in one bioassay the  $F_1$  colonies were intermediate; and in two bioassays there were no significant differences between the groups. In 17 field colonies surviving with original queens after 10 months, tracheal mite infestation was highly variable, with many colonies showing no detectable levels of infestation. Nevertheless, mite prevalences in colonies showed distributions supporting the findings of the bioassay. All three resistant colonies had undetectable mite levels, while prevalences in 4  $F_1$  colonies ranged from 0% to 53%, and prevalences in 10  $S \times S$  colonies ranged from 0% to 90%. These data sets do not support the hypothesis that a single dominant gene confers resistance to Russian bees. The trait most likely is regulated by a number of additive genes.

**19. Williams, G. R.<sup>p</sup>, Rogers, R. E. L.<sup>o</sup> & D. Shutler<sup>p</sup> – TALES FROM THE CRYPT: USING DEAD-BEE TRAPS TO MONITOR DEFORMED WING AND OTHER ASPECTS OF COLONY HEALTH** - Deformed wing virus is a positive-stranded RNA virus that has been detected in honey bees (*Apis mellifera*) in Europe, Asia, Africa, and most recently, the Americas (Allen & Ball, 1996 *Bee World* 77: 141-162; Ellis & Munn, 2005 *Bee World* 86: 88-101). Often associated with the parasitic mite *Varroa destructor* (Kevan *et al.*, 2006 *Am. Bee J.* 146: 694-697), this virus may cause wing deformity and shorten bee life-span (Yang, 2004 Ph. D. thesis, Pennsylvania State Univ. pp. 1-270); however, its effect on colony productivity and methods for monitoring need further investigation.

In July 2006, we carried out a 3-week bee health study using 10 colonies in north-central U.S. Colony strength was determined 4 times (July 10/11, 17/18, 22/23 and 27/28) by estimating coverage areas for adults, brood, honey, and pollen on each frame. Brood success was determined by following the egg to adult development of selected individuals ( $n \geq 26$  per hive). *Varroa destructor* was monitored using sticky boards during 24-hour sampling periods at the beginning, middle, and end of the experiment. To quantify intra-hive mortality, we used an underbasket dead-bee trap that consisted of a wooden frame, measuring 50 by 100 cm from the inside edge, and two wire screens, fine mesh on the bottom and coarse mesh on the top (Accorti *et al.*, 1991 *Ethol. Ecol. Evol. Special Issue* 1:123-126). Traps were checked daily and the number of recovered individuals was recorded.

During our evaluation of intra-hive mortality, we discovered a large proportion of individuals with wing deformities. All dead-bee traps recovered individuals with deformed wings, with the total proportion of affected workers and drones among the 10 colonies ranging from 2 to 44% and 30 to 84%, respectively, during the 3-week period. No relationships were found between the proportion of individuals with deformed wings recovered from dead-bee traps and any colony strength measurement (all  $R^2 < 0.15$ , all  $P > 0.27$ ); however, there was a strong relationship between the total proportion of workers with deformed wings and the total number of *V.*

*destructor* collected ( $F = 34.8$ ,  $R^2 = 0.81$ ,  $P < 0.001$ ), and a weaker relationship between drones recovered with deformed wings and *V. destructor* ( $F = 4.9$ ,  $R^2 = 0.38$ ,  $P = 0.06$ ).

Long-term studies on the effects of deformed wing virus are needed, especially since this virus appears to be prevalent in many regions of the U.S. Also, the usefulness of dead-bee traps to quantify deformed wing virus and its effect on colonies should be investigated. These preliminary data suggest that dead-bee traps can be used to quantify deformed wing virus, and may even serve as a non-invasive and cost-effective method to indirectly monitor *V. destructor* numbers in colonies that contain the virus.

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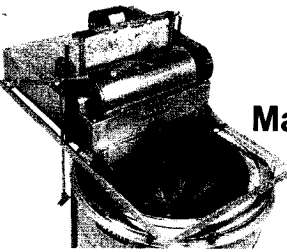
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