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INSIDE

A note on flight activity of 4-lb Australian package-bee colonies used for almond pollination

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Keywords: honey bees, *Apis mellifera*, foraging, *Prunus dulcis*

Increasing acreage of almonds (*Prunus dulcis*) in California has increased the demand for honey bee (*Apis mellifera*) colonies for pollination. Since 2005, domestic U.S. colonies have been supplemented with colonies started from package bees imported from Australia. The need for almond pollination in late winter in California fits well with the availability of bees in late summer in Australia. Little is documented, however, about how recently imported bees perform as pollinating units. We compared flight activity of Australian package bee colonies (APBCs) and overwintered colonies during almond bloom.

We measured overall flight activity and pollen collection of 28 APBCs and 28 overwintered colonies. Packages (Brown's Bees Australia Pty Ltd., Mendooran, NSW) were 4-lb. units imported and hived in mid January 2006. Overwintered colonies, which had been started as APBCs in spring 2005, were managed in southern California prior to being moved in early February together with the APBCs for pollination. All colonies were in 1½ story hives and fed two gallons of sucrose/fructose syrup and one pound of pollen patty. Bees were placed in 12-colony groups (one colony type per group) along a road between two 40-acre (0.162-ha) blocks of almonds near Delano, CA. In one block, 'Sonora', 'Nonpareil', and 'Mission' ranged from early bloom to initial petal fall during the observation period. The other block of 'Butte' and 'Padre' had little bloom. Colony populations were obtained by measuring the coverage of each comb (estimated to the nearest 10% of a deep Langstroth comb) by adult bees and by sealed brood.

Measurements of flight activity were made on 14, 17, 18, 20 and 24 February. Two observers used flight cones (Gary 1967) to count the number of bees exiting each colony for 30 sec once every hour from 0800 through 1600 h. Data were converted to bee flights per minute for analysis.

Pollen foraging was measured in a subset of 12 colonies of each type between 1100 and 1400 h each day. Hive entrances were screened closed for ca. 1 min and then 30-40 returning foragers

were swept into clear plastic bags. The percentage of bees carrying pollen pellets was recorded, and the bees were released.

Analysis of variance (ANOVA) and regression analysis were used to evaluate how flight activity was influenced by colony type, adult bee population, brood population, temperature and period of the day. Black globe temperatures were recorded at 5-min intervals at the test location. Period of day was assigned as morning (before 1100 h), midday (1100 – 1359 h) or afternoon (1400 h and later). Details of temperature measurements and statistical analysis are available elsewhere (Danka and Beaman 2007). Differences in pollen collection between the colony types and days were evaluated by ANOVA. Differences in bee populations between the colony types were evaluated with *t*-tests. Variation is reported as SE.

Temperature was the strongest regulator of flight activity. Flight rate increased with rising temperature but the increase was less at higher temperatures. This quadratic response to temperature differed for APBCs and overwintered colonies. Overwintered colonies had a greater rate of increasing flight through much of the observed temperature range (Fig. 1). Flight from overwintered colonies was nearly double that from APBCs at temperatures of peak flight activity (ca. 75 °F; 24 °C).

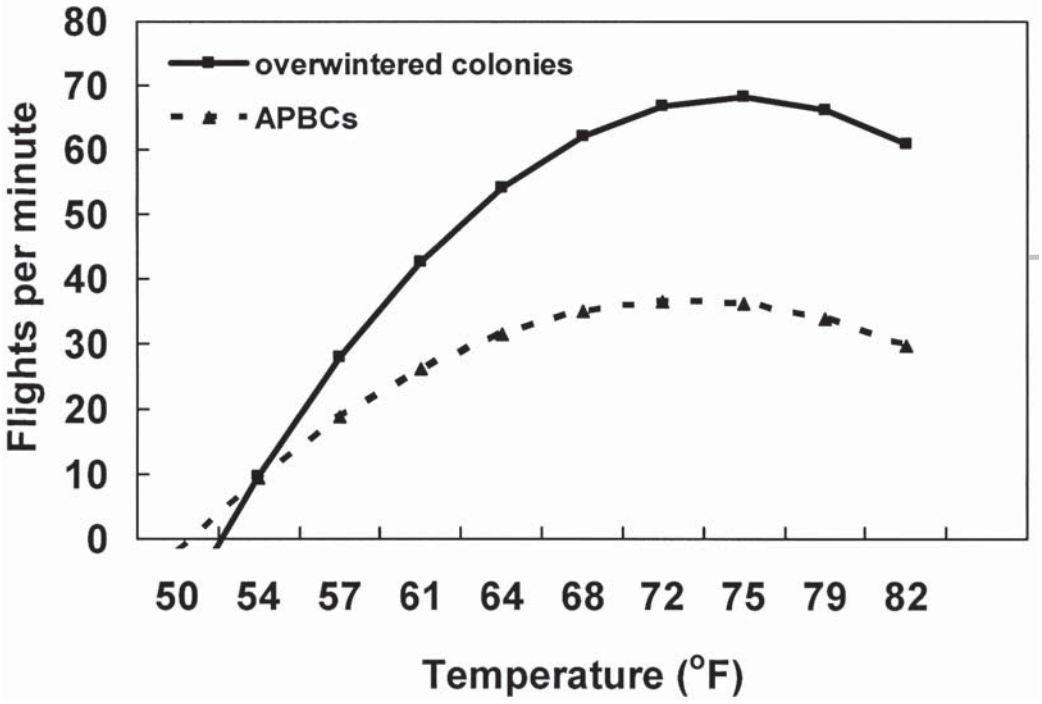

Figure 1. *Flight activity from Australian package-bee and overwintered colonies in relation to temperature. These flight responses are modeled using regression parameter estimates together with the average adult bee populations of each colony type and average response from the three periods of day.*

Colonies with larger populations of adult bees had more flight activity, but population size had a more pronounced effect in the morning and midday than it did in the afternoon. An additional comb, completely covered with bees, yielded about nine more flights per minute before 1400 h but only 4.5 flights per minute after 1400 h. We recorded a similar trend in a previous test of overwintered colonies during almond pollination (Danka *et al*. 2006). The area of sealed brood did not significantly influence flight activity.

Overwintered colonies were more populous than APBCs in both adult bees (4.7 ± 0.3 vs. 3.9 ± 0.2 combs fully covered with bees, respectively) and sealed brood (1.6 ± 0.1 vs. 1.1 ± 0.1 combs fully covered with brood, respectively). At these overall adult bee populations, average flight activity across the range of temperatures observed was 40 bees per minute from overwintered colonies and 34 bees per minute from APBCs.

The percentages of foragers returning with pollen did not differ between the colony types; overall, 59 ± 3% of foragers collected pollen. Pollen foraging differed between days but there was an inconsistent interaction between colony type and day, i.e., APBCs had a greater percentage of pollen collectors than overwintered colonies on 17 February, but the converse occurred on 18 February.

APBCs were less responsive to changes in temperature and fielded fewer foragers than overwintered colonies, especially at higher temperatures when most flight occurred. APBCs were smaller (i.e., they had 17% fewer adult bees and 34% less sealed brood), and so had less flight activity. The combination of different colony sizes and temperature–dependent flight responses led to significantly more foraging flights from overwintered colonies (with an overall average of 47.4 ± 1.35 flights per minute) than for APBCs (27.4 ± 0.8 flights per minute). Thus, newly hived 4-lb (1.8-kg) APBCs had only 58% of the flight activity of overwintered colonies. This finding is consistent with other recent measures of comparative foraging activity of APBCs (Eischen 2006). The lower foraging activity of APBCs should be considered when these units are used for almond pollination.

Acknowledgements

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Overwintering of Russian honey bees in northeastern Iowa

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Summary

Colony survival, levels of tracheal mite infestation, worker population size and weight loss of colonies from Russian test lines were evaluated during three winters (2001, 2002 and 2003) in Cresco, Iowa. Overall, 90% of the colonies survived the period from November to April with all lines showing good survival. The percentage of bees infested with tracheal mites in most Russian colonies in August, November and April was below the economic threshold of 20%. Surviving Russian colonies had good populations at the end of each winter [cluster volumes at *ca*. 50ºF(10°C) averaging at least 750 cubic in (12 *liters*)]. Colony weight loss from November to April was on average less than 20 lbs. (9 kg). The use of a screened bottom board increased weight loss by 20% compared to a standard wooden bottom board while additional top insulation had no effect. Russian bees provide a viable alternative for beekeepers needing to overwinter colonies in northern states.

Keywords: *Apis mellifera, Acarapis woodi*, honey bees, survival, tracheal mites

Introduction

Tracheal mites have made overwintering of honey bee colonies more difficult in the United States and Canada. Until the middle of the 1980s, high colony survival (about 90%) was possible for colonies which were disease-free, had large populations of workers, had a productive queen, had adequate honey stores and were physically protected from rapid heat loss due to wind or extreme cold (Furgala and McCutcheon 1992). In those days, colony losses were generally caused by small populations of workers or by inadequate honey stores (e.g. Johansson and Johansson 1971). Since the arrival of tracheal mites, increased winter mortality has been associated with high levels of tracheal mite infestation in the autumn (Furgala *et al*. 1989, Otis and Scott-Dupree 1992, De Guzman *et al*. 2006).

Tracheal mite resistant stocks prevent mites from reaching harmful levels. Colonies resistant to tracheal mites have a much greater chance of successfully overwintering, especially if they have other characteristics such as frugal food consumption and good clustering ability. Honey bees imported from the territory of Primorsky in far-eastern Russia are highly resistant to tracheal mites (De Guzman *et al*. 2002) and have shown good overwintering attributes (De Guzman *et al*. 2006). Beekeepers in Primorsky report good survival of colonies through winter, especially when colonies are held in wintering barns. We report on the overwintering performance in northeastern Iowa during 2001, 2002 and 2003 of colonies from queen lines being tested in the USDA, ARS breeding program of Russian bees. The main aims of this program are to select for resistance to varroa mites and honey production. However, the program also selects between and within queen lines to maintain resistance to tracheal mites and overwintering ability. The objectives of these tests were:

1) To confirm that Russian bees can overwinter successfully without treatment for tracheal mites in climates where beekeepers tend to have large problems with tracheal mites.

2) To evaluate possible differences between Russian queen lines in their overwintering performance prior to their inclusion in the breeding program.

Materials and Methods

Test Queens – Queens in colonies placed into overwintering tests were produced in the spring of each of three years. Queens produced in 2001, 2002 and 2003 were from lines in blocks C, A, and B of the breeding program, respectively (Rinderer *et al*. 2000). Queens were mated each year with drones from groups A and B, B and C, and A and C, respectively. Additionally, queens from a standard Russian queen line ("White-Yellow/Blue" from block A) were used each year.

Colony Conditions – Colonies chosen for the wintering experiments met several criteria, as follows: (1) the presence of the original queen introduced in the spring had been verified in August or September, (2) they were treated in August with Apistan® to eliminate possible confounding effects of varied rates of varroa mite infestation, (3) they had worker bee populations which were adequate for successful overwintering in that area (bees occupying at least the equivalent of 6 standard Langstroth sized frames), (4) they were housed in two standard Langstroth boxes and had been fed high fructose corn syrup (three to five gallons, 11 to 19 *liters*) so that most of the top box was filled with feed and honey and the total hive weight was above 90 lbs.

Data Collection – Colonies were overwintered in the same one or two apiaries near Cresco, Howard County, Iowa. Samples of workers for dissection of tracheal mites were taken in August of two of the three years (2002 and 2003). Colony weight, cluster dimensions (length, width and depth) at 45 to 55°F(*ca* 7 to 13°C), and worker samples were taken in November of 2001, 2002 and 2003. Colonies were placed on a metal platform attached to a load cell (SP4-100, HBM Inc., Marlboro, MA) connected to electronic digital displays for monitoring of weights. Colonies were then fitted with three sheets of insulation board (R-10, Dow-Corning) over the inner cover, and covered with corrugated cardboard wraps (WT-150, Mann Lake Ltd., Hackensack, MN). The same cluster measurements and samples were taken in each colony in April after removing the winter wraps. Summary weather data from a station 20 mi SSE (KDEH, Decorah, Iowa) were used to assess the severity of the winter periods.

Winter 2001-2002 – In the fall of 2001, 49 colonies representing nine test lines, and the Russian standard line (W-Y/B) were set up in two apiaries. In this test, the possible value of screened bottom boards and additional top insulation [25 lbs. (11.3 kg) of dry oats in a screened box] also were evaluated. Beekeepers in the area had used this technique decades ago because of possible value for humidity control and insulation. Cluster dimensions of colonies were used to produce size classes to which random assignments were made of four treatments (combining either a standard bottom board or a screened bottom board with a standard cover or a screened rim with oats).

Winters 2002-2003 and 2003-2004 – In 2002 and 2003, few-

er colonies with original queens were available after evaluations in the autumn. The emphasis of the tests changed to comparing possible differences between Russian queen lines. A few colonies with queens of Italian origin were also included these two years (two and three colonies, respectively). In 2002, 21 Russian colonies from six test lines and a standard line (W-Y/B) were established in one apiary with screened bottom boards. In 2003, four test lines, plus colonies from the standard line were replicated for a total of 16 colonies in one apiary. Due to smaller bee clusters in 2003, this experiment was conducted with standard bottom boards.

Statistical Analyses - The weight loss and final cluster volume of colonies surviving the winter of 2001-2002 were compared between treatments by analysis of covariance of a randomized block design with a factorial treatment arrangement of bottom board (screen vs. solid) and top insulation (addition of a layer of dry oats vs. the standard 3 layers of R-10 insulation board). Initial colony cluster volume was added as a covariate, and apiary was considered a random effect. Weight losses and final cluster volume in April for the two other winters (2002-2003 and 2003-2004) were analyzed as a completely randomized design with queen line as a fixed effect and initial cluster dimensions as a covariate.

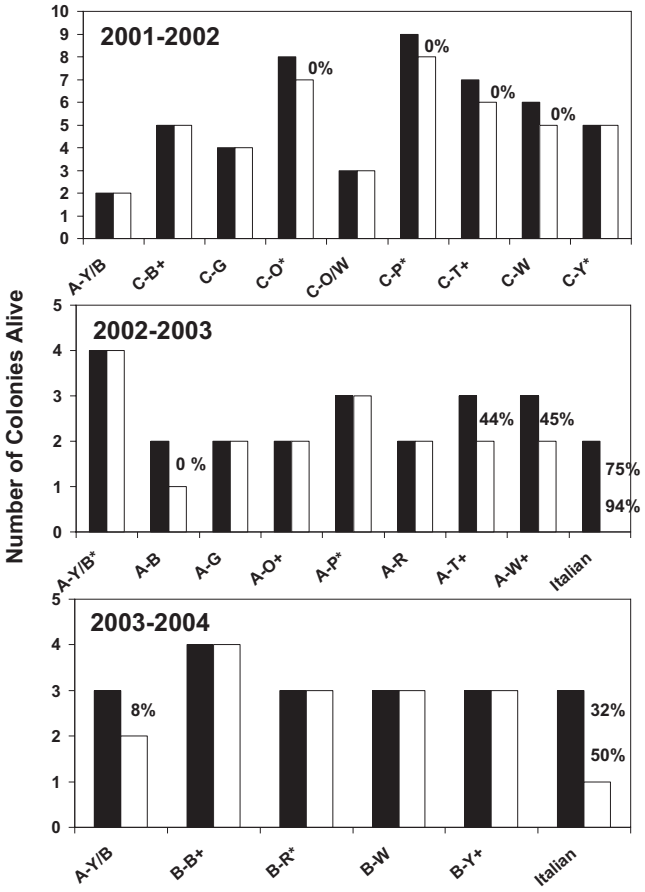

Figure 1. *Number of colonies from Russian queen lines placed into three tests in November (solid bars) and alive in April (clear bars) of the following year. Eight out of 86 colonies of Russian origin died through the winter, while four out of five Italian origin hives died. Percentage of workers infested with tracheal mites in November is indicated in the position corresponding to death of a colony recorded in April. Russian queen lines are indicated by group and color code within group. Queen lines followed by an asterisk were maintained in the breeding program. Queen lines followed by a cross were removed from the breeding program.*

Results and Discussion

Colony Survival and Tracheal Mite Infestation

The survival of Russian colonies ranged from 86 to 94% (Fig. 1) during three typical winters for the area. These survival rates were comparable to the 71 to 94% survival observed the previous two winters with Russian bees in the same apiaries (De Guzman *et al*. 2006). The survival of these Russian colonies matched the levels recorded in Minnesota for bees from different sources in the U.S. prior to the arrival of tracheal mites (Haydak 1958, Sugden and Furgala 1982, Duff and Furgala 1986, Sugden *et al.* 1988).

Most Russian colonies maintained negligible or very low levels of tracheal mites during the winter (Fig. 2). Colony mortality the first year was clearly associated with smaller colony sizes and not due to tracheal mites. The four Russian colonies that died had the smallest cluster volumes in November, and no detectable tracheal mites (Fig. 1). In contrast, the death of both Italian and Russian colonies during 2002 and 2003 were clearly associated with high tracheal mite loads in November: two of four Russian colonies that died those two winters and all four Italian colonies had more than 30 % of the bees infested prior to the wintering period (Fig. 1). Dead workers could be recovered from four of these six colonies at the end of the winter evaluation. From 90 to 100% of the recovered workers in each colony were infested with tracheal mites. Similar associations of high mite infestation with mortality had been found in Russian and Italian colonies in the same apiaries (DeGuzman *et al*. 2006).

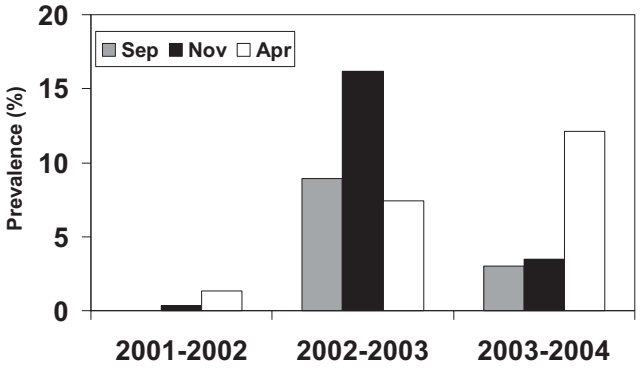

Figure 2. *Average colony tracheal mite prevalence (percentage of bees infested in a colony) in Russian colonies during three overwintering seasons. No data was collected in August of 2001. Data in April include infestation of dead workers recovered from the eight colonies which died between November and April.*

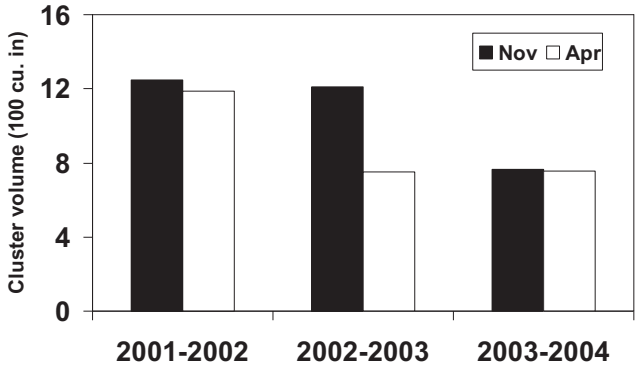

Figure 3. *Average volume of the cluster (cubic inches) estimated in each Russian colony at temperatures from 45 to 55°F in November and April. Analysis of covariance for each year showed no differences between lines.*

Colony Populations

Colonies which started the winter with low levels of tracheal mites and adequate populations, survived the most critical part of winter with good populations of bees (Fig. 3). Predictably, there were effects of the initial cluster volume in November on the final cluster volume in April (*P*=0.276, 0.007, 0.014 for 2002, 2003 and 2004 respectively). No significant differences between queen lines were found in the estimated final cluster volume in April (*P*= 0.66, 0.13, 0.46 for 2002, 2003 and 2004, respectively) when the initial volume in November was used as a covariate. Although Russian lines vary in other characteristics, they have similar good abilities to survive winter.

Colony Weight Loss

Weight loss per colony during the period from November to April averaged less than 20 lbs., 9 kg. (Fig. 4). De Guzman *et al*. (2006) found weight losses of Russian colonies in these same apiaries during the winter of 1999-2000 to be very low (about 9 lbs., 4 kg.), and found significantly higher weight losses in larger colonies of Italian origin (about 15 lbs. 7 kg.). Colony weight loss did not differ between Russian queen lines any of the three years (*P*=0.12, 0.97, 0.80 for weight loss through April 2002, 2003 and 2004, respectively), when initial colony volume was taken into account. Not surprisingly, a larger colony volume in November tended to increase weight loss (*P*=0.0013, 0.79, and 0.08 for 2002, 2003 and 2004, respectively). While we had no simultaneous comparisons with typical U.S. colonies of Italian origin, we have observed that Russian colonies tend to maintain lower populations during the winter. This attribute may prevent early starvation during the period of intense buildup in April and May, where honey stores and possible incoming resources are consumed at a very high rate (Furgala and McCutcheon, 1992).

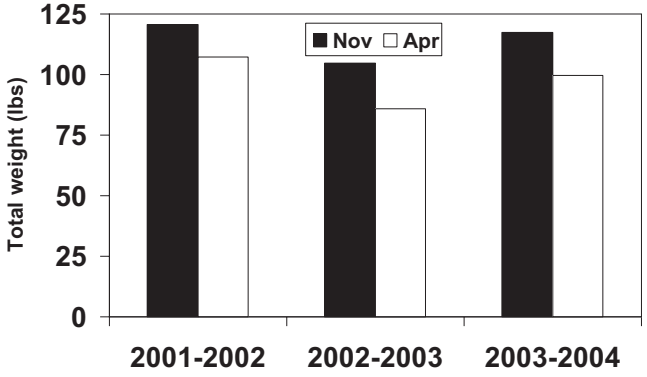

Figure 4. *Average weights of Russian colonies (lbs) in November and April during three overwintering periods. Analysis of covariance showed no significant differences between lines in the weight losses each year.*

Effect of Screened Bottom Boards and Additional Insulation

The test evaluating the benefits of extra top insulation [a layer of 25 lbs. (11.3 kg.) of dry oats above the hive] and of screened bottom boards did not show any clear colony survival advantage. The test did demonstrate that the screened bottom produced an increase in food consumption of about 20% (*P*=0.04, Fig. 5), but that the additional insulation from the layer of oats did not significantly decrease food consumption (*P*=0.37). There was no significant effect of the type of bottom board or of the extra insulation on the final cluster volume of bees in April (Fig. 5).

Conclusions and Recommendations

Russian colonies can survive well through the winter and are a valuable genetic resource for North America. Russian colonies tend to maintain tracheal mite levels below critical levels even through harsh winters, and this gives them an advantage in surviving winter periods with good populations for the spring buildup period. Additionally, Russian colonies use honey stores frugally during the overwintering period, decreasing the need for feeding in the spring.

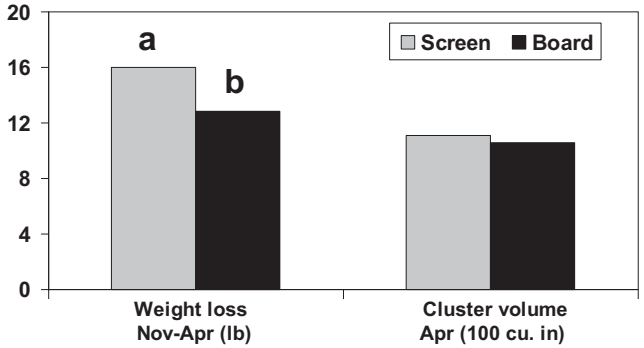

Figure 5. *Weight loss and final cluster volume in April of 49 colonies overwintered in 2001-2002. Colonies were assigned either a screened bottom board or a regular bottom board, combined with either standard insulation from three sheets of insulation board (R10) or a screened box holding 25 lbs of dry oats below the standard insulation boards. Analysis of covariance indicated no effect of the extra insulation, so the data are summarized for the effects of screened vs. solid bottom boards only. Different letters above the bar indicate significantly different means (P<0.05).*

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Effect of GSM Cellular Phone Radiation on the Behavior of Honey Bees (*Apis mellifera*)

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SUMMARY

This study consists of a series of experiments that investigate the effects of radiation emitted by GSM cellular phones (Global System for Mobile Telecommunications) on the behavior of harnessed and free-flying forager honey bees. A unique aspect of this study is that three subspecies – *Apis mellifera carnica*, *Apis mellifera caucasiaca*, and *Apis mellifera syriaca* – were employed. In the first series of experiments, we investigated its effects on proboscis extension and feeding in harnessed foragers. Relative to control animals, exposure did not influence proboscis extension or feeding. In a second series of experiments, free-flying foragers were trained to visit a target with the question of interest being whether exposure to cell phone radiation would influence flight navigation. Relative to control animals, the results indicated that a 45 minute radiation exposure did not influence return to the target. In the final series of experiments, the effect of GSM radiation on aggression was investigated. As in the previous experiments, no effect of radiation exposure was found. To our knowledge, this is the first study to investigate the effects of GSM cellular phone radiation on honey bees.

Keywords: aggression, insect, microwave radiation, navigation, proboscis

INTRODUCTION

The primary objective of this study was to elucidate the effects of GSM cellular phone radiation on the behavior of honey bees. GSM (Global System for Mobile Telecommunications) is one of the most common and fastest growing wireless network standards. Recent research has revealed that there are currently 2.8 billion GSM cellular phone subscribers worldwide (Wireless Intelligence, 2007). A pervasive media report asserted that cellular phones were a possible cause of honey bee colony collapse disorder in April 2007 (Good Morning America, 2007; Lean and Shawcross, 2007). An investigation of this report revealed that the media misinterpreted the findings of a study conducted by Kimmel *et al*. (2007) at Koblenz-Landau University in Germany. The study made no reference to CCD and did not look at the effects of cellular phone radiation on honey bees. It did, however, demonstrate a decrease in the number of return visits made by bees to observation hives into which active DECT (Digital Enhanced Cordless Telecommunications) phone base stations had been placed. However, caution must be employed when generalizing the findings of the Kimmel et al. (2007) study to other wireless communication technologies, namely those employed by cellular phone networks (i.e., GSM technology).

GSM technology utilizes bands of non-ionizing microwave frequencies in the range of 850, 900, 1800, and 1900 MHz. This study, which was conducted in the Republic of Turkey, looks at the 900 and 1800 MHz bands utilized by European GSM networks (Hamid et al. 2003; Ozyalcin et al. 2002). In addition to the ICNIRP (International Commission on Non-Ionizing Radiation Protection) emission limit guidelines, government-sanctioned wireless telecommunication regulations, and stringent handset tests carried out by cellular phone manufacturers have helped to ensure the safety of these devices. In fact, many recent studies have demonstrated that various tissues, cellular activities, memory, and learning in humans, rats, and mice are not affected when subjected to GSM or GSM-like microwave radiation (Cobb *et al*. 2004; Dasdag *et al*. 2003, 2004, 2008; Dubreil et al. 2002, 2003; Forgacs et al. 2006; Joubert et al. 2007; Kumlin *et al*. 2007; Sienkiewicz *et al*. 2000; Smith *et al*. 2007; Thorlin *et al*. 2006; Tillmann *et al*. 2007). Nevertheless, these findings should not downplay the potential health hazards involved with the use of cellular phones.

GSM or GSM-like radiation has been found to negatively affect the neural and reproductive tissues in both vertebrate and invertebrate species including humans, mice, rats, snails, and fruit flies (Atli *et al*. 2006; Erogul *et al*. 2006; Field *et al*. 1993; Lopez-Martin *et al*. 2006; Panagopoulos *et al*. 2004, 2007a, 2007b; Salford *et al*. 2003; Zhao *et al*. 2006). These studies provide compelling evidence that GSM radiation could in fact have negative biological effects on honey bees. Although the invertebrate data are insufficient to justify a directional argument, the potential for negative biological effects on learning and behavioral processes in honey bees caused by GSM radiation has important ecological ramifications: the honey bee is a keystone pollinator species. Sharp declines in honey bee populations due to GSM radiation could considerably weaken the infrastructure of food webs across the globe.

Another potential behavioral change resulting from GSM exposure could include an increase in aggression (e.g., increased potential for colony defense behavior). Aside from this risk factor alone, 22.2% of individuals surveyed in the United States report an intense fear of animals (Curtis et al., 1998). Specific phobias of animals including insects were reported by 5.7% of these individuals. According to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders), specific phobias can invoke powerful involuntary responses including intense anxiety or distress, panic attacks, or avoidance behavior. The elicitation of such responses could place individual cellular phone users who suffer from insect phobias at an elevated risk of suffering from multiple stings.

Trash receptacles found in frequented areas such as zoos, amusement parks, and university campuses are likely to contain discarded soft drinks that are attractive to foraging honey bees (Abramson *et al*. 1997). It has been estimated that nearly 1% of children and 3% of adult sting victims have systemic reactions to insect venom (Golden *et al*. 1989). Multiple stings can culminate in acute renal failure (Bresolin *et al*. 2002; Daher 2003; Ramanathan 1990), rhabdomyolosis (Hiran 1994), hepatic complications (Kini 1994), and although rare, even death. A case study conducted by Thiruventhiran (1999) revealed that 25% of individuals afflicted by acute renal failure induced by insect stings ultimately died, and it

has been estimated 0.4% of bee stings in the United States are fatal (Reisman 1992).

In an attempt to establish a definitive investigation of the potential consequence of GSM radiation on honey bee health, we have proposed three fundamental questions: (a) Does GSM radiation exposure affect the proboscis extension reflex or the ability to imbibe a sucrose solution? (b) Does GSM radiation exposure affect flight navigation and foraging ability? (c) Does GSM radiation elicit aggressive behavior?

MATERIALS AND METHODS

Four experiments were conducted during the months of June and July 2007 at the main campus of the Middle East Technical University (Orta Doğu Teknik Üniversitesi) in Ankara, Turkey. In Experiment 1, three different makes and models of cellular phone handsets were utilized, including the Motorola SLVR L7, Samsung SGH-i670, and Sony-Ericsson J100i. The respective specific absorption rate (SAR) values reported by each phone's manufacturer are as follows: Motorola SLVR L7 = 1.34, Samsung SGH-i670 = 0.95, Sony-Ericsson J100i = 0.96. A SAR rating is the ratio of the number of watts of energy absorbed per kilogram of living tissue. A brief review of the results from Experiment 1 and a revaluation of the employed design revealed it unnecessary to utilize all three cellular phones in remaining experiments. Only the Motorola SLVR L7, was utilized in the remaining experiments. This decision was reached, in part, by an incompatibility with the use of non-domestic cellular phones on the Turkish wireless telecommunications network.

The mean power density of the Motorola SLVR L7 was determined to be 1.41 ± 0.483 μW/cm2 at the 1900 MHz frequency band. It is important to note, however, that we were unable to obtain the mean power density readings of the cellular phones in Turkey due to a lack of necessary equipment. Measurements were obtained in the United States using a radio frequency power density meter developed by Alpha Lab Detector Technologies, Inc. The RF power density meter was placed 2 cm from the top of the cellular phone, which was the same distance at which the bees were placed in all harnessed forager experiments. The mean power density emission was calculated by averaging the values of 25 separate measurements collected during a 5 minute period. The methodology we employed for these calculations is similar to that utilized by Panagopoulos *et al*. (2007). Refer to this article for more information on expected field strength emissions of cellular phone handsets operating on 900/1800 MHz European GSM networks. Despite this setback, it is critical to note that actual power density emissions of cellular phones are never constant and depend on a number of technical variables (Hyland 2000, Panagopoulos *et al*. 2007).

We opted to utilize GSM handsets instead of GSM base stations for multiple reasons. First, handsets are easily accessible and they have been employed as radiation sources in previous radiobiology studies (Barteri *et al*. 2005; Diem *et al*. 2005; Panagopoulos *et al*. 2007; Salford *et al*. 2003; Weisbrot *et al*. 2003; Zhao *et al*. 2006). Second, base stations are static objects that nearly continuously emit radiation which makes it difficult to employ experimental controls. Third, we felt that the use of GSM handsets most closely mirrored real-life situations involving honey bee exposure to cellular phone radiation. Honey bees are arguably more likely to encounter a motile human using an active GSM handset than individual static GSM base stations, especially in densely populated areas. Other wireless communication network standards including CDMA, WCDMA, UTMS, OFDM, DECT, etc. were not considered in the present study because of the ubiquity of GSM wireless networks throughout the globe.

Harnessed Forager Experiments:

Experiment 1 - Effects of GSM Radiation on the Proboscis Extension Reflex

A minimum of four forager bees were collected from five observation hives of each subspecies for both the sham-exposed (control) and experimental groups. In total, 217 bees were collected and included this experiment: *A. m. caucasica* (control, n = 34; experimental, n = 50); *A. m. carnica* (control, n = 30; experimental, n = 36); *A. m. syriaca* (control, n = 37; experimental, n = 30). Bees in this experiment were harnessed in plastic straws cut into tubes approximately 2.5 cm in height. Plastic straws were selected as a substitute to the metal casings traditionally used in this harnessing procedure (Abramson *et al*. 2001). The rationale behind the use of plastic tubes was to prevent deflection of the radiation emitted by the cellular phone that would otherwise occur. A 2M sucrose solution, 1 cm x 1 cm filter paper squares, and plastic forceps were also employed in this experiment.

Each day between late morning and early afternoon, forager honey bees were captured in glass vials from hives located in the biology department's apiary and placed into appropriately labeled plastic storage bags. To prevent overheating and possible mortality, the bees were periodically transported to the laboratory and immediately placed into an ice bath. After the bees were rendered motionless they were harnessed in the plastic tubes with a strip of duct tape placed behind the head and thorax as demonstrated by Abramson *et al*. (2001). After harnessing, bees were placed around the top half of the experimental cellular phone in such a way that a semi-circle was formed. The bees were then carefully positioned with their ventral surface facing the phone. The rationale for this arrangement was to ensure the bees were directly irradiated by the phone's antenna.

An ABA design with an 11-minute interval per phase was implemented. The bees were randomly divided into control and experimental groups. One group served as a control, in which the bees were placed around a sham and received antenna stimulation once every minute for the duration of 33 min. This was accomplished via direct contact of the filter paper saturated in the 2M sucrose solution to the antennae. In the experimental group, bees were subjected to GSM radiation during the treatment phase (phase B – onset of minute 12) by establishing a voice connection between the experimental phone and a second cellular phone. The second phone was removed from the experimental area to reduce the possibility of additional radiation exposure, and the connection was immediately terminated at the end of minute 22. The dependent variable in this experiment was the proportion of bees that extended their proboscis following sucrose stimulation. A clearly visible extension of the proboscis was recorded as a "1" and the absence of a response was recorded as a "0". This procedure was repeated once every minute for the duration of the experiment in both the sham-exposed and experimental groups.

Experiment 2 - Effects of GSM Radiation on Feeding

As in the proboscis extension experiment, a minimum of four forager bees were collected from five observations hives of each subspecies for both the sham-exposed and experimental groups. A total of 170 bees were collected and employed in this experiment: *A. m. caucasica* (control, n = 27; experimental, n = 31); A. m. carnica (control, n = 25; experimental, n = 26); *A. m. syriaca* (control, n =

27; experimental, n = 34). The materials and harnessing procedures were also identical to those utilized in the first experiment. However, to obtain a measure of feeding, the filter paper was immediately moved into contact with the proboscis upon its extension following antenna excitation. Thus, an additional dependent variable in this experiment was the proportion of honey bees that imbibed the 2 M sucrose solution. All responses were recorded visually. If the solution was imbibed a "1" was recorded. A "0" was recorded if the proboscis was immediately retracted following stimulation or if the bee did not imbibe the solution.

Free-Flying Forager Experiments:

Experiment 3 - Effects of GSM Radiation on Flight Navigation

Subjects for this experiment consisted of free-flying forager honey bees from two colonies of each subspecies. A total of 109 bees were observed: *A. m. caucasica* (control, n = 13; experimental, n = 25); *A. m. carnica* (control, n = 12; experimental, n = 22); *A. m. syriaca* (control, n = 19; experimental, n = 18). Two observation hives of each subspecies were moved from the biology department's apiary and placed adjacent to one another in isolated sites to prevent subspecies interactions. Each site was geographically distanced by approximately 2.5 km. Bees at each site were trained to forage from a petri dish placed 20 m in front of the hive entrance. To accomplish this, the entrance of each hive was first sprayed with a lavender scented sucrose solution. A petri dish filled with the scented solution was then placed near the entrance of the hives and slowly moved away as the bees began to forage from it. Once depleted of the scented solution, the dish was subsequently refilled with an unscented sucrose solution for the remainder of the experiment. Each bee was carefully demarcated with the enamel model paint so we would be able to identify individual bees. After the bees were marked, the experimental cellular phone was placed beneath the petri dish and experimentation began.

An ABA design with 45-minute intervals was employed. This 45-minute flight time interval was selected in an attempt to replicate the methodology employed by Kimmel *et al*. (2007). For all three phases of the experiment, each marked bee's return was visually recorded. At the onset of the treatment phase (phase B – onset of minute 46), a voice connection between the experimental cellular phone and a second cellular phone was established. The second phone was moved to a location 30 m away and positioned with its antenna pointing away from the bee colonies and experimental area. The connection was immediately terminated at the onset of the post-treatment phase. Control data (sham-exposed condition) was also collected by following the same procedure, with the exception that no voice connection was initiated during the treatment phase. As in the harnessed experiments, failure to implement such a control would make it impossible to rule out the effects of confounds. A confounding effect of particular interest in this experiment included the possibility of a naturally occurring decrease in the number of returning foragers over time. Experimental and control data collections were not simultaneously conducted. Instead, the condition was randomly chosen. The dependent variable in both conditions was the total number of return visits made per bee in each treatment condition.

Experiment 4 - GSM Induced Aggressive Behavior

Forager and guard bees from a total of five hives of each subspecies were observed for this experiment. A small foam block was used as an observation and rest platform for the cellular phone, and a six mega-pixel digital camera was used to measure the dependent variable. To investigate the effects of GSM radiation on aggressive behavior in honey bees, the experimental cellular phone was set on a foam block that was placed 10 cm from the hive's entrance. As in Experiments one and two, an ABA design with 11-minute intervals was implemented. To quantifiably measure aggression as a dependent variable, a photograph of the platform was taken from the side of the hive once every 30 s. Photography stopped 33 min after the first image was captured. Photographs taken from in front of the hive would have produced a possible confounding effect, as the bees would likely become excited by a blocked hive entrance.

During the treatment phase, bees were subjected to GSM radiation by establishing a voice connection between the experimental phone and a second cellular phone. The second phone was placed at a distance of 20 m from the experimental area with its antenna positioned away to reduce the possibility of additional GSM radiation exposure. This procedure was repeated for five hives of each subspecies. At the end of the experiment, the number of bees in flight, and the number of bees on the platform or phone in each photograph was recorded. This distinction was made, because of the difficulty in discerning the difference between flying worker and guard bees. Therefore, aggression was measured by counting the number of both free-flying worker and guard bees on and off the phone.

RESULTS

Harnessed Forager Experiments:

Experiment 1 - Effects of GSM Radiation on the Proboscis Extension Reflex

A 3 X 3 X 2 X (3) split-plot ANOVA with trials as the repeated measure and experimental treatment condition, cellular phone model, and honey bee subspecies as the between-subjects variables was performed. The relationship of interest, experimental treatment condition by trial, revealed no significant interactions, with the largest $\eta 2 = 0.034$. This effect size was obtained by the formula $\eta 2 = 1 - \text{Wilks' } \Lambda$. Figure 1 illustrates the trial by condition interaction.

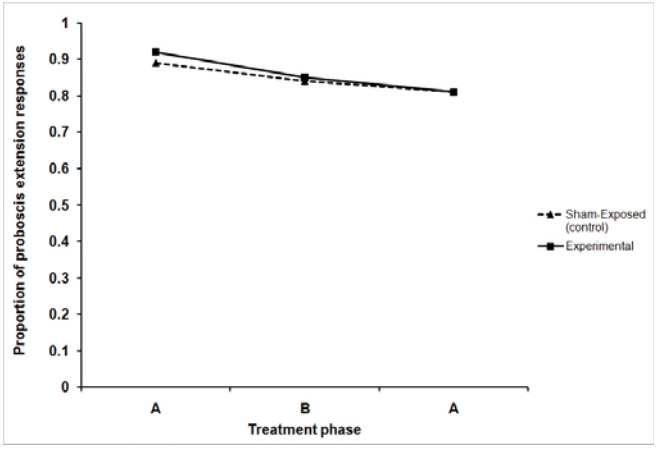

Figure 1. *Trials by experimental treatment condition reveals no effect on proboscis extension.*

Experiment 2 - Effects of GSM Radiation on Feeding

The primary dependent variable of interest in this experiment was whether the bees would feed on a 1.5 M sucrose solution when irradiated by the cellular phone. As a result of measuring feeding responses, the proboscis extension reflex investigated in Experiment one was again measured. Due to no significant main effects between the three utilized cell phone models in the Experiment one,

only the Motorola SLVR L7 was employed in this and the remaining experiments.

A 3 X 2 X (6) split-plot ANOVA with trials as the repeated measure and honey bee subspecies and experimental treatment condition as the between-subjects variables was performed. As in Experiment 1, the relationship of interest was the experimental treatment condition by trial interaction. No significant interactions were found, with the largest $\eta 2 = 0.034$.

Free-Flying Forager Experiments:

Experiment 3 - Effects of GSM Radiation on Flight Navigation

A 3 X 2 X (3) split-plot ANOVA with trials as the repeated measure and honey bee subspecies and condition as the between-subject variables was conducted in attempt to identify any possible main or interaction effects. As with the previous two experiments, the trial by experimental treatment condition relationship was of primary interest. The analysis revealed that this interaction was non-significant, with the largest $\eta 2 = 0.008$. The average number of return visits made to the artificial feeder can be seen in Figure 2.

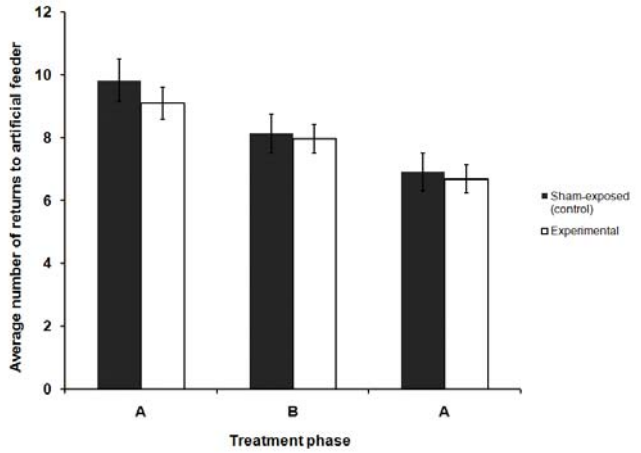

Figure 2. *Average number of return visits made by all marked bees to the trained feeding site (subspecies aggregated). Bars represent the standard error of the mean.*

Experiment 4 - GSM Induced Aggressive Behavior

A 3 X 3 univariate ANOVA was conducted to determine whether GSM radiation can elicit aggressive behavior. The dependent variable in this analysis was aggression as defined by the number of bees on the phone per thirty seconds. The subspecies [$F = 2.03$, $p = .13$, partial $\eta 2 = .004$, power = .42] and treatment condition [$F = 0.45$, $p = .64$, partial $\eta 2 = .001$, power = .12] main effects were not significant. A second 3 X 3 univariate ANOVA was conducted to analyze aggressive behavior as defined by the number of bees in flight per thirty seconds. Results of this analysis revealed a significant subspecies [$F = 6.93$, $p = .001$, partial $\eta 2 = .01$, power = .93] main effect. However, the experimental treatment condition main effect was not significant [$F = 2.15$, $p = .12$, partial $\eta 2 = .004$, power = .44]. These results are summarized in Figure 3, which shows the average number of bees on the phone and in flight during each treatment phase (trial).

DISCUSSION

The results of these experiments demonstrate that exposure to 900/1800 MHz GSM radiation does not influence the antennae response to sucrose or the feeding response in harnessed foragers. In experiments designed with free-flying foragers the ability of marked bees to return to a feeding location was also not affected. Moreover, unmarked foragers were continuously recruited to the experimental feeder throughout the duration of each experiment, thus indicating that exposure to GSM radiation at the feeding site did not affect communication within the observation hive. Despite this interesting observation, recruitment behavior was not included as a dependent variable in our study. Additional research is required to experimentally verify this finding. Finally, in an experiment designed to study whether GSM radiation acts as a stressor capable of inducing aggression in honey bees (i.e., increased propensity to attack and/or sting), no effect was found.

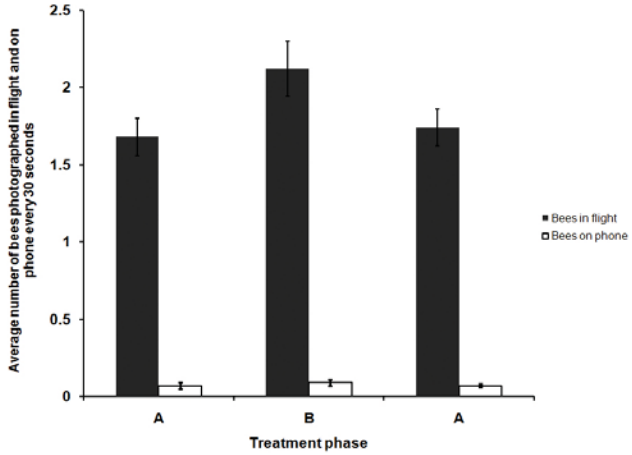

Figure 3. *Average number of bees photographed in flight and on the phone (subspecies aggregated). Bars represent the standard error of the mean.*

As previously mentioned, Kimmel *et al*. (2007) observed that DECT phone base stations activated from within bee colonies had fewer returning bees than colonies in which no base stations were placed. Our study suggests that GSM radiation does not have the same effect. Although we employed GSM cellular phones, the study of Kimmel *et al*. (2007) did not consider confounds attributed to the use of a non-ionizing radiation source, namely a potential increase in hive temperature caused by the base station's radiation emissions. The null results of our study suggest that it is not the radiation, but another stimulus that deterred reentry.

Negative results are never appealing; however, no uniform consensus on the effects of microwave radiation on biological processes has been demonstrated in the extant literature. Given the experimental parameters employed in our study (e.g., within and between subject designs, use of three subspecies, large sample sizes), we believe that any potential effects of GSM radiation on honey bee behavior should have been detected. Contrary to the media reports, our results also suggest that the 900 and 1800 MHz frequencies utilized by GSM technology are not a likely cause of, or a contributing factor in, colony collapse disorder. Other possible causes of CCD and factors contributing to honey bee population declines including biological pathogens (Cox-Foster *et al*. 2007), agrochemicals, climate change, and genetically modified crops must continue to be investigated. Moreover, the copious disagreement among published findings of the effects of cellular phone radiation on humans and other animals necessitates that researchers continue to investigate the biophysical interactions between microwave radiation and biological systems.

CONCLUSIONS AND RECOMMENDATIONS

Our experiments suggest that beekeepers are not at an in-

creased risk of being stung or initiating nest defense behavior while using cellular phones near hives. Our results also suggest that GSM cellular phone radiation emissions do not inhibit the foraging behaviors or navigational ability of honey bees, and are thus unlikely to affect colony health.

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Preliminary observations of autumn feeding of USDA-ARS Russian honey bees to enhance flight performance during almond pollination

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Summary

We attempted to increase bee populations of Russian and Italian honey bee colonies by feeding two pounds of patties of bee-collected pollen in October and November, and comparing fed colonies to unfed colonies of both types (n=16 per treatment group) in late winter. Flight activity of colonies in the four treatment groups was monitored electronically with ApiSCAN Plus® counters on 17-25 February 2006 while the colonies were used for almond pollination. At the beginning of almond bloom, the mean area of sealed brood was 56% greater in the fed colonies (both bee types) than in the unfed colonies. Adult bee populations were 17% larger in the fed group but this increase was not significant. Bee populations and brood populations both were similar for Russian and Italian bees (i.e., when feeding groups were combined). Changes in bee and brood populations did not differ statistically between Russian and Italian colonies. Flight activity during almond pollination was affected neither by feeding treatment nor by bee stock, presumably because these factors did not influence populations of adult bees. Flight activity was significantly affected by temperature, adult bee population and period of the day. The results showed that supplemental feeding maintained adult bee populations in Russian colonies through winter; more extensive or earlier feeding may increase bee populations.

Keywords: pollen supplement, foraging

Introduction

USDA-ARS developed Russian honey bees (*Apis mellifera*) primarily to provide U.S. beekeepers with a stock that resists parasitic mites and that has good honey production (Rinderer *et al*. 2005). These bees are now being used to help fill the recent increased demand for colonies to pollinate almonds in California in late winter. We previously found that Russian and Italian colonies that had equal adult bee populations during almond bloom had similar flight activity (Danka *et al*. 2006). However, Russian colonies often had less flight activity because they were less populous on average than Italian colonies. Those observations raised the issue of whether stimulative feeding of Russian colonies before almond pollination can enhance bee populations and flight activity during almond bloom. The situation is of practical importance for beekeepers who need to meet rental contracts. It also is of interest from the standpoint of behavioral ecology, as it is unknown whether these northern-adapted bees can be made to expand their populations in the autumn or winter. Supplemental protein supplied in the autumn or winter previously has been shown to boost non-Russian bee populations during the time of almond bloom (Peng *et al*. 1984, DeGrandi-Hoffman *et al*. 2008).

We began to address this question with exploratory feeding trials with colonies overwintering in Louisiana in two years prior to the results presented here. Mid-winter (January) feeding of two formulations of a commercial pollen substitute yielded no appreciable population expansion in either Russian or Italian bees in February or March 2004; Italian colonies were larger than Russian colonies whether fed or not. We then chose to evaluate natural bee-collected pollen as a supplement. A test of feeding one pound of pollen in November 2004 indicated that fed Russian colonies had greater populations of bees and brood than unfed Russian colonies in February and March 2005. The trial described here is a larger investigation of the effect of feeding pollen in autumn on population expansion and resultant flight activity of Russian bees during almond pollination. There is no standard regime among beekeepers for feeding supplemental protein in autumn. We chose to feed two pounds of supplement because this amount sometimes is given by commercial beekeepers, and also was the amount used in a recent autumn feeding test of a new protein supplement (Feedbee®; Saffari *et al*. 2004) which resulted in larger bee populations in the following April (A. Saffari, pers. comm.).

Materials and Methods

Colonies were established in spring and summer 2005 in cooperation with a commercial beekeeper in central Louisiana. Russian queens from four commercially available lines (Rinderer et al. 2005) were mated to Russian drones at isolated research mating stations maintained by our laboratory. Italian queens were reared from commercial stock [Bordelon Apiaries (Moreauville, LA) and Ohio Queen Breeders (Worthington, OH)] and open-mated to drones of the same sources. All colonies were kept on six-way pallets, with colonies of one stock per pallet and with colonies of both stocks kept in two apiaries.

The population of adult bees and the area of sealed brood of each colony were measured to the nearest 10% coverage of a deep Langstroth comb in late October 2005. The 32 Russian and 32 Italian colonies selected for use, and the fed and unfed groups within these stocks, initially had equal populations of adult bees and equal amounts of sealed brood (Fig. 1). Half of the colonies of each stock were given supplemental pollen in the form of patties made of bee-collected, autumn pollen mixed with 15% sucrose syrup (66% solids) by volume. The 'fed' colonies each were given

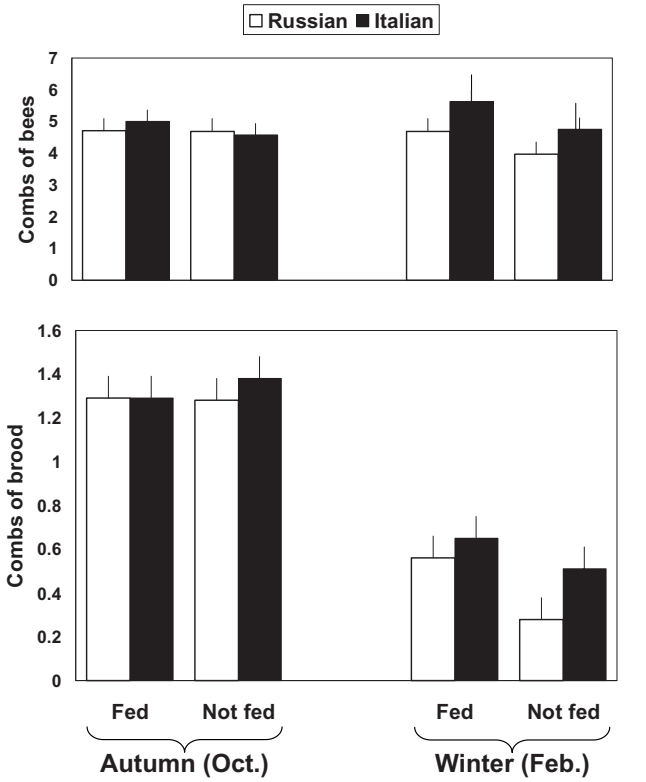

Figure 1. *Mean populations of adult bees and brood in colonies of Russian and Italian bees either fed two pounds of supplemental pollen in autumn or not fed supplement. Error bars represent one SE. The only difference between fed colonies and unfed colonies occurred in brood populations in winter. For reference, one deep comb has about 273 sq in (1760 sq cm) of surface area.*

1-lb (454-g) pollen patties on 27 October and 9 November. This feeding schedule coincides with the last normal cycle of brood rearing in the region. All colonies were given 2 gal (7.6 liters) of high fructose corn syrup during the feeding period. The bees were held over winter in the two Louisiana apiaries until they were moved for almond pollination.

The colonies were trucked directly to an almond orchard near Lost Hills, CA, and distributed along a quarter mile (ca. 400 m) of one central orchard road on 30 January 2006. The orchard had ca. 500 acres each of 'Nonpareil' and 'Monterey'. Bee and brood populations were measured on 16 February at the start of bloom. Six colonies were not included further because their queens had superseded.

Flight activity was measured during nine days of the major bloom period (17-25 February) using ApiSCAN-Plus® electronic counters (Lowland Electronics; Leffinge, Belgium). These counters are mounted at the hive entrance and register interference of infrared light beams to quantify the activity of outgoing and incoming bees. The principal and design were described by Struye *et al*. (1994). The data obtained from 0700 through 2000 h were converted to an average hourly count of bee flights per minute. ApiSCAN counts were adjusted by multiplying by 0.67 to account for the average effect of bees clustering at the hive entrance (Danka and Beaman 2007). Temperatures were recorded at 5-min intervals as black globe temperatures (Corbet *et al*. 1993) 39 inches (1 m) above ground using HOBO dataloggers (#H08-00804) and thermocouples (# TMC6-HB) (Onset Corp.; Bourne, MA). Temperatures were converted to hourly averages for analysis.

Populations of adult bees and sealed brood were evaluated with analysis of variance (Proc Mixed; SAS 2000) for effects of stock and feeding treatment; season was included in the analysis of adult bee populations. Bee populations were transformed to $\log_{10}$ counts to make variances homogeneous. Comparisons of means following a significant ($\alpha = 0.05$) *F*-test were made by Fisher's protected least significant difference (LSD) test. Flight activity was analyzed as a completely randomized design involving a split-plot treatment arrangement (colonies within type as the main unit; repeated measures of colonies through time as the subunit). Regression analysis followed analysis of variance to evaluate how flight activity was influenced by bee stock, feeding treatment, adult bee population, brood population, temperature and period of the day. Period of day was a classification variable that segregated observations into morning (before 1100 h), midday (1100 – 1359 h) and afternoon (1400 h and later) counts. The full model analysis evaluated the main effects, squares of main effects and all 2-way interactions. Effects found to be highly significant at $P < 0.01$ were retained in the reduced model. The model was further reduced by eliminating terms found to contribute to less than 10% of variation for each main effect according to Type I sums of squares. Retained terms were used as regressor variables to model the number of bees leaving a colony under defined conditions of the significant effects.

Results

At the beginning of almond pollination (three and a half months after feeding), the mean area of sealed brood was 56% greater in the 28 fed colonies (0.61 ± 0.09 (SE) combs of brood) than in the 30 unfed colonies (0.39 ± 0.06 combs of brood) (Fig. 1); this was a significant increase (Table 1). (Note that here 1.0 comb means a comb that is fully covered with bees, not a comb that is as little as 2/3 covered, as is commonly used for strength inspections during pollination rentals.) The brood increases of fed colonies within the two bee stocks did not differ statistically despite varying by nearly four-fold (100% for Russians, 27% for Italians). When feeding treatment groups were pooled within each stock for analysis, brood populations were statistically similar for Russian and Italian bees.

Populations of adult bees were 19% larger in the fed group but this increase was not significant (Table 1, Fig. 1). Feeding increased adult bee populations similarly in Russian and Italian colonies, and bee populations were similar for Russian and Italian colonies of

Table 1. Results of analysis of variance of the effects of bee stock and feeding treatment on populations of adult bees and brood. There were 32 colonies each of Russian and Italian colonies, and half of each type were given 2 lbs (0.91kg)of supplemental pollen beginning in late October 2005. Bee and brood populations were measured at feeding and at the beginning of almond pollination in mid February 2006.

Parameter	Effect	*F*	df	*P* > *F*
sealed brood	stock	2.26	1,54	0.139
	feeding	4.05	1,54	0.049
	stock × feeding	0.42	1,54	0.521
adult bees	stock	0.10	1,55	0.754
	feeding	2.10	1,55	0.153
	season	1.43	1,55	0.237
	stock × feeding	0.36	1,55	0.553
	stock × season	0.04	1,55	0.839
	feeding × season	1.31	1,55	0.184

combined feeding treatment groups. Bee populations in February did not differ significantly from those in November (P=0.237 for all colonies; P=0.883 for fed colonies; P=0.128 for unfed colonies), but some trends we apparent. First, bee populations of fed colonies (combined bee stocks) were about 7% larger in February than in November, while unfed colonies were about 6% smaller. Second, fed Italian colonies were 12% larger in February than in November while fed Russian colonies showed no change in bee population. Third, unfed Russian colonies showed a loss of about 16% while unfed Italian colonies gained about 4%.

Flight activity during almond pollination was affected neither by feeding treatment nor by bee stock, presumably because these factors did not influence populations of adult bees. Flight activity was significantly affected by temperature, adult bee population and period of the day (Table 2). Flight was greater at higher temperatures, and temperature and colony size interacted such that flight responses to temperature were more pronounced in large colonies. For example, Fig. 2 shows responses of colonies grouped as "large" (average of 6.55 combs of bees) and "small" colonies (average of 2.84 combs of bees) by segregating all colonies at the overall average size of 4.76 combs of bees. A 1.8° F (1° C) rise yielded 6.6 more flights per minute in large colonies and 2.5 more flights per minute in small colonies when all other factors were equal. Temperature effects, regardless of colony size, were more pronounced in the morning than in the midday and afternoon (Fig. 3). An additional comb of bees yielded 8.0 more flights per minute in the morning, but only ca. 3.6 more in the midday and afternoon. Flight was inhibited by factors other than temperature later in the day (e.g., depletion of nectar and pollen), as activity at all but the lowest temperatures recorded in the afternoon was much lower than at the same temperatures earlier in the day.

Table 2. Test results from analysis of variance of a reduced model of honey bee flight activity. Shown are Type 1 SS, F-tests from Type 3 tests from GLM, and parameter estimates for regression equations that describe the influences of temperature, adult bee population and time of day on flight.

Effect	SS (× 1000)	F	df	$P > F$	Parameter estimate
bees	1,398	24.19	1,4402	<0.001	-5.36
temp	10,005	7.58	1,4402	0.006	-1.37
time	1,262	45.17	2,24	<0.001	am=-28.81[1]
bees*temp	792	1165.84	1,4402	<0.001	0.91
temp*time	742	390.71	2,4402	<0.001	am=4.29[2]
col(type)	719	NA	NA	NA	NA
time(day)	639	NA	NA	NA	NA
day(col, type)	250	NA	NA	NA	NA
residual	2,996	NA	NA	NA	NA

[1] midday = 61.19, pm = 4.26
[2] midday = -0.17, pm = -0.00

Discussion

Feeding two pounds of bee-collected pollen in autumn affected brood populations more than it affected adult bee populations in late winter. This suggests that bees stored the food, ceased brood rearing as usual in late autumn, and then used the stored nutrients when brood rearing resumed in mid winter. Feeding appeared to stimulate brood rearing in the Russian colonies in particular. Fed Russian colonies had twice as much brood as unfed Russian colonies, whereas the feeding yielded a 27% increase in brood of Italian colonies (Fig. 1). This is interesting because it might be expected that bees from northern areas, where the brood rearing cycle around winter is expected to be highly programmed, would be less likely to respond to winter feeding. The value of feeding Russian colonies was further supported by the observation that fed Russian colonies tended to maintain their adult bee populations through the winter, while unfed colonies tended to lose bees (Fig. 1). The current high rental fees being paid for colonies in almond pollination thus may make more intensive feeding regimes cost effective.

Flight activity of Russian colonies was consistent with what we observed previously in comparisons of Russian and Italian bees during pollination of almonds (Danka *et al*. 2006) and lowbush blueberries (Danka and Beaman 2007). The environmental effects of temperature, colony population and period of the day affected flight, but bee stock did not. Furthermore, Russian and Italian bees

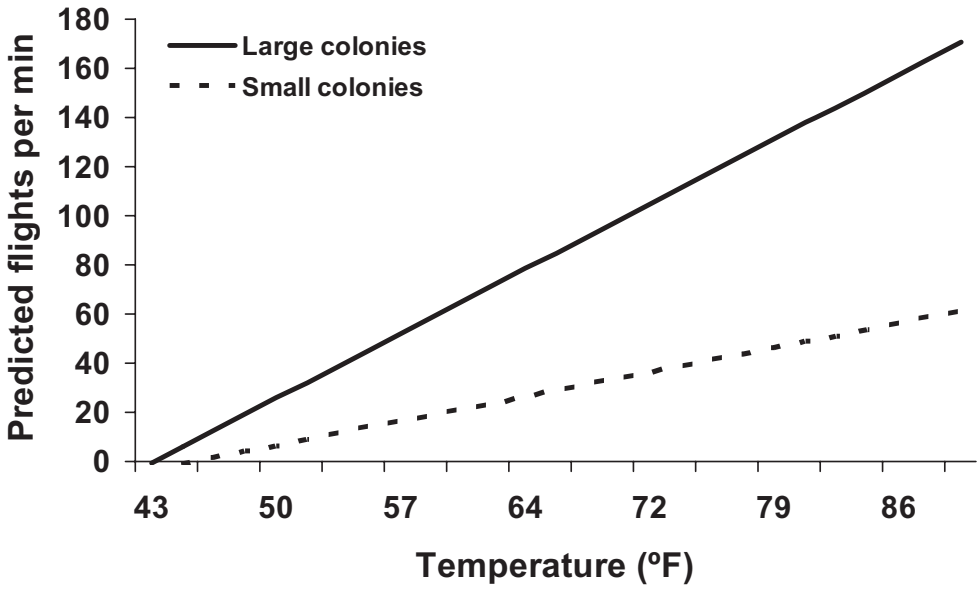

Figure 2. *Flight activity of large and small colonies of honey bees in relation to temperature. These are flight responses modeled using regression parameter estimates and the average adult bee populations of "large" (mean of 6.55 combs fully covered with bees) and "small" (mean of 2.84 combs of bees) colonies.*

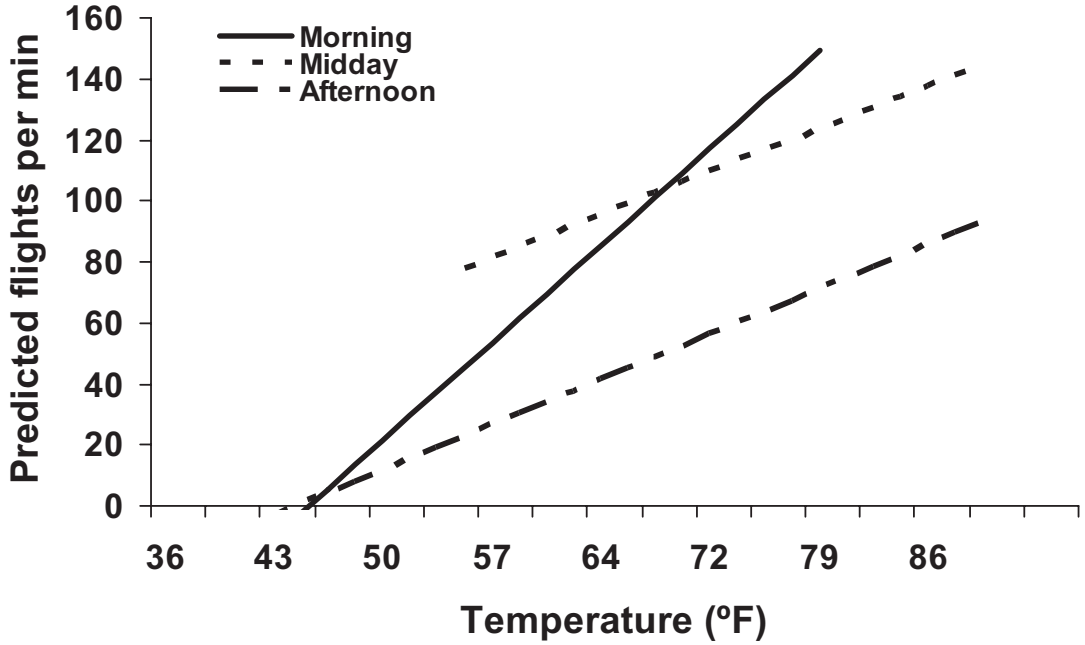

Figure 3. *Honey bee flight activity as influenced by temperature and time of day. Colonies responded more strongly to varying (rising) temperatures in the morning than they did later in the day. The results shown are predicted responses of colonies that have an overall average population of adult bees (4.76 combs of bees).*

responded similarly to varying environmental effects. Our cumulative observations indicate that Russian colonies of adequate size are useful pollinators of almonds.

Conclusions and Recommendations

Russian colonies that were fed two pounds of supplemental pollen in the autumn had larger brood populations than unfed colonies in late winter. Although this feeding program did not increase the populations of adult bees, the results indicate the potential to do so. We recommend feeding a minimum of two pounds of supplemental protein to maintain bee populations in Russian colonies. Population increases perhaps could be obtained if feeding is begun earlier than late autumn and if more than two pounds of supplement is given.

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