High Levels of Cotton Pollen Collection Observed for Honey Bees (Hymenoptera: Apidae) in South-Central Louisiana[1]

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J. Entomol. Sci. 40(3): 316-326 (July 2005)

Abstract Honey bees, *Apis mellifera* L., typically reject pollen of upland cotton, *Gossypium hirsutum* L., as a resource. This study evaluated the potential of stimulating bees for enhanced pollen collection in overcoming this rejection. In 2002, 16 equal-sized colonies of each of two commercial stocks of bees (Italian and Russian) were placed adjacent to cotton fields at Rosedale, LA, and manipulated so that half of the colonies of each bee type had high stimulus to collect pollen and half had low stimulus. Differential stimuli were achieved by interchanging combs having relatively large amounts of brood with combs having pollen, between colonies of the two treatment groups (i.e., high stimulus colonies donated pollen and received brood, whereas low stimulus colonies had the converse). Stimulus manipulations resulted in more general pollen collection, but not cotton pollen collection, in the high stimulus group on days 1 and 6 after treatment. Foraging responses of the treatment groups equalized by 11 days after treatment. Collection of cotton pollen was minimal (≤2% of all foragers) during this period and was not affected by stimulus treatment. Italian colonies had greater total foraging activity and pollen collection effort on day 1 after treatment, but the bee types foraged similarly on days 6 and 11. There were no interactions of the effects of stimulus treatment and bee type. After the treatment effects dissipated (by day 11, 5 August 2002), collection of cotton pollen increased substantially. Approximately one-fourth of all foragers and 80% of pollen collectors carried cotton pollen pellets during a 2-wk period in midAugust. In 2004, observations of 11 colonies at a cotton planting near Fordoche, LA, showed that foragers again carried notable amounts of cotton pollen during the middle of bloom but little cotton pollen earlier or later. At the peak, a mean of 26% of pollen loads of all colonies and 50-59% of pollen in two colonies were of cotton. The reason for this unexpected willingness to gather cotton pollen is undetermined and warrants investigation because of the potential importance for cotton pollination by honey bees.

Key Words Honey bees, *Apis mellifera*, upland cotton, *Gossypium hirsutum*, pollination, foraging

Upland cotton (*Gossypium hirsutum* L.) generally is described as a self-fertile and auto-pollinating plant, but production may increase by 3-30% as a result of pollination by honey bees (*Apis mellifera* L.) (reviewed in Free 1993, Rhodes 2002, Ward and Ward 2002). For hybrid types of upland cotton involving male-sterile fruiting lines,

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[1]Received 28 October 2004; accepted for publication 29 November 2004.

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commercial production relies on insects, typically honey bees, to vector pollen between male-sterile and male-fertile cultivars.

Honey bees avidly collect cotton nectar but often forage at extrafloral nectaries and thus fail to enter the corollas and contact pollen. Those bees that do enter flowers are said to only "rarely" (Moffett et al. 1975) or "seldom" (McGregor 1959) collect upland cotton pollen, and instead tend to groom and rid themselves of pollen grains before returning to the colony. Published observations indicate a maximum of 15-25% of foragers with pollen in cotton fields (Kaziev 1956), although these bees may not have been packing pellets. At the hive, Waller et al. (1985) reported only four of more than 10,000 pellets taken from returning foragers came from cotton. Beekeeper anecdotes (pers. comm.) generally concur with published references to very little collection of cotton pollen. It has been suggested that upland cotton pollen is not collected because the large, echinate grains are difficult to pack successfully in the corbiculae (Vassiere and Vinson 1994). McGregor (1976), Eisikowitch and Loper (1984), and Loper and Davis (1985) suggested that cotton pollen will be collected only if more favorable pollen sources are not available. Active pollen foraging presumably would be beneficial because of an increase in pollen vectoring and pollination.

Honey bee colonies can be stimulated to collect pollen through genetic selection (Hellmich et al. 1985) and by manipulating the amounts of brood and pollen (e.g., Fewell and Winston 1992) or brood pheromone (Pankiw et al. 1998) in the nest. The aims of the first test reported here were to see if cotton pollen collection could be increased by stimulating colonies to collect pollen, and whether this pollen collection response differed for two genetic stocks of honey bees. Simple observations of pollen foraging in a second season evaluated whether the unexpectedly high amount of cotton pollen collection seen in the first experiment occurred in another foraging situation.

Materials and Methods

Bees. Two commercial stocks of honey bees were used in the 2002 study. Italian colonies were derived from queens obtained from Wooten Apiaries (Palo Cedro, CA). Russian honey bee stock was developed from bees originally imported from far-eastern Russia and selected for resistance to the parasitic mite *Varroa destructor* Anderson and Trueman and for other favorable beekeeping traits (Rinderer et al. 2000). These bees were not selected for increased pollen collection but have life history traits that differ from Italian bees and may have a greater propensity to collect pollen (T. Rinderer, pers. comm.). Russian colonies came from queens of selected, commercially available breeding lines (00B-775, 00B-795, 02A-557 and 02A-933).

Italian colonies were housed in Langstroth hives each having two deep brood chambers and approximately 30,000 cm^2 of comb surface area on 17 combs. Russian colonies were each in three medium-depth brood chambers and had approximately 30,200 cm^2 of comb surface area on 27 combs. Hives were kept on pallets; the four colonies on each pallet were all of one bee type. Hive entrances were restricted to 10 × 2.2 cm and covered with 1.25-cm-mesh screen to slow access by returning foragers and thus facilitate counting these bees.

Comb contents were measured on 18-20 July, shortly before the colonies were moved to the cotton site. In each of 20 colonies of each bee type, the areas of comb with brood of all stages, with pollen, with honey or nectar and with empty cells were measured to the nearest 100 cm^2 using a 10- × 10-cm grid. Sixteen colonies of each

bee type that had similar mean amounts of brood (Table 1) were chosen for further use.

Colonies were moved to the test site on 22 July. Hives were located between two adjacent fields totaling approximately 70 ha of 'DPL 458' cotton near Rosedale, Iberville Parish, LA. The location had a restricted assemblage of pollen-source plants because most of the surrounding hectarage was planted with sugarcane (*Saccharum officinarum* L.), a plant which normally does not flower in Louisiana. Small amounts of a variety of plants that yield pollen were present in noncultivated field borders and drainage ditches.

Stimulus manipulations. Colonies within each bee type were ranked and paired according to their brood areas irrespective of pollen stores. Within each pair, combs with large areas of brood from one colony were exchanged with combs having large areas of stored pollen from the other colony on the evening of 25 July. Averages of 1320 ± 760 (mean ± s) cm^2 of brood and 1060 ± 720 cm^2 of pollen on 2-4 combs were exchanged within pairs of colonies. This exchange created a group of high pollen collection stimulus colonies with averages of 18% more brood and 40% less pollen than they initially had, and a group of low pollen collection stimulus colonies that conversely had similarly less brood and more pollen.

Foraging measurements. Total flight activity in each colony was estimated 1, 6 and 11 days after the stimulus treatments were applied. The numbers of foragers returning during 4-min intervals were counted twice daily for each colony by two different observers. All foraging counts were made between 1000 and 1400 h, which is the period when cotton flowers are most fully open and have the greatest visitation by foragers (Moffett et al. 1975). Observations in the field confirmed that flowers were not fully open before approximately 1000 h and that bee activity began to wane after approximately 1300 h as flowers senesced.

Pollen collection was measured two ways. First, during each count of total foraging, the number of returning bees that had visible pollen pellets also was recorded. Second, on days 7 and 12 after treatment, hive entrances were blocked and 30-40 returning bees were swept from flight into clear plastic bags. The percentage of bees with pollen was recorded and bees were released. Two such samples were analyzed by different observers for each colony on each day.

The rate of collection of cotton pollen among returning foragers was measured by three methods. First, the percentage of returning bees carrying cotton pollen loads during the entrance counts described previously was recorded on day 1 after treatment. Cotton pollen pellets were identifiable because of their white color and distinctive, loosely packed form. These observations were extended as pollen collection trends changed over time. Second, bees with cotton pollen among the bees sampled in bags were counted on day 6 after treatment and later. Third, pollen traps (Sundance model, Ross Rounds, Albany, NY) were used on each colony to capture some incoming pollen for 24 h on two occasions (26-27 July and 12-13 August). Pollen pellets from traps were examined at 63× magnification to determine the percentage of cotton pollen pellets among all pollen types present. Up to 200 pellets per colony were examined, and samples having fewer than 30 pellets were disregarded. Pollen types were compared with reference pollen taken from pellets on bees foraging on local plants.

Data on total foraging activity and the percentage of total bees carrying pollen were evaluated by repeated-measures analysis of variance (PROC MIXED; SAS Institute 1987) having a 2 × 2 factorial arrangement of treatments (stimulus × bee type). Mean

Table 1. Mean (±s) cm^2 of comb area of nest components in honey bee colonies subjected to stimulus manipulations, and quantities of brood and pollen exchanged to form stimulus treatment groups. Sixteen colonies of each bee type were used, and high and low stimuli were applied to half of each group. NA, not applicable.

	Brood	Pollen	Honey	Empty
Overall	7170 ± 1260	2630 ± 1520	11955 ± 3290	8210 ± 3115
High stimulus group	7130 ± 1250	1760 ± 700	11044 ± 3317	8175 ± 2957
Low stimulus group	7210 ± 1310	3500 ± 1640	12850 ± 3938	8169 ± 3543
Exchanged to create high and low groups	1320 ± 760 (18%)*	1060 ± 720 (40%)	NA	NA
Russian	7020 ± 1060	2020 ± 1240	12850 ± 3230	8390 ± 3380
Exchanged among Russian colonies to create high and low groups	1210 ± 540 (17%)	540 ± 480 (27%)	NA	NA
Italian	7320 ± 1460	3240 ± 1570	11060 ± 3350	8030 ± 2850
Exchanged among Italian colonies to create high and low groups	1430 ± 960 (20%)	1590 ± 510 (49%)	NA	NA

* Values in parenthesis are quantities given as percentages of initial quantities.

separation was based on *t*-tests of least squares means. For each foraging trait, the two counts on each day for each bee type were averaged. Because the main effect of day (i.e., day 1, 6 or 11 after stimulus manipulations) was significant, results of the two foraging responses are presented separately for each day. Differences in rates of cotton pollen collection between bee types on 7, 11, 16 and 26 August were evaluated with *t*-tests.

Pollen foraging observations in 2004. Eleven colonies of moderate size (9.9 ± 3.2 deep combs covered with bees) and mixed genetic stock were moved to a 304-ha planting of 'DPL 458' cotton near Fordoche, Point Coupee Parish, LA, on 6 August 2004. Cotton pollen collection was evaluated by blocking hive entrances, capturing returning foragers in clear plastic bags, counting bees with loads of cotton and with other pollen, and releasing the bees. Three groups of 50 bees each were taken from each colony between 1030 and 1330 h; summed data from these counts were used. Pollen foraging was monitored every 3-4 days from 9-27 August. Drainage ditches in the fields contained blooming plants that probably provided more alternative pollen sources than were present in the 2002 test.

Four of the colonies were fitted with pollen traps; pollen was collected during 14-17 and 28-30 August. For each colony, a 10-ml sample of pollen was removed, and the pellets were sorted into cotton and noncotton groups. The percentage of cotton pollen was quantified by weight.

Results

In 2002, pollen collection was greater in high stimulus colonies than in low stimulus colonies (Table 2, Fig. 1). Pollen collection was greater on day 6 after applying stimuli than on day 1 ($t = -11.05$; df = 28; $P < 0.001$) and day 11 ($t = 8.55$; df = 28; $P < 0.001$); it also was greater on day 11 than on day 1 ($t = -3.36$; df = 28; $P = 0.002$). Total flight was similar in high and low stimulus colonies. Total flight was greater on day 6 after

Table 2. Probabililty values from analysis of variance of the effects of pollen collection stimuli, bee type and day after treatment that potentially regulated foraging of honey bee colonies placed adjacent to a cotton field at Rosedale, LA, in 2002

	Effect on total foraging			Effect on pollen collection		
	df	*F*	*P*	df	*F*	*P*
Stimulus treatment	1, 28	1.53	0.228	1, 28	10.82	0.003
Bee type	1, 28	0.40	0.534	1, 28	2.44	0.129
Type × treatment	1, 28	1.66	0.208	1, 28	1.70	0.202
Day	2, 28	16.46	<0.001	2, 28	62.26	<0.001
Treatment × day	2, 28	0.56	0.579	2, 28	1.83	0.180
Bee type × day	2, 28	6.00	0.007	2, 28	4.86	0.016
Bee type × treatment × day	2, 28	0.23	0.797	2, 28	0.48	0.621

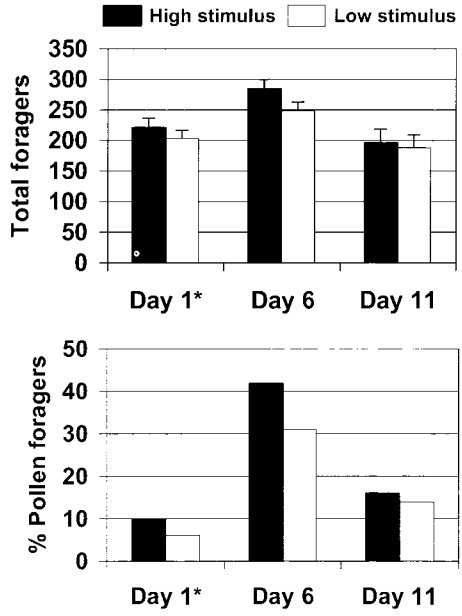

Fig. 1. Total foraging activity (i.e., foragers returning during 4 min) and pollen collection activity for the two stimulus groups of honey bees during the 2002 experiment. Data are mean ± SE. Means within a day that differed at $P < 0.05$ are denoted by an asterisk

applying stimuli than on day 1 ($t = -5.59$; df = 28; $P < 0.001$) and day 11 ($t = 4.03$; df = 28; $P < 0.001$).

Italian colonies had a greater percentage of pollen collectors ($t = -2.25$; df = 28; $P = 0.033$) and more total flight ($t = -2.67$; df = 28; $P = 0.013$) than Russian colonies on day 1 after stimulus treatment (Fig. 2). Italian and Russian colonies then had similar pollen collection and total flight on days 6 and 11 after treatment. No significant statistical interaction occurred between the effects of stimulus treatment and either bee type or day for total foraging or for pollen collection (Table 2).

Collection of cotton pollen was meager (≤2% of all foragers) during the early phase of our observations (Table 3). Data on cotton pollen foraging from day 1 unfortunately were incomplete because rain developed before most bees were sampled. On days 6 and 11 after applying stimulus treatments, cotton was poorly but equally represented in the pollen loads of bees from high and low stimulus colonies, and also in pollen loads of bees of both types. Brood and pollen combs were reconfigured after the stimulus effects dissipated (day 11), but observations continued because an increase in incoming cotton pollen became apparent. Surprisingly high levels of cotton pollen were collected during mid August, when approximately one-fourth to one-third of all returning foragers and 80% of pollen collectors carried cotton pollen during a period of nearly 2 wks (Table 3). Italian bees tended to collect cotton pollen at a greater rate (Table 3). This difference between bee types, however, was not nearly as strong as the overall pattern of increased cotton pollen gathering. Pollen

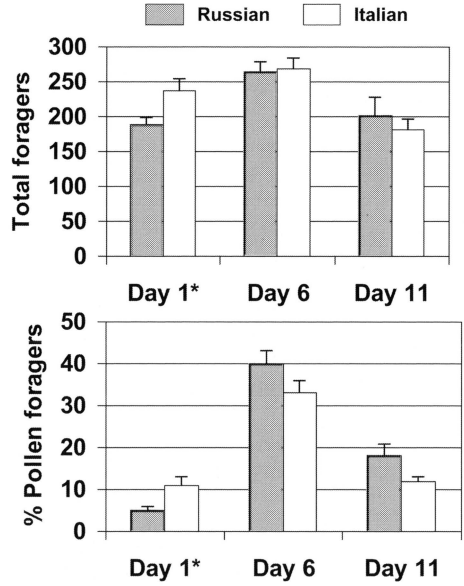

Fig. 2. Total foraging activity (i.e., foragers returning during 4 min) and pollen collection activity for the two honey bee types during the 2002 experiment. Data are mean ± SE. Means within a day that differed at $P < 0.05$ are denoted by an asterisk.

traps were used twice for 24-h periods. Almost no pollen was recovered on 26-27 July, but on 12-13 August about 40% of pellets that were recovered intact were of cotton pollen. Cotton pollen pellets often disintegrated when they were scraped from the corbiculae as foragers passed through the screens in the traps. Other pollen sources common in pollen trap samples were Johnsongrass (*Sorghum halepense* (L.) Persoon), teaweed (*Sida rhombifolia* L.) and Brazilian vervain (*Verbena brasiliensis* Vellozo). Insecticide treatments and diminished bloom prompted curtailment of observations at the end of August 2002.

In 2004, foragers again carried notable amounts of cotton pollen during the middle of bloom (Table 4). At the peak on 17 August, a mean of 26% of pollen loads of all 11 colonies were of cotton. Two of the colonies had 50-59% of pollen foragers carrying cotton pollen, and in six colonies cotton accounted for at least one-fourth of the incoming pollen. Some cotton pollen was collected before and after this peak, but none was seen on 9 August and very little was collected on 27 August.

The percentage of cotton pollen in trap collections varied between colonies and times. During 14-17 August, the four colonies collected 83, 45, 5 and 3% cotton pollen. Later, on 28-30 August, cotton composed 0-0.2% of the pollen trapped from these colonies.

Discussion

The results of the 2002 study confirm the intended effect of stimulating differential pollen collection between two groups of treated bees. The fluctuation in the percent-

Table 3. Collection of upland cotton pollen by honey bee colonies at Rosedale, LA, in 2002. Pollen collection stimuli were applied from 26 July to 7 August. Data are mean ± s from 16 colonies of each bee type

	26 July*	1 August	7 August	11 August	16 August	21 August*	26 August
% of all foragers with cotton pollen	—	<1	2 ± 3	25 ± 14	24 ± 11	—	21 ± 12
Italian	—	<1	3 ± 4	31 ± 15 a	26 ± 9	31 ± 8	27 ± 12 a
Russian	—	<1	1 ± 2	19 ± 2 b	21 ± 14	—	14 ± 9 b
% of pollen foragers with cotton pollen	—	2 ± 4	12 ± 19	82 ± 22	81 ± 23	—	67 ± 25
Italian	—	3 ± 6	21 ± 23	89 ± 21	91 ± 14 a	86 ± 10	78 ± 19 a
Russian	—	<1	3 ± 8	76 ± 22	72 ± 26 b	—	56 ± 27 b

Means for the two bee types within a column and within a foraging parameter followed by different letters differ significantly ($P < 0.01$, t-test).

* Data are incomplete for 26 July and 21 August because of the onset of rain before all colonies were sampled; eight Italian colonies were sampled successfully on 21 August.

Table 4. Collection of upland cotton pollen by honey bee colonies at Fordoche, LA, in 2004. Data are mean ± s from 11 colonies

	9 August	13 August	17 August	21 August	24 August	27 August
% of all foragers with cotton pollen	0	0 ± 1	6 ± 6	3 ± 3	1 ± 1	<1
% of pollen foragers with cotton pollen	0	2 ± 5	26 ± 19	7 ± 7	3 ± 6	<1

age of pollen foragers, but not in total foraging, also was expected based on prior studies showing that manipulations of brood and pollen tend to shift the ratio of nectar to pollen gatherers within the foraging population (Fewell and Winston 1992, Fewell and Page 1993). The treatment of stimulation for more pollen collection did not, however, result in foragers switching to the apparently less desirable cotton source. The proportion of pollen collected from cotton was small during the period of about 1 wk that the treatment stimulus effects lasted. McGregor (1976) related that even an apparent pollen deficiency in colonies did not cause bees to gather cotton pollen; he did note some bees collecting cotton pollen "freely" late in the season, but that this pollen may have come from Pima cotton (*G. barbadense* L.).

Italian bee colonies had greater total foraging and pollen collection efforts than equal-size Russian colonies had 1 d after applying the stimuli. There was a trend of the Russian colonies reacting relatively more slowly; the percentage of bees gathering pollen was nearly greater ($t = 1.95$, df = 28; $P = 0.061$) in Russian colonies on day 11. Thus, the bee types may differ in the timing of their responses to foraging stimuli or to manipulations of nest contents.

The most significant finding was the unexpectedly large rate of collection of cotton pollen that began about 2 wks after the bees were moved to the test site. This appears to be the highest rate of upland cotton pollen collection recorded for honey bees. Its cause, however, is undetermined. There apparently was no relationship to the pollen collection stimuli which were applied; these stimuli dissipated before the major increase in cotton pollen gathering. Others have suggested that cotton pollen is collected only when other pollen sources are not available (McGregor 1976, Eisikowitch and Loper 1984, Loper and Davis 1985). However, it is unlikely that already limited alternative floral resources became significantly less available during the month-long course of our observations.

The appearance of the loosely packed pollen pellets fits closely with the explanation by Vassiere and Vinson (1994) that spines interfere with the physical adhesion of cotton pollen grains within a pellet. They found that bees were less inclined to collect pollen types that did not pack easily. Perhaps the high humidity of our study environment during cotton bloom (79 ± 15% average daily relative humidity recorded at Baton Rouge during the 32 days of observations) (LSOC, 2002) contributes to pollen pellet formation. This environment largely differs from the more arid regions where the bulk of research on cotton has occurred. Eisikowitch and Loper (1984) anecdotally cite bees collecting cotton pollen under humid conditions. High humidity could enhance the adhesiveness of the liquid that bees regurgitate from their crops and add

to pollen when packing it into pellets. It might also dissipate electrostatic charges that often cause repellence between pollen grains (Erickson and Buchmann 1983). Neither of these possibilities, however, accounts for the dramatic shift in the relative cotton pollen income found through time because humidity levels did not trend upward during our study.

The observations from 2004 verify relatively high levels of foraging for cotton pollen, at least at times, in south Louisiana. Rates of cotton pollen collection in 2004 were not as great as those in 2002. This may have been due to a greater availability of noncotton pollen sources. Furthermore, a cold front that produced six record daily low temperatures on 13-18 August apparently also suppressed cotton flowering; eight, 25-m row transects had an average of 4.2 flowers per m row on 9-13 August, but only 2.3 flowers per m row on 17-27 August. Average relative humidity was still quite high (70% on 17 August and an average of 78% for all sampling dates).

Safe beekeeping opportunities near cotton in the southeastern United States and, thus, potential pollination input, are likely increasing as insecticide use diminishes owing to boll weevil eradication and expanded use of transgenic cotton varieties. Given the widely described behavior of honey bees rejecting pollen of upland cotton, our documentation of the ability of honey bees to collect this pollen warrants further research into underlying regulatory factors.

Acknowledgments

Lorraine Beaman contributed to many aspects of this research. Logistical support was provided by Gary Delatte, John Englert, Joshua Grimmett, Trey Guillory, Victor Rainey, Robert Spencer, Toby Trowbridge and Alexis Villegas. Ted Glaser granted permission to work at his cotton fields. Frank Eischen, Gerald Loper, Thomas Rinderer and José Villa made valuable comments on the manuscript. This research was completed in cooperation with the Louisiana Agricultural Experiment Station.

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