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Honey Bee Foraging and Resultant Seed Set among Male-fertile and Cytoplasmically Male-sterile Carrot Inbreds and Hybrid Seed Parents[1,2]

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Abstract. **A study of foraging by honey bees (*Apis mellifera* L.) among cytoplasmically male-sterile and male-fertile seed parents of carrot (*Daucus carota* L.) revealed that honey bee discrimination between the fidelity to carrot phenotype and genotype were evident and often extreme. Some lines were extensively visited while others were virtually ignored. Wide differences in seed set were evident among male-sterile F_1's and inbreds and male-fertile lines. Differences in seed yield were correlated with foraging preferences, but the quality of nectar from the stomachs of bees was not.**

A complex of problems ranging from nonrandom pollinator foraging activity to genetic incompatibility between seed parents has resulted in poor commercial production of hybrid seed from carrots. Observation has shown that various cytoplasmically male-sterile and male-fertile carrot lines bloom at different times and are probably differentially attractive to pollinators even when they are blooming simultaneously. This floral variation among maternal and paternal lines inhibits row crossing by honey bees, the principal pollinator of commercially produced carrots (1).

Honey bees, whether they are foraging for nectar or for pollen, readily discriminate between nectar and pollen sources on the basis of foraging stimuli (e.g. aroma, taste, color, size, and shape) and retain a marked fidelity to a single source (2, 3, 4). Apiculturists familiar with this behavior ordinarily think of discrimination as limiting interspecific foraging activity. However, such extreme differences are evident in the floral characteristics of selected parental lines of carrots that we must recognize the great potential for intravarietal discrimination by bees.

Since honey bees seemed to forage discriminately between carrot genotypes and some were rarely visited by bees and as a result set seed poorly, we compared levels of foraging activity between and the resultant seed set among selected male-sterile and male-fertile genotypes.

Methods and Materials

The studies were conducted from 1973 to 1976 in field isolation cages at Madison, Wisconsin. Initially we ascertained only whether bees discriminated between corolla color, since male-fertile maintainer (M) lines are white, and male-sterile (S) lines are either off-white or green. In later studies we evaluated foraging based on genotype and then compared seed yields.

Foraging. 1973. Two identical 3-row screened plots were studied. In each a center row (7.6 m) containing white corolla male-fertile (M) plants all of the same line was flanked

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by rows with a random assortment of blocks of petaloid male-sterile (S) lines of 6 to 10 plants each, having either off-white or green corollas. In a few blocks the S line was segregating for color and thus had plants with corollas of both colors. Each cage contained two 4-frame 16.7 cm (6-5/8") deep hives of honey bees.

At peak bloom, beginning at midday, we began marking foraging bees with quick drying enamel paint. Twenty bees (25 bees on the M lines) were marked as they visited plants of each corolla phenotype (S lines) in each cage. Then, at 15-min intervals for the following 2 hr, marked bees on each corolla type were counted for 3 min per row. The following day the process was repeated. Records were kept for bees marked on both days.

The data were totaled and the percent of bees crossing over or retaining fidelity to the original color phenotype was calculated.

1974. The experimental design was like that used in 1973 except that 50 bees were marked on each corolla phenotype in each of 2 cages on 4 consecutive days, weather permitting.

Concurrently (Aug. 1-6), an attempt was made to obtain similar data for genotypes irrespective of color. A third 3 × 12 m caged 3-row plot wherein a single M center row (30 stecklings) flanked by rows of S lines in short blocks (21 lines of 5 stecklings each) was used for this study. All rows were of equal length. On 5 consecutive days, weather permitting, 100 bees were marked (9-11 AM) as they foraged on the M row. Thereafter at 10- to 15-min intervals the number of marked bees visiting each of the S blocks during a 30-sec period was recorded as was the presence or absence of pollen in the corbiculae (pollen baskets). The data were summarized by carrot genotype.

1976. Three separate 3-row caged plots (3 × 12 m) were established, each with a different M line but with an identical series of thirteen S lines in 5- to 10-root blocks. Again, the center M row was of about the same length as the S rows flanking it. Each cage contained a 4-frame colony of honey bees.

From July 7 to August 10 between 11 AM and 1 PM, weather permitting, 50 bees were marked on the fertile row in one of the cages. Then a single observer used a cassette recorder to report the number of bees visiting each sterile line. As assistant timed the observer so that only 30 sec were expended per line. Data were taken at about 15-min intervals and summarized according to genotype.

Nectar. The percent dissolved solids (sugar concentration) in carrot nectar was studied to determine whether it varied significantly between corolla color phenotypes or genotypes. As many as 10 foraging honey bees were captured daily while they were gathering nectar in the cages during flowering in 1973 and 1974. These bees were frozen immediately. Later when they were thawed, their crops (honey stomachs) were excised, and the contents were removed. Nectar samples were also taken directly from flowers with 1 $\mu\ell$ pipets whenever this was possible. Eight such samples were removed from male-fertile carrot seed parent lines and 29 samples from male-sterile lines. These, too, were frozen and stored until analyzed for sugar content.

Before analysis for sugar content via gas-liquid chromatography, subsamples were weighed into a Reacti-Vial[3] and 20 $\mu\ell$ of inositol were added as the internal standard. Then all samples were placed in a 50°C oven at 1.8 kg/cm^2 (26 psi) vacuum until dry (overnight). Two hundred $\mu\ell$ of Tri-Sil[3] were added to each dried sample, and the samples were gently shaken for 6 hr. One-microliter samples were injected into a 1.8 m × 3.2 mm (6 ft × 1/8 inch) SS SE-30[3] column (3% on chromosorb W[3] (HP), 80/100 mesh) with 40 ml/min N_2 flow. The column was temperature programmed as follows: 1 min at 140°, then to 280° at 8.24°/min (total analysis time = 19 min). The area under the peaks was electronically integrated and then converted to mg of sugar on the basis of the response to the internal standard (5). Nectar samples were analyzed for total dissolved solids with a Bausch and Lomb[3] Model 3 L refractometer.

Seed yield. At harvest in 1974 and 1976, all umbels were removed, threshed and the seed cleaned. Yield data are expressed as the mean weight in grams of seed produced per plant.

Results and Discussion

Intra-phenotypic and -genotypic foraging constancy by worker honey bees was evident. Deviations appeared to be mainly incidental. The percent of dissolved solids in nectar could not be positively correlated with phenotype or genotypes. Seed yield per plant coincided closely with bee preference.

Foraging constancy and nectar analyses. Crossing over from the male-fertile to the male-sterile line, the principal direction of activity necessary if pollination is to occur, was low for both sterile phenotypes in 1973 and 1974 (Table 1). Of those bees marked while foraging on the fertile row, 86.5% continued to forage there; only 8.5% moved over to the off-white S plants, and 5.0% moved to the green S plants. Bees initially marked on the off-white and green S plants tended to continue to return to similar plants (63.3 and 65%, respectively). Crossover to the fertile line was about the same in both groups (8.7% from off-white and 9.5% from green) as was crossover to the opposite S corolla color (28%, off-white to green and 25.3% green to off-white). These data are based on a total of 85 observations. Crossing over between sterile lines would likely be higher since they were in the same rows. It is also significant that the tendency to crossover from sterile to fertile lines was low. Our data therefore provide further evidence of discrimination between S and M lines. On the second day, discrimination and fidelity were less marked; nevertheless, the foraging preferences were similar. A difference of nearly tenfold in the level of visitation between S lines was evident in 1974 (Table 2) (see also evaluation of these lines by Erickson and Peterson (1). Pollen-collecting bees and those collecting both nectar and pollen were similarly ranked.

In 1976, the percent crossover from M to S lines was 19.7% (n = 40). Further, selected inbred and F_1 S genotypes were ranked (high to low) according to the number of bees previously marked on the fertile row observed foraging on each S block (Table 3). There was as much as a sixfold difference between the numbers of marked bees foraging on the various sterile lines. (The third plot was omitted from these summaries as the data were atypical due to the fact that the M line was segregating for sterility.) As in 1974 (Table 2), nectar sugar content appeared to be generally higher in the less frequently visited lines, probably due to evaporation concentration in the absence of collections.

The percent dissolved solids in nectar taken from bees and summarized by corolla color is as follows (value in parentheses is number of samples):

	White M	*Off-white S*	*Green S*
1973	36.0% (38)	38.5% (43)	34.1% (37)
1974	26.0 (185)	24.7 (751)	28.9 (53)

Thus, there was no discernible tendency for one phenotype to exceed the others in percentage nectar sugar. However, the differences may have been obscured by mixing of nectar loads by the bees.

The percent dissolved solids (sugar concentration) in the nectar from flowers or from the crops of bees foraging on the various lines appeared least in those lines most favored by the bees (Tables 2 and 3). We cannot be sure that these data show a cause and effect relationship since it may be that the low rates of bee visitation among some lines may have prolonged evaporation at the air-nectar interface, thereby concentrating the nectar. Also, in some plant species, nectar replenished by

Table 1. Indices of the frequency of foraging bee crossover between caged fertile and sterile carrot phenotypes (based on corolla color).

Year	Frequency of bee crossover (%)									n
	Fertile to			Off-white to			Green to			
	white M	off-white S	green S	white M	off-white S	green S	white M	off-white S	green S	
Same day data										
1973[z]	87.6	3.3	9.1	3.5%	34.1	62.4[y]	3.6	10.8	85.6	31
1974[x]	86.2	10.1	3.7	10.4	73.1	16.5	11.0	28.9	60.1	54
Combined data	86.5	8.5	5.0	8.7	63.3	28	9.5	25.3	65.0	85
Subsequent day's data										
1973	63.7	12.1	24.2	0	30.0	70.0[y]	0	30.0	70.0	12
1974	59.8	33.1	7.1	15.9	64.4	19.7	20.0	40.8	39.2	38

[z]Data for 2 consecutive days.

[y]High value probably due to predominance of green corolla color among male-sterile blocks.

[x]Data for 4 consecutive days.

Table 2. Frequency of foraging bee visitation, nectar-sugar content, and seed yields of sterile carrot genotypes (1974).

Selected lines	No. bee visitations			Dissolved solids in nectar[y]		Seed yield (g/plant)
	Collector of[z]					
	Nectar	Pollen	Nectar and pollen	Percent	n	
B3640 x B3363 S	27	6	6	22.2	60	36.6
B3640 S	16	5	1	23.3	54	23.4
B3640 x B3316 S	16	1	1	26.8	40	14.6
B3640 x B3421 S	12	2	2	29.7	38	20
B3640 x B3080 S	11	1	2	23.7	44	25.5
B3316 x B3363 S	8	1	1	22.8	102	12.8
B3412 x B3363 S	5	1	1	22.6	29	16.5
B3430 S	3	2	1	25.7	64	17.9
B3403 S	5	1	0	27.7	44	11.7
MSU5931 S	4	1	0	25.3	30	9.3
B3615 S	3	1	1	32.9	15	12.8

[z]Bee visits/plant. All from single cage w/21 S lines, M line B2158, 93 observations.

[y]Expressed from bees.

Table 3. Frequency of foraging bee visitation, nectar sugar content, and seed yields of sterile carrot genotypes caged with two pollen parents (1976).

Selected lines	B4367 M cage		B3080 M cage		Combined nectar-sugar[w] (%)
	Foraging bees[z]	Seed yield[y]	Foraging bees[x]	Seed yield	
Pollen parent					
4367 M		11.0 g			
3080 M				13.7 g	
Male-sterile parent					
(B3640 S x B3080 M) x B3640 M BC_1	6.2	40.3	1.0	33.6	
(B3640 S x B3363 M) x B3640 M BC_1	5.3	32.7	0.5	33.3	4.1
B3640 S x B3316 M F_1	2.3	16.2	2.3	27.5	19.9
B3640 S x B3080 M F_1	3.4	11.5	4.4	20.2	14.4
B3615 S BC_3	2.4	13.6	0	6.0	
B3430 S BC_4	3.4	8.6	0	7.5	34.5
B3640 S BC_4	1.0	7.9	1.0	5.0	
MSU5931 S BC_4	3.0	7.2	1.5	0	35

[z]Bee visits/plant. 12 observations.

[y]Grams per plant.

[x]14 observations.

[w]Taken from plant by micropipet when quantity sufficient.

Table 4. Sugar chemistry of carrot nectar.

Carrot genotype	n	Fructose (%)	αglucose (%)	βglucose (%)	Sucrose (%)
Male-fertile	8	52.6	22.8	22.9	1.7
Off-white male-sterile	14	52.8	21.6	23.7	1.9
Green male-sterile	15	51.9	22.7	23.4	1.7

flowers (after the first nectar produced has been removed) has a substantially lower content of dissolved solids (Erickson, unpublished data). The extent to which nectar is replenished in carrot florets is unknown. Another possibility is that the higher concentration of dissolved solids may be the result of low seed set, which makes available a greater surplus of photosynthate in these lines.

Erickson and Peterson (1) did detect phenotypic and genotypic differences in the quantities of nectar taken directly from carrot flowers and also differences in the percent dissolved solids between corolla phenotypes. However, in the present study, we were unable to detect differences in the sugar percentages for sucrose, fructose, and glucose between color phenotypes within a genotype or between genotypes when nectar expressed from bees was analyzed by gas-liquid chromatography. However, the sample size (5) was too small to permit a definitive statement about genotype. The sugar fractions obtained are summarized by phenotype in table 4. We consider it likely that the various lines perform differently when environmental conditions are different – for example, under the stress of inadequate soil fertility, moisture, or sunlight. There is some evidence for this in other plant species.

Seed yield. There was a positive relationship between bee visitation rates and yield of carrot seed, which tended to favor F_1 and BC_1 generations though not exclusively (Tables 2 and 3). Variability between F_1 lines was evident just as it was in our previous series of investigations (1).

We also observed differences in the ability of M lines to self and outcross, and these differences were apparent when the M lines for 1974 were ranked (Table 5). Generally poor selfing ability is associated with poor outcrossing, but this was not entirely the case since a number of the lines consistently set more seed per plant on the sterile lines than on themselves. Also, in 1976, line B4367 M selfed produced only 11 g seed/plant and line B3080 M produced 13.7 g, but as pollen parents they were measurably better across most but not all S lines. Line B4367 M had generally higher levels of interline crossing over by bees than line B3080 M (Table 3). Most S lines were more intensively visited by bees in the plot where B4367 M was the male parent than in the other two plots. Even so, occasionally sterile parents like (B3640 S × B3363 M) × B3640 M still set large quantities of seed though little visitation was observed.

Table 5. Carrot seed yield (g/plant) of selected male-fertile genotypes (sibbed) with combined mean yield of male-steriles (outcrossed) by plot, 1974.

Line	Mean seed yield of pollen parent (g/plant)	Mean yield of combined steriles (g/plant)	n
B3080	26.7	41.1	2
B2163	20.9	15.3	11
B2158	19.0	17.3	20
B3430	14.2	15.9	2
B3407	13.6	11.3	6
B3069	10.4	8.8	9
B4367	9.7	18.0	7
B3073	7.2	9.4	14
B3316	2.8	7.5	5
B3230	1.5	5.4	7

Conclusions

On the basis of existing knowledge of honey bee foraging behavior, the floral differences between male-fertile and male-sterile carrot lines described by Erickson and Peterson (1) and the foraging and yield data presented herein, nonrandom honey bee foraging between M and S parents does occur. Thus, successful implementation of a cytoplasmic male-sterile hybrid seed production system must include minimizing floral differences between parent lines. Screening of available pairs of lines should be attempted. Perhaps a measure of developmental success can be accomplished when open-plot seed-yield data are gathered with bees present. However, in those instances in which suitable floral characteristics are missing from an otherwise desirable seed parent, identification, breeding, and/or selection for compatible characteristics are essential.

Improved sampling and analytical techniques are needed to better evaluate the extent of nectar and aroma differences between carrot genotypes.

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