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Asynchrony of Floral Events and Other Differences in Pollinator Foraging Stimuli between Fertile and Male-sterile Carrot Inbreds[1,2]

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Abstract. **The inability to achieve adequate pollination of seed parents has slowed the development of hybrid carrots (*Daucus carota* L.) and dampened industry acceptance. Thus, cytoplasmically male-sterile inbreds and F_1 seed parents were compared with their fertile counterparts for synchrony of floral events and character of pollinator foraging stimuli. Usually, but not always, male-sterile plants were visually different, bloomed later, and exhibited delayed nectar and aroma production compared to male-fertiles. The quality and quantity of nectar and aroma were also different, with male-sterile flowers often inferior to fertile flowers in amounts of nectar produced. Successful use of any cross-pollinated entomophilous hybrid crop system should involve selection for similar floral characteristics early in the breeding program to insure maximum transfer by insects of pollen from male-fertile to male-sterile parents.**

Controlled cross-pollination is essential in commercial production of hybrid seed crops, and with entomophilous plant species, this control can best be achieved via cytoplasmic male sterility (CMS). Jones and Davis (7) were the first to describe commercial hybrid production that makes use of CMS (in onions). However, yields of hybrid seed from entomophilous crops in CMS systems have been characteristically low and seed crop failures are commonplace though unexplained. Several causes have been suggested for this diminished productivity, among which, poor pollination is believed to be of major importance.

Open-pollinated cultivars of carrot are attractive to many pollinating insects, and adequate cross-pollination of these is easily achieved in commercial production (8). However, commercial production of seed from cytoplasmically male-sterile carrots, first described by Welch and Grimball (11), have been beset by disasters. These failures probably result from non-random pollinator foraging patterns (1).

Carrot florets are protandrous. Pollen dehiscence lasts 1-2 days per floret and 6½ days for the umbel. If one assumes that stigma receptivity of the individual floret begins when the two styles start to separate, the stigma becomes receptive on the 3rd or 4th day after anthesis and may remain receptive for a week or longer. Five to six orders of umbels may be produced, and these open in waves at 9- to 13-day intervals. The 1st three orders produce 90% of the seed harvested (1, 3, 4, 5, 6, 8). The period of bloom for one plant may last for a month to 6 weeks.

Nectar of carrot florets, though not abundant, is secreted on the upper surface of the ovary wall (6, 8). It is reflective under ultraviolet light, which suggests that foragers are able to see it as a colored mass (10). Nectar-collecting honey bees, *Apis mellifera* L., are the principal pollinators of hybrid carrots. However, little is known regarding the quality or quantity of nectar or other floral characteristics including aroma of different carrot cultivars, inbreds, or F_1 hybrid seed parents. It is known, however, that in male steriles, the anthers are either distorted and brown in the domestic cytoplasm or absent in the wild cytoplasm with petaloid structures in their place. Most of the present commercial F_1 hybrids are from the petaloid type, which seems to produce inferior seed yields compared with the brown-anther type. Stable brown-anther steriles have been more difficult to establish and maintain than petaloid steriles in wild cytoplasm (9, 11).

In the present investigations we followed the progression of floral events in cytoplasmic male-sterile inbred lines having the wild cytoplasm discovered by Munger in 1953 (9), and their F_1 male-sterile progeny (maternal seed parents), and in male-fertile (paternal parent) lines. Floral differences were then compared with seed yield data. We here attempt to describe the way floral stimuli and reward (and timing of floral events) contribute to bee activity. Our purpose is not to characterize individual lines, but rather to demonstrate by example the inherited floral diversity between parental lines and between sublines insofar as these differences reduce random foraging of insect pollinators.

Materials and Methods

The studies were conducted during 1974-75 in the field and during 1975-1977 in a greenhouse where we were able to control environmental conditions. The roots were produced in the field, harvested, subjected to cold induction for 8-10 weeks at 2°C (35°F) and cut to ca. 13 cm (length) before planting or potting for flowering and seed production.

Design

Field studies. 1974. Ten 3-row caged breeding plots of various sizes (3 × 3 to 12 m) were selected for study (Fig. 1). In each plot, a central fertile row was flanked by rows of equal length that contained a random assortment of blocks of male-sterile lines. Male-sterile lines included both inbred and F_1 hybrid seed parents. Each block consisted of from 4 to 7 stecklings. None of the S lines appeared in all cages (see also Erickson, et al. (2).

During bloom one 4-frame (16.8 cm deep) colony of honey bees was placed in each cage.

1975. Twenty 1.8 × 1.8-m plots caged to exclude flower-visiting insects were established, each containing a single pair of carrot lines. Each pair consisted of 6-10 stecklings of a fertile maintainer (M) line and of its isogenic (S) counterpart.

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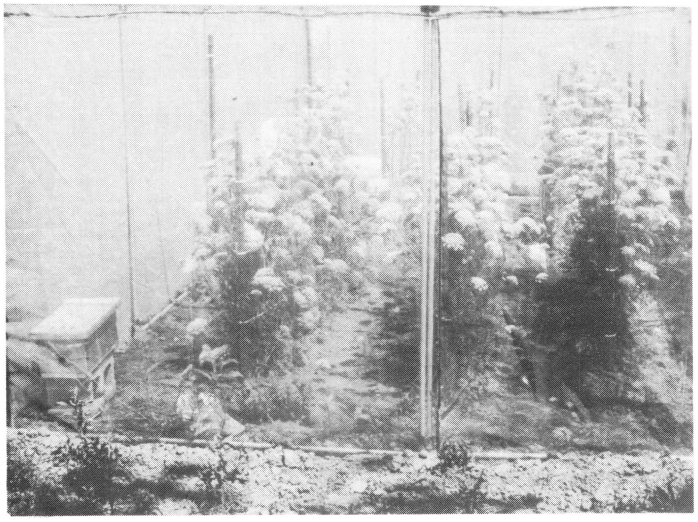

Fig. 1. Three-row caged breeding plots.

Greenhouse studies. 1975. Six M and 14 S lines (both inbred and F_1 seed parents) were selected for study on the basis of field observations made in the previous years. Six roots of each line were planted in early November in 15- to 18-cm clay pots with soil from a single source (standard greenhouse soil mix). Greenhouse temperature was maintained at 16°C daytime and 10° nights until bolting was observed. During bloom, the temperature was increased to 24° daytime and 18° nights on a 15-hr photophase. All pots were uniformly watered daily.

1976. Ten isogenic pairs selected on the basis of 1975 field observations were potted and maintained as in 1975.

Procedures

Field studies. On the day of the onset of flowering and on alternate days thereafter, all lines in each cage were examined between 10 a.m. and 3 p.m. The sequence of observation was varied among cages from day to day to avoid time-of-day bias. Color and stage of bloom were recorded. Also, the onset and duration of foraging cues (nectar, reflectance of ultraviolet light by the nectar, and aroma) were noted and the quantity of each was scored on a 0 to 5 scale. Nectar is normally difficult to remove from carrot florets, so we elected to quantify it on the basis of specular reflectance. Aroma was quantified and characterized by olfaction by two observers who worked as a team and reconciled any differences of opinion. Aroma-type dominance was determined on the basis of frequency among all observations. Results were adjusted to means for the entire block. Anthesis data were summarized as the average of block means over 2 years.

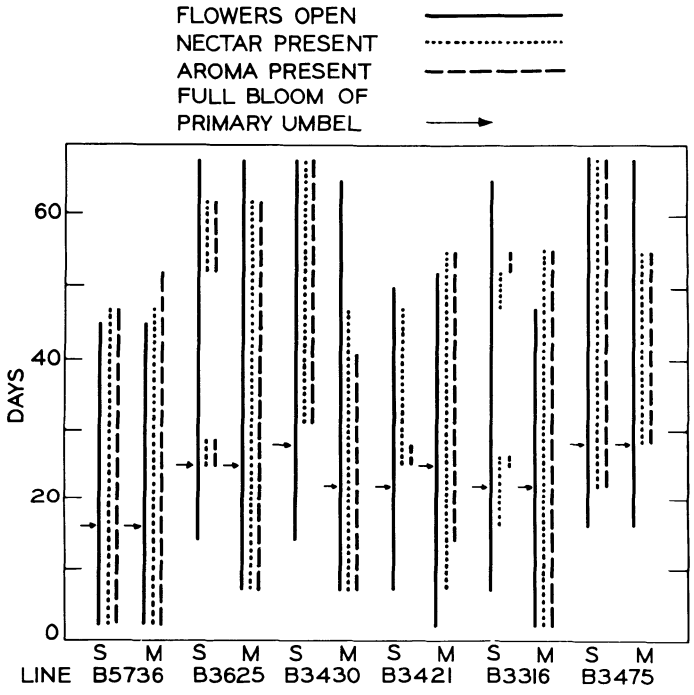

Fig. 2. Floral development among selected isogenic S and M carrot lines grown in field cages.

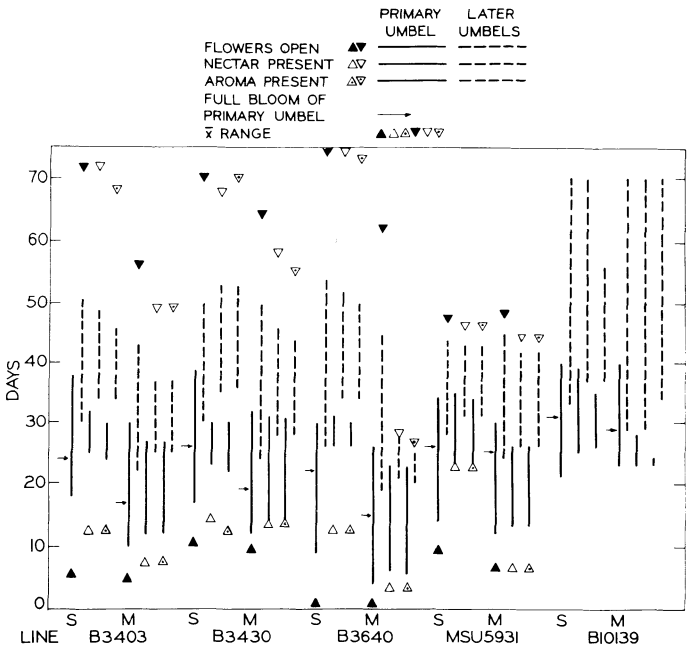

Fig. 3. Floral development among selected isogenic S and M carrot lines grown in the greenhouse.

Also, nectar sugar concentration was determined from samples obtained, whenever possible, directly from individual florets by using 1 $\mu\ell$ pipets (Microcaps)[2]. Phenotypes were compared on the basis of corolla color and genotypes. Umbels were bagged for several days before bloom. Nectar samples were analyzed for total dissolved solids (sugars) with a Bausch and Lomb Model 3L refractometer.

Seed yields were obtained at harvest by removing umbels from all plants then threshing and cleaning the seed. Values are expressed as the mean weight of seed per plant.

Greenhouse studies. In both years, all plants were monitored individually throughout anthesis, but particular attention was given to first flowering, pollen dehiscence (M lines), duration of anthesis of the primary umbel, and then of all remaining umbels, duration and quantity of nectar and aroma produced, and aroma quality. Methods of evaluation were similar to those of the field tests except that a single observer was used, since experience had shown that the differences in anthesis were obvious. A second observer was consulted on the few occasions when differences were slight. Also, some S lines were segregating for flower color (green or off-white). In this case, data were summarized on the basis of corolla color. Data presented represent the means for all plants of each line over 2 years.

The work with greenhouse-grown carrots in 1977 came about when unequal numbers of roots of 3 M and 17 S lines

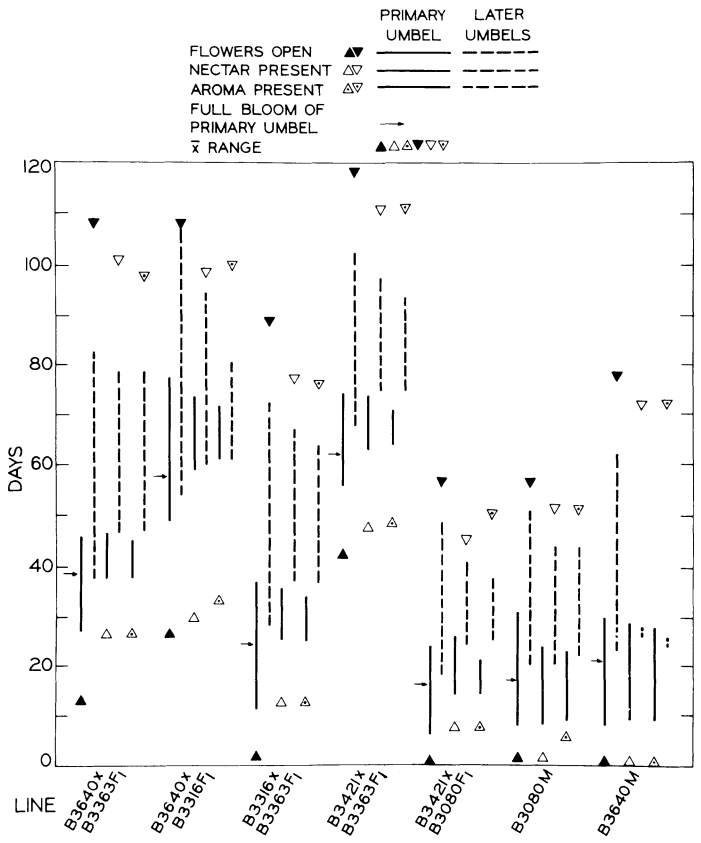

Fig. 4. Extremes in floral development among hybrid carrot seed parents.

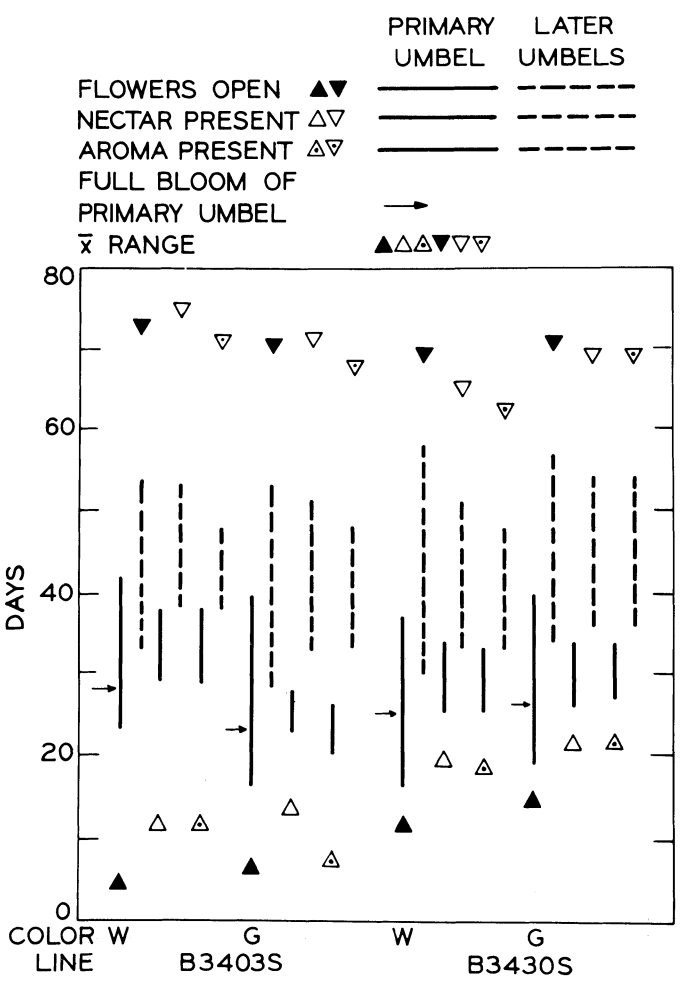

Fig. 5. Comparative floral development among carrot sublines segregating for corolla color.

were potted in the greenhouse and randomized on 3 benches. Subsequently, unknown environmental circumstances precipitated exceptional nectar secretion, thereby permitting accurate quantification of the relative ability of the various lines to secrete nectar. All nectar that could be removed from each plant via 1-$\mu\ell$ micropipets was taken and quantified, and volumes in excess of 6 $\mu\ell$ were removed via 100-$\mu\ell$ pipets. When the quantity was great enough, the percent dissolved solids was determined. All plants were sampled without knowledge of pedigree once a week for 3 weeks during midbloom when all lines were flowering.

Results and Discussion

Synchrony of floral events. A graphic illustration of anthesis as it occurs in selected isogenic inbred S and M lines grown under field conditions is shown in Fig. 2. The lines depicted are representative of the extremes of variability evident among and within lines. Generally, primary umbels required up to 15 days to reach full bloom; later umbels sometimes reached full bloom in no more than 3 days.

Occasionally isogenic S and M pairs, as in line B5736, bloomed simultaneously, a demonstration of synchrony of floral events. However, from our many observations, this synchrony appears to be the exception rather than the rule. More typically, the S inbreds bloomed later and had a shorter period of attractiveness to the bees since nectar and aroma production are delayed until the primary umbel is in full bloom. (The actual duration of bloom varied only slightly.) At the other extreme, as for B3430, there was little overlap of nectar and aroma production between isogenic pairs. Rarely, the span of nectar and aroma production in the S line exceeded that of the M line (B3475). Nectar and aroma production in the inbreds was poor (B3421S) and there were frequent periods when neither was produced (B3625S and B3316S). Similar differences were evident among isogenic pairs grown in the greenhouse (Fig. 3).

Although there appeared to be some correlation within pedigrees, variability among floral characteristics was also evident among selections out of the same parent (Fig. 2). B3430 and B3421 were selected from the same line and B5736 and B3625 were selected from a second unrelated line.

Seed yield (g per plant) was closely related to floral synchrony (note particularly B5736 in the following tabulation):

	B5736	*B3625*	*B3430*	*B3421*	*B3316*	*B3475*
M	32.1	6.5	4.1	4.2	1.3	0.4
S	19.2	5.2	0.7	2.8	0.4	7.9

Certainly some of the asynchrony evident in Figs. 2 and 3 may be the result of inbreeding, but the fundamental differences were evident among sterile F_1 seed parents evaluated in the greenhouse. Fig. 4 shows the broad range of variability (expressed as annual mean) evident among individual plants representing hybrid seed parents. This is not characteristic of the fertiles. The asynchrony of anthesis between certain of the S lines and the typical fertile lines shown in Fig. 4 is striking and undoubtedly contributes significantly to poor seed yield. It has been demonstrated in other plant species that pollinator foraging is dependent upon the availability of nectar and the concomitant stimuli of UV reflectance and aroma, hence the most effective period of synchrony in carrots must be the period when all these floral characteristics are present on both the S and M parent lines.

Table 1. Volume and % dissolved solids of nectar secreted by selected carrot lines grown in greenhouse, 1977.

Line	Total plants	Plants with nectar	Nectar quantity (μℓ)	Dissolved solids (%)	(range)	(n)
Fertile						
B4435M	11	4	> 250	21.0	–	1
B10138S x B10138M	13	8	12	–	–	–
(B3440S x B3316M) x B3316M	20	none				
Sterile						
B4435S x B4435M	5	none				
B4435S x B10138M	22	17	> 6,000	18.8	14-23	14
B10138S x B4435M	27	24	> 12,000	23.2	10-37.5	23
(B10138S x B4435M) x B10138M	28	none				
(B4435 x B10138M) x (B4435 x B10138M)	104	34	> 1,800	20.5	10-36	9
B10138S x B10138M	10	8	> 750	18.2	17.5-19	2
B10138S x B3316M	6	none				
(B10138S x B3615M) x B3615M	8	5	> 250	25	–	1
B10138S x MSU5931M	13	11	> 2,850	20.2	11-38	8
B10138S x (B10138M x MSU5931M)	9	5	> 450	24.9	19-38	4
B3316S x MSU5931M	9	none				

There are 2 additional observations that should be noted. Among the S lines there seemed to be a tendency for the presentation of nectar and aroma to cease between the flowering of the primary umbel and that of the remaining umbels. This, of course, contributed to further asynchrony of floral events. This tendency was not apparent in any M lines studied except B10139M. In the M lines, pollen began to dehisce on the first or second day of bloom, and nectar and aroma production occurred simultaneously. Because there is a delay in aroma production in the S lines, there may be some genetic linkage between the ability to produce pollen and ability to present foraging cues before the stigmata of protandrous flowers become receptive.

Differences were evident in the synchrony of anthesis between sublines differentiating in flower color (white, W; green, G) (Fig. 5). In some lines, these differences were minimal (B3430) and, for the most part, seemed less pronounced than between selections out of a single cross. However, this deduction should be studied further because the numbers of plants in each group were limited (B3403 W = 3, G = 7, B3430 W = 6, G = 3).

Nectar. Empirical (visual) quantification of nectar secretion among carrot lines proved less than satisfactory. Our 0-5-scoring system did indicate some differences but not as clearly as the volumetric comparisons made in 1977 (Table 1). Ability to secrete nectar varied widely among lines. Also, in 1977 some decline in percent dissolved solids seemed evident (March 2, $\bar{x}$ = 23.5%, n = 9; March 10 , $\bar{x}$ = 2.5, n = 44; March 16, $\bar{x}$ = 19, n = 3) but statistical analysis was not attempted because of variation in sample sizes.

A limited amount of data obtained in 1975 in the field suggests differences in the percent dissolved solids between M and S white- and green-flowered lines:

	White fertile	*White sterile*	*Green sterile*
$\bar{x}$	30.7%	11.0%	20.1%
$S_{\bar{x}}$	7.4	1.1	4.7
n	4	15	9

The green-flowered types plainly had high nectar-sugar content, but this may not relate to reproductive potential. It could result from the photosynthetic rate of the plant relative to its physiological state (e.g., low seed set).

No differences could be detected in the reflectance of ultraviolet light by carrot nectar except those imposed by volume and concentration. Figure 6 clearly shows that nectar-bearing florets differ in this respect from those that do not bear nectar, as the greatest reflectance comes from those umbels with the greatest quantity of nectar. If bees use ultraviolet light as a means of locating a nectar source, it is easy to see how important this information might be. We have known for some time that anthers too are UV reflective, and their absence would then contribute to differentiation and discrimination between S and M lines by bees.

Aroma. With our method of evaluation, we were unable to mathematically define the levels of aroma production by smelling individual lines and plants. Nonetheless, certain lines consistently produced far stronger aroma than others. The production of aroma was tied closely to the presence (visually determined) of nectar even though peak nectar and aroma production do not coincide with peak bloom (that point when all umbels have opened). We found that there were frequently several peaks in aroma, each associated with a later umbel(s).

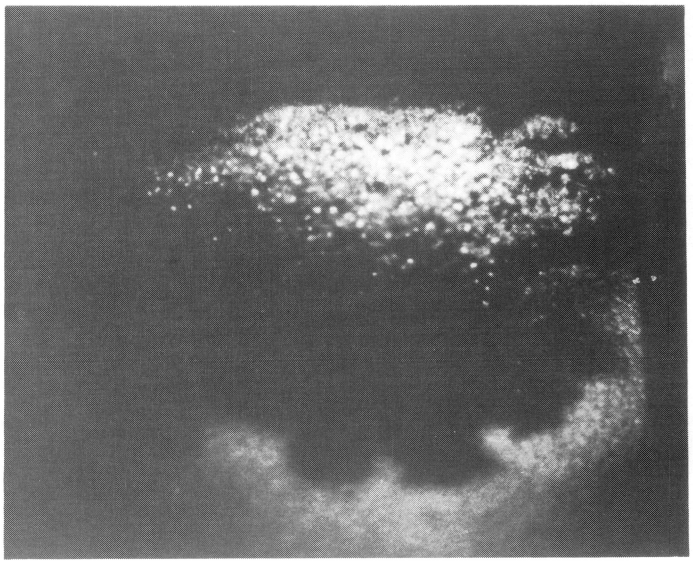

Fig. 6. Fluorescence of carrot nectar under ultraviolet light (F 2.8/1 sec, 18-A filter and high speed Ektachrome film).

These seemed to be of declining intensity, but additional data are needed to verify the impression since differences encountered between years or perhaps between lines may be the result of environmental differences.

We were able to empirically detect 5 distinctly different aromas produced by the various lines. Observers characterized these differently (e.g. one observer found a particular aroma "fragrant," and another thought it "offensive"), but all observers noted each as different from the others. One aroma, by far the most common and characteristic of nearly all M and some S lines, we call the "common carrot perfume" (CCP). This perfume seems to have several components including the other aromas that might be described as "fruity," "foul," "carrot," and "sweet."

The various lines could be characterized by the dominance of a particular aroma or group of aromas in which several components were detected. Notably, the individual components were least frequently associated with M lines, which had the CCP complex as the dominant fragrance. The individual components often seemed to appear in succession during bloom of the S lines (e.g. carrot → fruity → foul). On occasion, a plant aroma would be consistently fruity. Infrequently, aroma was never detected. The multicomponent CCP aroma was detected, if only briefly, on most but not all S lines. In the lines segregating for flower color, we detected differences in aroma between plants within lines.

Conclusions

Certain anatomical and physiological differences characteristic of S and M carrot flowers lead to preferential honey bee foraging on plants (see also Erickson et al. (2). On the basis of information concerning bee foraging behavior, the characteristics that affect row crossing from M to S plants by bees gathering either nectar and/or pollen are:

1. Flower color. It varies from white in M lines to a range of white or offwhite to green among S lines. A few green corolla M lines do exist but were unavailable for testing.
2. Flower aroma. It varies from perfume-like (noted in most fertiles and some steriles) to indistinct, carrot-like, or absent (noted in some white and green corolla S lines). Chemical analyses are underway.
3. Timing and duration of anthesis. These appear to be distinctly dissimilar between most S and M lines used as components in a hybrid seed production. Preliminary studies indicate that some S lines may bloom as long as 30 days later than the same M lines.
4. Carrot nectar. It appears to be highly variable in quantity between S and M lines. In general, nectar quantity among S plants was below that of the M plants. There also appears to be a tendency for a majority of S lines to secrete nectar and release volatiles (odor) much later than M lines. The petaloid type of the S lines is the result of early cell differentiation wherein the anthers become petal-like structures. The brown anther sterile type results from late cell differentiation. The impact of timing of cell differentiation on the delay of floral events has not been studied. Nectar from all M and S lines reflects ultraviolet light. Intensity of the fluorescence is variable depending in part upon the quantity of nectar secreted. Fluorescence in carrots is probably a visual cue to foraging honey bees.
5. Male-fertile plants contain anthers that fluoresce, and this presumably provides another visual cue for pollen-collecting honey bees. This cue is, of course, absent from S plants.
6. Plant height was noted to be variable between lines (not studied). Generally M plants are shorter and less vigorous than S plants of the same line; the sterile F_1 parents show striking expression of hybrid vigor and are taller than those used as pollen parents.

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