

Genetic Variability in Flower Attractiveness to Honeybees (*Apis mellifera* L.) within the Genus *Citrullus*

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Abstract. Fruit and seed set in insect-pollinated agricultural crops rely primarily on honeybees because of their ease of management and transportation. In many fruit and vegetable crops, the number of bee visitations can be the limiting step in obtaining optimal yield. Increasing the attractiveness of flowers to honeybees could, therefore, provide a useful means of improving fruit yield and seed production. Genetic variability in attractiveness to honeybees was found within the genus *Citrullus*. The number of daily visits per flower ranged from six to 12 among cultivars. Moreover, most of the visits to the more attractive cultivars occurred in the first hour of bee activity, whereas visits to the less attractive cultivars started later in the morning. A positive relationship was found between the frequency of bee visitations and seed number per fruit. Analyses of floral attributes indicated no genetic variability in flower size, amount of pollen grains, or nectar volume; however, differences were observed in the concentration of sucrose and total sugars in the nectar. A positive relationship was found between attractiveness to bees and nectar sugar concentration, suggesting that this characteristic is one of the parameters responsible for variability in attractiveness to honeybees.

Honeybees are the most important pollinators in the production of several agricultural crops. The value added to crop production by honeybee pollination in the United States alone is estimated at >U.S. \$9 billion (Robinson et al., 1989). Efficient pollination has a direct impact on both fruit yield and quality. In addition, efficient pollination can be crucial for seed production (McGregor, 1976). If few pollen grains are transferred to the stigma, fruits will contain few seeds.

Little information is available regarding the pollination requirements of watermelon. Adlerz (1966) reported that in watermelon, normal fruit development requires a minimum of eight to 10 bee visits to the flower. This number may be even higher for hybrid seed production and especially in the production of triploid (seedless) watermelons (Maynard, 1992).

The frequency of bee visitations depends on genetic as well as environmental factors. Because of the negative influence of high

temperatures on bee activity, bee visits to watermelon flowers usually peak in the morning hours (Ambrose et al., 1995). Another factor affecting the number of bee visits is competition between the various species in a given area. Some plant species are more attractive to honeybees than others and, therefore, the proportions of the various species at any given time play an important role in the relative attractiveness of each species. Flower attributes such as size, color, flower organs, and nectar guides on the petals, nectar volume, nectar composition, and amount of pollen are considered to be important factors attracting honeybees and as such can affect visitation frequency (Dobson et al., 1990; Fahn, 1979; McGregor, 1976).

Recent studies indicate that the chemical components contributing to a flower's fragrance also play an important role in the attractiveness of flowers to honeybees (Henning et al., 1992; Loughrin et al., 1991; Masson et al., 1993; Matile and Altenburger, 1988; Pham-Delegue et al., 1989). Collectively, these reports indicate that bee behavior is controlled by the integration of both perceived cues, such as color and/or fragrance, and the amount of a reward, such as pollen and nectar. Current studies have focused on defining the importance of each factor, as well as the interaction between them, in the attractiveness to honeybees of flowers from various species.

A high variation in the attractiveness of flowers from different species reflects diverse genetic variability. The study reported here was designed to characterize genetic variability in the attractiveness of flowers within the genus *Citrullus* to honeybees and to define the potential parameters controlling this trait.

Plant material. Genetic material included accessions from four *Citrullus* species: *C. lanatus*, *C. colocynthis*, *C. ecirrhosus*, and *C. rhemii*. Two cultivars ('Sugar Baby' and 'Mallali') of *C. lanatus* were crossed with accessions of the three species. Altogether, the first set of observations included >70 cultivars, F1 crosses, and back crosses between the various F1s and either parent.

Bee visitations and the number of seed. The frequency of bee visitation was determined in two successive years (1996–97) based on the method used by Elmstrom and Maynard (1990), with some modifications. Watermelon seedlings were transplanted in rows of loamy sand soil (agricultural farm, Rehovot) at 1-m spacing with centers 1.8 m apart. Each plot was 10 m long and the experiments were designed as randomized blocks with five replications. The average density of staminate flowers at anthesis was ≈10 per m². Since some of the cultivars contained a small number of pistillate flowers, the data presented include visitations to staminate flowers only. To eliminate variation due to the number of flowers, observation plots were selected such that each one included an area of 1 m² with exactly 10 staminate flowers. Bee visits were counted for 2 min in each plot.

To determine the relationship between bee visits and number of seed per fruit, mature fruits were collected in the end of the growing season. The first fruits setting on four plants in each plot were collected (20 fruit for each cultivar) and the number of seed were counted in each fruit.

Flower diameter and pollen counts. Flower diameter was determined on the day of flower opening on four different dates. Fifteen flowers from each cultivar were measured on each date.

To establish the quantity of pollen available to foraging bees during the morning visitation hours, 10 flowers of each genotype were sampled every 30 min during these hours. Anthers were removed with scissors into a vessel containing 10 mL of a 10% aqueous sucrose solution and shaken thoroughly to remove the mature pollen grains. Four samples, 10 mL each, were taken from each vial and pollen grains were counted in a hemacytometer using a Leitz optical microscope at a magnification of 100×.

Nectar analyses. Flower buds were covered just before anthesis with either gelatin capsules or gauze pads. Nectar was collected the following morning with a graduated 5-mL glass capillary tube (Brand, Werheim, Germany) into microfuges and kept on ice. Samples were stored at –18 °C for further sugar analyses. Sugars were separated in an analytical HPLC system (Kontron 325; Zurich, Switzerland) fitted with a Sugar-Pak column (6.5-mm i.d., 300 mm long; Waters Associates, Milford, Mass.) using an LDC refractive-index detector (Refractor Monitor IV; LDC Anal., Riviera Beach, Fla.). Samples were diluted with H₂O and then filtered through a 0.45-mm membrane (Watman, Maidstone, England) before injection into the HPLC system.

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Statistical analysis. Data were tested by one-way analysis of variance or simple regression using the option fit $y \times x$, and by stepwise use of the option fit model of the JMP program (SAS Institute, Inc., Cary, N.C.) for analysis of multiple variant regression.

Results

Genetic variability in flower attractiveness within the genus *Citrullus*. Initial observations included ≈ 60 cultivars of *C. lanatus*, several accessions of wild-type species within the genus *Citrullus* and several F1s between the commercial cultivars ('Sugar Baby' and 'Mallali') and *C. colocynthis*, *C. ecirrhosus*, or *C. rhemii*. Based on these observations, five cultivars were selected for more detailed analyses. The cultivar BAG (*C. lanatus*) and the wild-type *C. colocynthis* (cultivar CC-10) received a significantly higher number of bee visitations than did 'Sugar Baby' (Fig. 1) and visits to flowers of L-224, which is BC₁F₃ of the cross between *C. colocynthis* and 'Mallali', was lower than that of 'Sugar Baby'. The average number of bee visits in the morning hours was 12, 7.5, and 6 to BAG flowers, 'Sugar Baby', and L-224 flowers, respectively. Perhaps the most interesting result was that most visits to plants with a high number of bee visits were made in the early morning hours, while the peak visitations to the other lines occurred ≈ 60 to 90 min later (Fig. 1).

Floral attributes that attract honeybees. Further study was designed to characterize floral attributes attractive to honeybees, including flower's diameter, pollen production, and the volume and sugar content of nectar. This set of experiments included the earlier analyzed cultivars BAG, 'Sugar Baby', L-224, and several of their F1s.

Flower diameter was measured on the day of anthesis. All flowers were 20 to 30 mm in diameter, with no significant differences between the cultivars tested (Fig. 2). The correlation coefficient between the frequency of honeybee visits and flower diameter was only -0.05 , indicating that flower diameter is not responsible for the differences in the number of bee visits per cultivar.

When pollen was collected during the early morning hours, prior to bee visitation, no significant differences were observed among all the tested cultivars (Fig. 3). Another experiment was aimed at quantifying the fluctuations in pollen quantity during the bee visitation hours. As seen in Fig. 4, a sharp drop in the percentage of pollen grains remaining, relative to the number measured before bee visits, was observed in BAG flowers within the first 30 min of bee visitation, but not in 'Sugar Baby' or L-224 flowers. Pollen grain numbers declined in the last two cultivars after ≈ 60 min of bee visitation, with a sharper decline in 'Sugar Baby' than in L-224.

Nectar volume was highest in BAG and lowest in 'Sugar Baby'. Similar results were obtained when flowers were covered with either gelatin capsules or gauze pads (Fig. 5). Values were higher when flowers were covered with capsules, probably because of lower

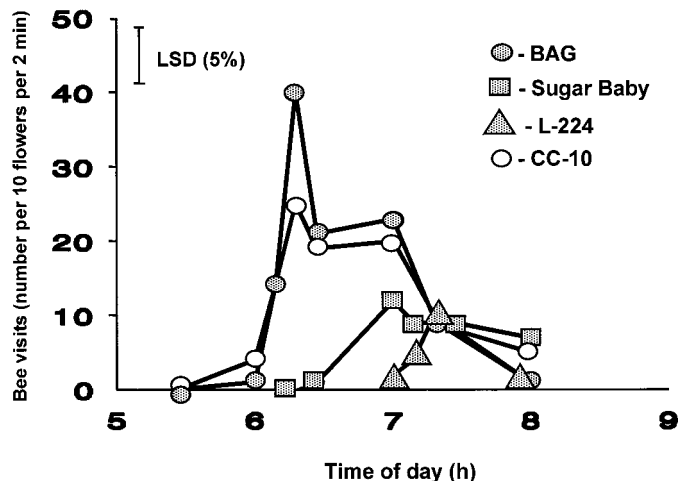


Fig. 1. Number of bee visits to male flowers of watermelon cultivars BAG, Sugar Baby (*C. lanatus*), CC-10 (*C. colocynthis*), and L-224 (derived from a cross between *C. colocynthis* and the commercial variety 'Mallali'). Values are the number of bee visits to 10 flowers during 2 min.

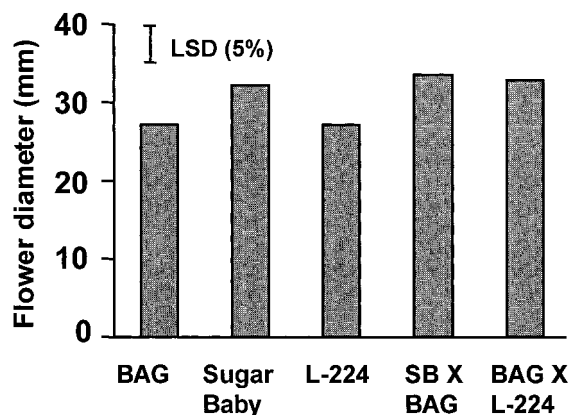


Fig. 2. The average diameter (mm) of watermelon flowers of cultivars BAG, Sugar Baby, L-224 and two of their hybrids. Values are mean of 60 flowers, at anthesis, measured on four different days (15 flowers per day).

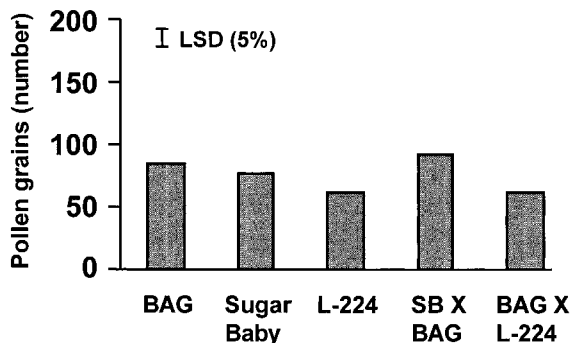


Fig. 3. The average number of pollen grains of watermelon cultivars BAG, Sugar Baby, L-224, and two of their hybrids. Values present the means of grains per hemacytometer field using a light microscope in measurements that were taken on four different dates.

evaporative losses. The volume of nectar was small and the variability relatively high, resulting in nonstatistical differences between the cultivars tested.

Analysis of sugar composition in the nectar indicated the presence of only sucrose, glucose, and fructose (Table 1). Sucrose accounted for $>50\%$ of the total sugar. The percentages of total sugar in BAG, 'Sugar Baby', and L-224

nectar were 32%, 24%, and 21.5%, respectively.

Discussion

Our study revealed a relatively wide genetic variability within the genus *Citrullus* with respect to flower attractiveness to honeybees. Differences of up to 100% in the number

of bee visits were recorded between the various watermelon cultivars (Fig. 1). Within one field, these differences were reflected in both the total number and the diurnal frequency of visits. Peak bee visitation occurred 1 h earlier in the more attractive cultivars than in the less attractive ones. This phenomenon can be explained by the amount of reward remaining. Visits to the attractive flowers caused early depletion of pollen and nectar, resulting in a shift in visitations toward less attractive cultivars that still contained these rewards. As indicated in Fig. 4, the depletion of pollen grains was more rapid in flowers of the attractive lines, whereas the reduction in the amount of pollen in the less attractive 'Sugar Baby' and L-224 started only when pollen was almost absent from flowers of the former.

The amount and availability of pollen grains is considered a limiting factor for optimal seed set in watermelon (Adlerz, 1966; Elmstrom and Maynard, 1990; Maynard, 1992). Because honeybees are the main pollinators of watermelon in commercial fields, low visitation frequency may cause reduced seed production. A direct relationship between the number of bee visits per flower and the number of seeds set per fruit was found in this study. Seed set was higher in BAG (400 seeds per fruit), than in 'Sugar Baby' (225) or L-224 (190). These data confirm earlier reports indicating that normal fruit development may be impaired if flowers receive less than eight honeybee visits (Adlerz, 1966; Maynard, 1992). Furthermore, these results suggest that the process of seed production may be limited by the number of bee visits.

What are the important parameters controlling attractiveness to honeybees? Some flower attributes, such as color, size, and pattern, are perceived as cues by honeybees and may influence their behavior (Dafni, 1992; Giurfa et al., 1994; von Frisch, 1967). However, we found no significant differences in flower size or pattern among the cultivars (Fig. 2). Although we did not characterize the typical absorption spectrum for flowers in each cultivar, we found no visible differences in color, suggesting that these cues cannot explain the differences in attractiveness to bees.

Pollen and nectar are the two food rewards that supply the honeybee's requirements for amino acids and carbohydrates. Although some variation in the amount of pollen was observed in the current study (Fig. 3), it cannot explain the observed variability in attractiveness to honeybees.

Nectar is generally believed to be the most important factor attracting bees to flowers (Dafni, 1992; Free, 1993). Several studies have stressed the importance of nectar volume, while others emphasize the importance of the concentrations or relative amounts of specific sugars (Baker and Baker, 1983). The volume of nectar secreted by flowers of watermelon cultivars is low. Given the high standard error values, we found no significant differences between the genotypes (Fig. 5). On the other hand, nectar sugar concentration and sugar composition differed significantly among the cultivars (Table 1). Waller (1972)

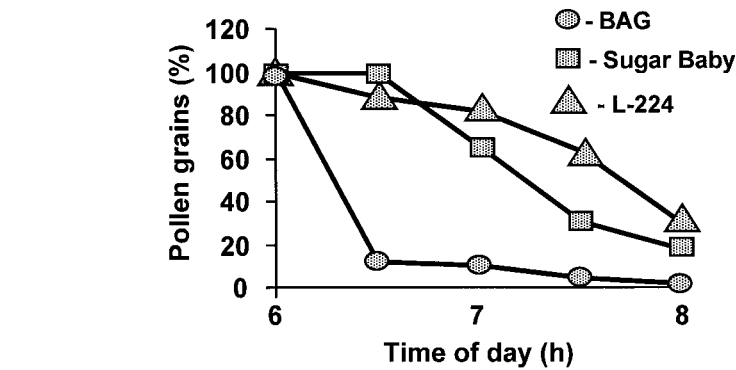


Fig. 4. Changes in the percentage of pollen grains, relative to the number obtained before bee visits, in flowers of watermelon cultivars BAG, Sugar Baby, and L-224. Values present the means of grains per hemacytometer field using a light microscope in measurements that were taken on four different dates.

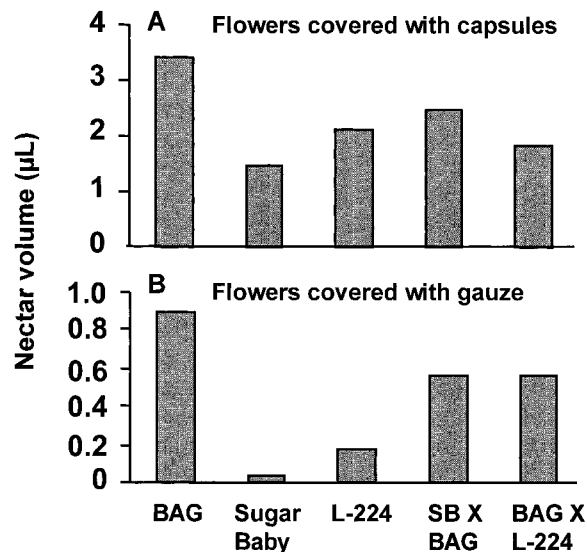


Fig. 5. Nectar volume (mL) per staminate flower in watermelon cultivars BAG, Sugar Baby, L-224 and two of their hybrids. Values are mean of 15 flowers. The flowers were either covered with (A) gelatin capsules or with (B) gauze pads on the night before anthesis and the nectar was collected during the following morning hours.

Table 1. Sugar concentrations (%) in staminate flowers of three watermelon cultivars. Flowers were covered with gauze pads in the evening and nectar was collected early the following morning. Values represent the mean \pm SE of eight flowers from each plant line.

Plant line	Sucrose	Glucose	Fructose	Total sugar
BAG	20.5 \pm 5.2	6.0 \pm 1.2	5.6 \pm 1.1	32.1 \pm 4.1
Sugar Baby	15.2 \pm 1.2	4.4 \pm 1.1	4.5 \pm 1.1	24.1 \pm 2.1
L-224	11.7 \pm 3.3	5.0 \pm 0.9	4.8 \pm 0.8	21.5 \pm 4.2

suggested that honeybees prefer nectar with a sugar concentration in the range of 30% to 50%. Within this range, there was no advantage to specific compositions of the three major sugars (sucrose, glucose, and fructose). Analysis of nectar secreted by staminate watermelon flowers confirmed the presence of sucrose, fructose, and glucose only. However, only BAG nectar contained sugars at a concentration $>30\%$. Note that in all cultivars the proportion of sucrose was $\approx 60\%$, those of glucose and fructose $\approx 20\%$ each. Thus, sugar concentration in nectar may be partially responsible for the differences among watermelon cultivars in attractiveness of flowers.

The chemical composition of flower volatiles may also affect bee behavior. Olfactory signals are rapidly learned, indicating that foraging behavior results from the association of plant allelochemicals acting as chemosensory cues for the honeybee (Pham-Delegue et al., 1990). Moreover, the fact that in some cases bees are more attracted to flowers with a meager level of nectar than to those with high levels indicates that the olfactory signal(s) may be a more dominant factor controlling bee behavior (Cruden et al., 1983; Dafni et al., 1988). Preliminary analyses of the chemical composition of headspace volatiles collected from watermelon flowers indicate that varia-

tion does exist among our various watermelon cultivars (data not shown). A current study is aimed at characterizing the profiles and chemical compositions of these volatiles.

Hybrid seed production can be facilitated by mechanisms that enhance the transfer of pollen from one selected parent line to another. Increasing the number of bee visits per flower may lead to higher seed yields and crop production. The observed genetic variability in the attractiveness of watermelon flowers to honeybees validates the potential for improving seed production. The challenge now is to characterize the major factor(s) responsible for this variability.

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