Seed Production in Watermelon: A Comparison between Two Commercially Available Pollinators

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Additional index words. bumble bees, *Bombus impatiens*, honey bees, *Apis mellifera*, pollination, *Citrullus lanatus*

Abstract. The number of honey bees (*Apis mellifera* L.) continues to decline due to parasitic mite pests and other factors. Honey bees and bumble bees (*Bombus impatiens* Cresson) were therefore compared for their effects on the seed set of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] in a 2-year field experiment. The experiment was a $2 \times 4 + 2$ factorial, comparing bee type (honey bee or bumble bee) at four visitation levels (1, 6, 12, and 18 bee visits) to pistillate flowers, with two controls: a no-visit treatment and an open-pollinated treatment. Bee visitation level had a strong positive influence on seed set ($P \leq 0.0001$). All flowers bagged to prevent insect visitation aborted, demonstrating the need for active pollen transfer between staminate and pistillate watermelon flowers. Flowers visited by *B. impatiens* consistently contained more seed than those visited by *A. mellifera*, when compared at equal bee visitation levels ($P \leq 0.0001$). We conclude that bumble bees have great potential to serve as a supplemental pollinator for watermelon when honey bees available for rental are in limited supply.

The use of non-honey bee pollinators in crop production is not new; since 1947, the U.S. Department of Agriculture, Agricultural Research Service, has supported research programs devoted to the development and management of native and imported bee species for use as alternative pollinators of specific crops (Torchio, 1990). For example, commercial seed production of alfalfa (*Medicago sativa* L.) is now largely accomplished by the alkali bee, *Nomia melanderi* (Hymenoptera:

Halictidae) and the alfalfa leafcutting bee, *Megachile rotunda* (Hymenoptera: Megachilidae). Several species of *Osmia* (Hymenoptera: Megachilidae) have proven to be efficient and manageable pollinators of orchard crops such as almonds (*Prunus amygdalus* Batsch), apples (*Malus domestica* Borkh.), and pears (*Pyrus communis* L.) (Free, 1993; Kuhn and Ambrose, 1984). More recently, bumble bee colonies [*Bombus* spp. (Hymenoptera: Apidae)] have become commercially available in the United States and have had a dramatic effect on the greenhouse tomato (*Lycopersicon esculentum* Mill.) industry, replacing costly and labor-intensive hand pollination and/or mechanical vibration (Kueneman, 1995).

In many cases, development of manageable non-honey bee pollinators has focused on the few crops that are inadequately pollinated by honey bees (Torchio, 1990). However, honey bees, which were introduced to North America from Europe during the colonial period, are generally recognized as the most important pollinators for commercial production of the vast majority of U.S. crops that require insect pollination (Free, 1993; McGregor, 1976; Oertel, 1980). Honey bee colonies have certain attributes that make them very efficient and reliable pollinators for most crops: they have large population sizes (up to 60,000 individuals per colony), exist on a perennial basis, can be managed using established practices, and can be transported over

considerable distances to selected locations. In the United States, the number of honey bee colonies rented for crop pollination exceeds 2 million annually and the value added to crop production by honey bees is estimated to be U.S. \$9.3 billion (Robinson et al., 1989).

Unfortunately, in the last several years the availability of honey bee colonies has become a serious limiting factor in the pollination of many bee-pollinated crops. The accidental introductions of two parasitic mite species [*Acarapis woodi* (Rennie) (Tarsonemidae) and *Varroa jacobsoni* Oudemans (Varroidae)] have decimated honey bee populations in North Carolina, reducing the feral and managed honey bee colonies by 90% and 30%, respectively, and other states have experienced similar reductions (Ambrose, 1997; Harbo and Hoopingarner, 1997). These recent losses to the pollinator force available for crop production are compounded by steadily declining populations of wild bees and managed honey bees, which has been attributed to increased herbicide and pesticide use over the last half-century and depletion of hibernation and nesting sites due to large-scale monoculture and urban sprawl (Martin and McGregor, 1973).

Due to the detrimental impact the parasitic mites have on colony survival, obtaining rental honey bee colonies for pollination may become a limiting factor in the production of many crops that are dependent upon bee pollination for fruit and seed set. With the advent of commercial bumble bee hives, a potential alternative or supplemental pollinator is now available when the numbers of honey bee colonies are in limited supply. Our objective in this 2-year study was to compare the effects of honey bee and bumble bee pollination on seed and subsequent fruit production in watermelon under field conditions. A previous article (Stanghellini et al., 1997) dealt with the effects on fruit set and abortion. In this paper we present data for seed production.

Materials and Methods

Cultivation practices and experimental design. From mid-April to early October in 1995 and 1996, studies were conducted to compare the effects of honey bee and bumble bee visitation and bee visit number on seed set in watermelon. Bee type was also compared with two controls; a no-visit (no entomophilous pollination) treatment and an unrestricted-visit (open pollination) treatment. The field, located at the Horticultural Field Laboratory in Raleigh, N.C., measured 28 × 50 m. Sequential plantings of 'Royal Jubilee', a recommended cultivar in North Carolina (Schultheis, 1995), were made each year on raised, plastic-covered beds 50 m in length. Soil type in the field was an Appling Sandy Loam (Clayey Kaolinitic Thermic Typic Kanhapludult). The plants were drip irrigated and fertilized in accordance with standard commercial practices (Sanders et al., 1995).

The experiment was a randomized complete block design with four replicates. Containerized transplants were grown in a greenhouse for 21 d, hardened for about a week, then

Received for publication 1 May 1997. Accepted for publication 26 Aug. 1997. This research was funded in part by the North Carolina Agricultural Research Service (NCARS Projects NC 6355 and 6187) Raleigh, N.C., Koppert Biological Systems, 2856 S. Main Street, Ann Arbor, MI 48103, and the North Carolina State Beekeepers Association. Use of trade names in this publication does not imply endorsement by the NCARS of products named or criticism of similar ones not mentioned. We gratefully acknowledge Drs. Cavell Brownie and Mary Peet for their advice, and Dennis Adams, Leslie Newton, Jason Doherty, Lane Tabor, Mark Scepaniski, Paul Lineberger, Clifton Ryan, and Watson Hall for their technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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planted into the field with a row spacing of 1.5 m and in-row spacing of 0.9 m, giving 53 plants per bed (two beds and 106 plants per replicate). In 1995, the field planting dates for the four replicates were 18 May, 30 May, 13 June, and 27 June, respectively. Bee visitation dates for the four replicates were as follows: 3 July through 20 July (replicate 1); 24 July through 26 July (replicate 2); 28 July through 3 Aug. (replicate 3); and 4 Aug. through 6 Aug. (replicate 4). In 1996, another four replicates were planted on 21 Apr., 6 May, 22 May, and 7 June. The dates of treatments (bee visitation) were 7 June through 1 July and 7 July through 18 July for replicates 1 and 2, respectively.

Treatment. In mid-May 1995 and 1996, two honey bee colonies ($\approx 20,000$ bees per colony) and two commercial bumble bee colonies ($\approx 50\text{--}75$ bees per colony) were placed along the edge of the field when most of the watermelon plants had reached anthesis; hives of both species were replaced periodically throughout the season. The honey bee colonies were from the North Carolina State Univ. (NCSSU) apiary. The bumble bee colonies contained a common native American species (*Bombus impatiens* Cresson) and were provided by Koppert Biological Systems, Ann Arbor, Mich. The bumble bee colony housing was designed for in-greenhouse use (cardboard exteriors); therefore, they were placed in protective wooden stands 1 m in height. Voucher specimens for both bee species used in these studies were placed in the NCSSU Insect Collection.

Pistillate flowers were bagged in the early evening before anthesis to control bee visits the following day. Bags (26×26 cm closed) were fashioned from nylon pollination cage netting. The bags were edged by Velcro-like strips to enclose the pistillate flower and thus prevent unwanted visitation prior to treatment. Aluminum wire was shaped to keep the sides of the bag from contacting the blossom.

Bee visitation treatments began when temperatures reached 21°C on the day of anthesis; bags were removed and 1, 6, 12, or 18 bee visits were allowed on each flower by either honey bees or bumble bees. Each time a bee landed on the flower, it was counted as a visit. An individual bee could be responsible for more than one visit. An approaching forager of any other species was prevented from landing on the treatment flower by using an aluminum pole with plastic forestry ribbon attached to the end. Flowers were discarded if they were visited by an insect other than the one designated for that particular flower. Foragers from wild bumble bee colonies were present in the field and had a variable range in body size. These foragers were sometimes not readily distinguishable from the Koppert (commercial) colonies; thus, uniformity of *Bombus* treatments was established by restricting flower visitation to those bumble bees that had a body length similar to that of commercial bumble bee foragers (1.5–2 cm).

After each flower had received the desired number of visits, its bag was resealed and tagged with treatment type and date. Bags on

the open-pollinated controls were left open for the duration of the sampling period, and then resealed when the controlled visitation treatments for that day were completed. The no-visit controls remained bagged the entire day of anthesis. All bags were removed from the flowers after 1930 HR on the day of treatment after bee activity in the field ceased. Bags were placed on new pistillate flowers in the pre-anthesis stage at this time for treatment the following day.

All previously set fruit were removed before treatments were made, since developing fruit inhibit additional fruit set in watermelon (Cunningham, 1939; Hibbard, 1939). All pistillate flowers that developed after treatment were removed from the test plants for the next 5 to 7 d, insuring that treated flowers were given an optimum chance for development as they constituted "first-set" fruit.

The sampling period per day was restricted to a morning interval starting at 21°C and ending between 1200 and 1300 HR, based upon research on anther dehiscence (Seaton and Kremer, 1939), pollen viability and stigmatic receptivity (Poole and Porter, 1933; Sedgley and Buttrose, 1978), and peak bee foraging activity (Ambrose et al., 1995; Brett and Sullivan, 1972). Individual plants of the test cultivars were chosen randomly each day for treatment. Treatment type was also allocated randomly; however, those with the same bee visit number, e.g., 6 honey bee vs. 6 bumble bee visits, were done simultaneously or back-to-back to alleviate or reduce any time-of-day effects. Each of the 10 treatments (honey bee or bumble bee at bee visit numbers 1, 6, 12, and 18, plus the two controls) were repeated on 10 plants per replicate.

Harvest. In both years the developing fruit were allowed to mature to maximum size in order to record viable seed set. All treatment and control flowers that aborted were recorded. Upon harvesting, the seeds were extracted from the fruit by hand and placed in a container of water. All seeds that sank to the bottom were counted as viable; those that floated were considered nonviable (empty seed coats).

Statistical analysis. Seed set comparisons between bee types were made using analysis of variance (Type I SS) in both experiments (SAS Institute, ver. 6, 1989; Steele and Torrie, 1980). Linear components of the regression of mean seed set on bee visit number (1 to 18 visits) for both bee types were evaluated. Seed set comparisons for each of the eight bee type by visit number combinations (i.e., honey bee at one visit, bumble bee at one visit, etc.) were then tested individually against the two controls (no entomophilous visit and open-pollinated treatments) using the least significant difference procedure ($P \leq 0.05$) (SAS Institute ver. 6, 1989; Steele and Torrie, 1980).

Results

Bee visitation was from 3 July to 6 Aug. in 1995 and from 7 June to 18 July in 1996. Of the four plantings (replicates) in 1996, no data were collected from the latter two (22 May and 7 June), as persistent and excessive rains hin-

dered bee activity and resulted in diseased plants. The two-replicate 1996 data were combined with the 1995 data, as neither the year nor replicate (year) main effects, nor the year $\times$ bee, year $\times$ visit, year $\times$ bee $\times$ visit interactions were significant ($P > 0.10$ in all cases). Rain prevented treatments on the following days: 5, 7, 8, 11, 18, 19, and 31 July in 1995, and 10–14, 17, 18, 23–25 June, and 11–15 July in 1996. Air temperature ranged from 16.8 to 35°C during the experiment, but bee visits were only recorded at $\geq 21^\circ\text{C}$.

As the number of bee visits increased from 1 to 18, mean seed set increased linearly for each bee type ($P \leq 0.0001$) (Fig. 1). Linear regression equations for $1 \leq x \leq 18$ (where x = visit number) were as follows: for honey bee treatments, seed set = $5.84x + 162.7$; for bumble bee treatments, seed set = $5.28x + 217.3$. All fruit bagged to prevent bee (or other insect) visitation aborted within a week of anthesis, resulting in no seed set for this control group. Averaging over years and replicates, both the bee type main effect and bee visit number main effect were highly significant (for both main effects, $P \leq 0.0001$); when compared over visit number, seed sets were significantly higher following visits by bumble bees than following visits by honey bees, and increased bee visits by either bee type significantly increased mean seed set.

Fruit receiving 12 or more bumble bee visits or 18 or more honey bee visits did not differ significantly from the open-pollinated control at the 5 percent probability level (LSD) (Fig. 1). Seed set in fruits from flowers receiving bee visits, including the open-pollinated controls, were significantly different from the no-visit control ($P \leq 0.0001$), as no fruit were obtained from this treatment group.

Discussion

Numerous studies have been made of the influence of honey bee visitation on seed production in cucurbit crops other than watermelon (Avila et al., 1989; Bohn and Davis, 1964; Collison, 1976; Connor, 1969; Eischen and Underwood, 1991; Fisher and Pomeroy, 1989; Gaye et al., 1991; Iselin et al., 1974; Jaycox et al., 1975; Mann, 1953; McGregor et al., 1965; McGregor, 1976; McGregor and Todd, 1952; Stanghellini, 1996). However, a correlation between fruit set and seed set would provide evidence that increased bee visitation increases watermelon seed production (Adlerz, 1966; Brewer, 1974; Goff, 1937; Rao and Suryanarayana, 1989; Rosa, 1925; Spangler and Moffett, 1979). Adlerz (1966) attempted to relate seed production in watermelon to increased bee visits by exposing flowers to two, four, six, or eight honey bee visits. Unfortunately, most flowers visited less than six times aborted and the relationship between the number of visits and the number of seeds per fruit was not established. However, he did note that seed number was greater following eight visits than following six.

In our study, seed set was strongly influenced by the number of bee visits ($P \leq 0.0001$); as visits by either honey bees or bumble bees

Fig. 1. Mean seed set per watermelon fruit for honeybees, bumble bees, and open-pollinated and no-visit controls, averaged over 2 years and six replicates.

increased from 1 to 18, there was a highly significant increase in the number of mature seeds that developed (Fig. 1). When bee visitation was prevented (the no-visit control), all flowers aborted. The fact that unpollinated cucurbit flowers, with the exception of those of parthenocarpic cultivars, will not produce fruit has been well established (Free, 1993; McGregor, 1976). Averaged over bee type, seed set ranged from 196 for single visits to 293 for 18 visits, while the open-pollinated control produced an average of 305 seeds per fruit.

Bee type also greatly influenced watermelon seed production ($P \leq 0.0001$). Fruit from bumble bee-visited flowers had consistently higher seed sets than did those visited by honey bees when compared at equal bee visit numbers (Fig. 1). Analysis of the individual bee type by visit number combinations (LSD; $P \leq 0.0001$) showed that at least 18 honey bee visits, but only 12 bumble bee visits, were required to produce mean seed sets comparable to those of the open-pollinated control (Fig. 1; LSD, $P \leq 0.0001$).

These results agree with our previous studies on fruit abortion rates (Stanghellini, 1996; Stanghellini, et al., 1997) and further support the use of cultured bumble bees as alternate pollinators to honey bees, at least for the crops used in these studies, watermelon and cucumber. Our future research will examine the effects of whole colony levels (honey bee vs. bumble bee) on the pollination of selected vine crops to determine if the results found in these studies at the individual and multiple bee level translate to increased crop and/or seed production.

This study clearly demonstrates: 1) the need for honey bee or other entomophilous pollination in the production of vine crops such as watermelon, 2) the value to increased seed production resulting from efficient and abundant pollinators, and 3) the potential value of bumble bees in the field pollination of watermelon and possibly other vine crops.

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