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ORIGINAL RESEARCH ARTICLE

# An evaluation of Fruit-Boost™ as an aid for honey bee pollination under conditions of competing bloom.

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## Summary

An experimental situation was created in which watermelon and sunflower were blooming simultaneously and competing for pollinators. Measures of watermelon flowering and bee visitation were made for three consecutive weeks of bloom and followed by measures of fruit at harvest. The honey bee attractant Fruit Boost™ was tested for its efficacy in focusing honey bees onto the target crop (watermelon) and improving pollination. Fruit Boost did not significantly affect total number of honey bees visiting watermelon flowers, the proportion of honey bee visits that occurred on female flowers, fruit-set, or fruit weight. The onset of sunflower bloom on week 2 was associated with a sequential drop in honey bee numbers on watermelon between weeks 1-3, a near absence of fruit set after week 1, and significant decrease in melon size between weeks 1 and 3. More broadly, this study suggests that pollination is compromised in agro-ecosystems where crops or feral plants are competing for limited pollinators.

## Evaluación de Fruit-Boost™ como una ayuda para la polinización de las abejas en condiciones de competencia por las flores.

### Resumen

Se diseñó un experimento en el cual la sandía y el girasol florecían simultáneamente y competían por los polinizadores. Las medidas de la floración en la sandía y las abejas visitantes se realizaron durante tres semanas de floración consecutivas y posteriormente se tomaron medidas de la fruta cosechada. La atracción de abejas por Fruit Boost™ fue ensayada por su eficacia en orientar a las abejas sobre el cultivo diana (sandía) y en mejorar la polinización. Fruit Boost no afectó de forma significativa al número total de abejas que visitan las flores de sandía, a la proporción de abejas visitantes sobre las flores femeninas, al cuajado, o al peso del fruto. El inicio de la floración del girasol en la semana 2 se asoció con una caída secuencial del número de abejas sobre la sandía entre las semanas 1-3, una ausencia casi total del cuajado del fruto después de la semana 1, y una disminución significativa en el tamaño de los melones entre las semanas 1 y 3. En términos más generales, este estudio sugiere que la polinización se ve comprometida en los ecosistemas agrícolas donde los cultivos o plantas silvestres compiten por los polinizadores limitados.

**Keywords:** *Apis mellifera*, seedless watermelon, *Citrullus lanatus*, sunflower, *Helianthus annuus*, pollinator competition

## Introduction

As the number of honey bees (*Apis mellifera* L) in North America declines due to parasites, pests and diseases (Cox-Foster *et al*., 2007), there is a parallel interest in improving the pollinating efficacy of those bees that remain. A pollinator deficit is especially acute if neighbouring crops must compete for limited pollinators (Levin and Anderson, 1970). Under conditions of compromised pollinator efficacy, honey bee attractants may help focus limited pollinators onto the crop of interest (Delaplane and Mayer, 2000). Of a handful of tested bee attractants (Ambrose *et al*., 1995), those based on queen mandibular pheromone (QMP, Fruit Boost™, Phero Tech, Inc.; Delta, B.C., Canada) have had the most promising research record (Currie *et al*., 1992a,b; Naumann *et al*., 1994).

As a model crop requiring pollination, hybrid seedless (triploid) watermelons (*Citrullus lanatus* Thunb.) are attractive because of their high consumer appeal (Maynard, 2003). Standard commercial cultivars have separate male (staminate) and female (pistillate) flowers, large sticky pollen grains, and an adhesive stigma, all of which implicate insect pollination (Stanghellini *et al.*, 1997). Pistillate diploid watermelon flowers require more than six honey bee visits to set fruit (Adlerz, 1966). This requirement is even greater for triploid watermelon which, because of its unviable pollen, must receive viable pollen from staminate diploid donors (Stanghellini, 2002). Between 16 and 24 honey bee visits are required to achieve maximum triploid watermelon fruit set (Walters, 2005).

The objective of this study was to determine whether application of a honey bee attractant to seedless watermelons during bloom promotes pollination by honey bees under conditions of pollinator competition. The model target crop was seedless watermelon and the model competitor sunflower (*Helianthus annuus* L).

# Materials and methods

## Culture

The experiment was conducted at the 36 ha Horticulture Research Farm of the University of Georgia, Oconee County, GA, USA, about 4 ha of which was serendipitously planted with sunflower. Seeds of triploid 'Sugar Heart' (main variety) and diploid 'Crimson Sweet' (polleniser) watermelons were germinated in a greenhouse. Six 20 × 18-m experimental plots with plant spacing of 1 m and row spacing of 2 m were set up throughout the farm. In each plot, seven rows were planted on 25 May with polleniser seedlings on the outer and centre rows. Plants were watered in with 20: 20: 20 (N: P: K) fertilizer. Strip irrigation was installed in each row in all plots (except for Plot 2 which was provided with overhead irrigation and included in the experiment after two weeks to replace an original plot with poor stand). Any dead or missing plants were replaced with reserve plants on 16 June.

## Treatment assignment and insect and flower parameters

Each plot randomly received one of two treatments (three plots per treatment): 1. application of label rates of Fruit Boost or; 2. a control spray of water. One active, full sized honey bee colony was positioned at the edge of each plot at a rate approximating 7.5 hives / ha.

On 18 July (Week 1) treatments were applied with a backpack sprayer. Fruit Boost was applied at a rate of 5.5 ml concentrate to 11.4 l water. Treated plots received 18.9 l of mixture; control plots received 18.9 l water. Applications were made after 16.00 to ensure that virgin flowers opening the next morning would be treated the whole duration of their anthesis. On the morning of 19 July we counted the number of honey bee flower visits. One observer was assigned to each plot (six observers) and observations synchronized to run between 08.15-09.00. Each observer walked down each row for five minutes, recording the number of honey bee flower visits and the sex of flower visited (7 rows × 5 min = 35 min per plot). A bee landing on an open flower was considered to be a 'visit.' Subsequently, 100 unopened female flowers were tagged and numbered in each plot. Honey bee observations were similarly recorded on 20 and 21 July except that observers rotated to different plots.

Two additional weeks of treatment, flower tagging (15 female flowers per plot) and bee observations were conducted on 25-28 July (Week 2) and 1-4 August (Week 3). Days of Fruit Boost application had the following climatic variables: temperature highs 28.6-34.6°C, precipitation 0-0.46 cm, and average wind speed 0.81-1.13 m/s. On one day during each of Weeks 2 and 3 the relative taxonomic diversity of insect visitors to watermelon flowers was measured. Using the protocol described above, each observer recorded by sight the number of insect flower visitors in the following taxa: honey bees, bumble bees (*Bombus* spp), squash bees (*Peponapis pruinosa*), butterflies and moths (Lepidoptera), beetles (Coleoptera), bugs (Hemiptera), sweat bees (Halictidae), flies (Diptera), carpenter bees (*Xylocopa virginica*), and hunting wasps (Vespidae). Additionally, during Weeks 2 and 3 we measured each plot for the sum of open flowers (male + female) available to bees and the ratio of female : male flowers by placing a 0.5-$m^2$ plastic pipe square over clusters of flowers and recording the numbers female and male; the average of ten counts per plot was determined.

## Harvest parameters

On 19 August the number of mature and developing fruit for both triploids and pollenisers was counted in each plot. On 22-23 August we harvested and weighed (to the nearest 0.01 kg) melons recovered from flowers tagged in Week 1; the ratio of number recovered fruit / number tagged flowers constituted fruit set for each plot. Fruit set and weight were determined similarly for Week 3; Week 2 yielded no melons, so harvest data were removed from analysis.

## Data analysis

All response variables were analyzed for treatment and week effects, with interactions, with the General Linear Models procedure (SAS, 1992). The main effects week and treatment were tested against their interaction term. Where necessary, means were separated with Tukey's test.

***Table 1.*** Relative taxonomic diversity of insect visitors to watermelon flowers; for each mean *n* = 12.

| **Taxon** | **Relative percentage abundance (± SE)** |
|---|---|
| Honey bees, *Apis mellifera* | 10.3 ± 2.0 |
| Bumble bees, *Bombus* spp. | 9.9 ± 2.4 |
| Squash bees, *Peoponapis pruinosa* | 9.3 ± 1.8 |
| Butterflies and moths, Lepidoptera | 4.1 ± 1.6 |
| Beetles, Coleoptera | 21.5 ± 4.2 |
| Bugs, Hemiptera | 7.7 ± 1.9 |
| Sweat bees, Halictidae | 40.0 ± 4.5 |
| Flies, Diptera | 1.1 ± 0.5 |
| Carpenter bees, *Xylocopa virginica* | 0.04 ± 0.04 |
| Hunting wasps, Vespidae | 0.08 ± 0.08 |

# Results

## Sunflower competition

The sunflowers began flowering throughout the farm during Week 2, creating an acute condition of pollinator competition with the watermelons. Large numbers of bees from numerous taxa were observed visiting the sunflowers for their pollen and nectar.

## Insect and flower parameters

In watermelon during Weeks 2 and 3, there were no differences between weeks ($F$=0.34; df=1,1; $P$=0.6659) or treatments ($F$=0.01; df=1,1; $P$=0.9443) for sum of flowers available to bees. Also, there were no differences between weeks ($F$=0.16; df=1,1; $P$=0.7578) or treatments ($F$=0.01; df=1,1; $P$=0.9420) for percentage of female flowers. The relative abundance of insect visitors to watermelon flowers is given in Table 1. Of the bees, arguably the most relevant taxon, the numeric ranking was (highest to lowest): sweat bees, honey bees, bumble bees, squash bees, and carpenter bees. The disproportionately high number of sweat bees (Halictidae) raises the possibility of further research on the usefulness of this group as watermelon pollinators. The proportion of insect visitors comprised of honey bees decreased from 12.6 ± 2.5 % (mean ± SE, $n$=6) in Week 2 to 8.1 ± 3.2 % in Week 3 ($F$=134.8; df=1,1; $P$=0.0547). Similarly, the proportion of insect visitors comprised of Lepidoptera decreased from 8.1 ± 2.3 % in Week 2 to 0.2 ± 0.2 % in Week 3 ($F$=236.8; df=1,1; $P$=0.0413).

***Table 2.*** Number of honey bee flower visitors counted in experimental watermelon plots for 35 minutes; for each mean $n$=18. Nearby sunflower began blooming Week 2. Different letters indicate means significantly different at α≤0.05.

| **Week** | **Number of honey bees (± SE)** |
|---|---|
| 1 | 81.7 ± 7.0 a |
| 2 | 33.4 ± 4.8 b |
| 3 | 6.2 ± 1.9 c |

There was a numeric increase in total number of honey bee flower visitors in plots treated with Fruit Boost (42.9 ± 8.4, $n$=27) compared to controls (37.9 ± 6.1, $n$=27); however, this effect was not statistically significant ($F$=0.74; df=1,2; $P$=0.4812). There was a significant and stepwise decrease in number of honey bee visitors from Week 1 to Week 3 ($F$=55.76; df=2,2; $P$=0.0176, Table 2). When we examined the proportion of honey bee visits that occurred on female flowers (potentially effecting fruit-set), there was no significant difference between Fruit Boost (6.3 ±1.2 %, $n$=126) and control fields (6.3 ± 0.9 %, $n$=129) ($F$=0.20; df=1,2; $P$=0.6973), nor were there differences between weeks ($F$=3.09; df=2,2; $P$=0.2444).

## Harvest parameters

On 19 August, each control plot had an average (± SE) of 185.7 ± 11.5 developing or mature triploid melons; each Fruit Boost plot had an average of 121.3 ± 34.2 triploid melons. By pooling data for Weeks 1 and 3, we found a numeric increase in percent fruit set in Fruit Boost plots (11.4 ± 2.6 %, $n$=6) compared to controls (9.3 ± 3.0 %, $n$=6); however, this effect was not significant ($F$=0.28; df=1,10; $P$=0.6069). Average weight per triploid melon was unaffected by treatment ($F$=54.50; df=1,1; $P$=0.0857) and ranged from 0.2-11.6 kg; however, triploid melons resulting from flowers tagged in Week 1 (5.3 ± 0.2 kg, $n$=78) were significantly ($F$=334.31; df=1,1; $P$=0.0348) heavier than those from Week 3 (2.6 ± 0.7 kg, $n$=4).

# Discussion

Our work did not demonstrate a consistent benefit of Fruit Boost honey bee attractant in promoting pollination of the seedless watermelon 'Sugar Heart' under conditions of pollinator competition with sunflower. There is weak (non-significant) evidence that Fruit Boost increased total number of honey bee flower visitors, but there was no difference between Fruit Boost and control plots (6.8 vs 6.8 %) in the proportion of honey bee visits to female flowers. There were no differences in the proportion of female flowers available to the bees, whether sorted by treatment or week; hence plot effects were sufficiently random. Such non-differences at the level of bee visitation translated into non-differences in harvest parameters. There was a numeric, but non-significant increase in fruit set in Fruit Boost plots, but no differences in average fruit weight. The number of harvestable melons was numerically higher in control plots.

The impact of the pollinator competition condition which serendipitously commenced in Week 2 was measurable at different levels. There was a sequential drop in honey bee numbers in watermelon between Weeks 1-3, a drop in the proportion of insect visitors comprised of honey bees between Weeks 2 and 3, a near absence of fruit from flowers blooming after Week 1 (Week 1 $n$=78 *vs.* Week 3 $n$=4), and a significant decrease in melon size between Weeks 1 and 3. The fact that there were no differences in either the number of flowers available to bees or the percentage of female flowers between Weeks 2 and 3 suggests that fruit loss was not a physiological effect. It appears that under these conditions of pollinator competition, Fruit Boost was unable to restore adequate numbers of honey bees to watermelon. It is noteworthy that the proportions of another nectarivorous taxon, the Lepidoptera, similarly declined in watermelon between weeks 2 and 3.

In summary, it appears that Fruit Boost did not sufficiently increase honey bee flower visitation in seedless watermelon to improve pollination performance over that in control plots. The experiment was conducted under conditions of acute pollinator competition, a situation that ordinarily justifies the use of bee attractants. More broadly, this study suggests that pollination is compromised in agro-ecosystems where crops or feral plants are competing for limited pollinators.

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