

Effect of Synthetic Queen Mandibular Pheromone on Pollination of Cotton by Honey Bees, *Apis mellifera*

M. Keshlaf, R. Mensah, O. Nicetic, R. Spooner-Hart

Abstract—The effectiveness of a commercial bee attractant, synthetic honey bee queen mandibular pheromone (Fruit Boost®) for enhancing pollination of *Gossypium hirsutum* was evaluated in a transgenic (Bt) cotton crop. The study assessed the number of bee visitations to blossoms of plants treated with Fruit Boost® as well, as effects on fruit set, yield, and lint quality. Bee activity on plots sprayed with pheromone concentrations of 50 and 500 queen equivalents (QEQ) /ha did not differ significantly from water-only control, on the day of application or the subsequent day. Application of the pheromone did not increase fruit set, yield, or lint quality. Two consecutive pheromone applications, applied two days apart, were not significantly different from a single application for any parameter.

Keywords—*Apis mellifera*, cotton, pollination, QMP pheromone.

I. INTRODUCTION

INSECT pollination is required to achieve higher yields and superior fruit quality in many economically important crops. Although cotton, *Gossypium hirsutum* L., is primarily self-pollinated, cotton flowers visited by the European honey bee, *Apis mellifera* L., have been reported to produce heavier bolls [1]-[4] with improved lint quality [6] and seed-oil content [5]. However, higher honey bee densities than for many other crops are required to achieve optimal production in cotton [1], [7], [8]. This is probably because its flowers are unattractive to honey bees, because of competition from blooms of more attractive plant species nearby, or due to unfavourable weather conditions during the pollination period [3], [7], [9].

A number of chemicals, including sugar syrup [10], citral, geraniol, and anise oil [11]-[13], Bee Lure® [14], Pollenaid-D® [13], and Beeline [13], [15]-[21] have been applied to flowering crops to increase their attractiveness to honey bees and as a result to increase pollination, but with generally disappointing results.

The discovery of a number of honey bee pheromones and the ability to synthesize them has led to their evaluation as honey bee attractants. Several commercial products based on worker bee nasov gland pheromone (e.g. Bee-Scent®,

BeeHere™) have been reported to increase honey bee foraging and/or fruit set in some horticultural crops [22]-[26] but not in others [18], [20], [27]-[29].

Queen mandibular pheromone, produced from the mandibular gland of mated queen bees, is a five-component blend which is highly attractive to worker honey bees at extremely low concentrations [30]. Its commercial product, Fruit Boost®, has been reported to increase bee activity, yield and/or quality of many crops [31]-[34]. Here we report on field evaluation of Fruit Boost® to attract foraging bees to cotton flowers and on subsequent boll (fruit) set, yield and lint quality.

II. MATERIALS AND METHODS

The investigations were conducted on a commercial farm at Narrabri, NSW Australia, (30.30° S, 149.8° E) in a 9 ha field planted with transgenic Bt (Bollgard®II) cotton, in full bloom. A small apiary of six strong, managed honey bee colonies in two-deck, eight-frame Langstroth hives was introduced two days prior to the experiment and placed adjacent to the crop. Nine, ~0.1 ha plots (43×24m) were established in the field in a completely randomised block design with a series of three plots at each of the distances 100, 200 and 300m from the apiary. The three plots in each line were separated from each other by a 60 m buffer.

The treatments were two concentrations (50 and 500 queen equivalents [QEQ]/ha) of synthetic queen mandibular pheromone, Fruit Boost® (Contech Inc., Victoria, BC), and a water-only control. One QEQ is equivalent to the amount of pheromone in an average pair of queen mandibular glands [30]. The treatments were applied in water by a tractor-mounted ground rig sprayer at rate of 500 L/ha, between 06.00 and 09.00 h. The same treatments were applied two days later, using the same methodology.

Subsequent bee foraging activity was determined by selecting four rows, 43 m long in the middle of each treatment plot for data measurements, and the row data were averaged to give a value for the plot. Visual counts of the number of honeybees visiting cotton flowers were carried out at 12.00 h and 14.00 h (to coincide with maximum bee activity) on the day of spray application and the following day, by counting the number of honey bees on cotton flowers [7]. Thus, four assessments were made for each treatment on the day and day after the first application and the day and day after the second application. Bee activity was measured by recording the number of bees visiting flowers during an observation while

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walking down the row. Pollinating bees were defined as those which visited and entered flowers (infloral bees) and therefore were likely to contact pollen. Each floral bee visit was scored as a pollinating bee, and results were expressed as mean number of pollinating honey bees per 100 flowers. In the meantime, any other potential pollinator present in flowers was recorded.

A. Yield Measurements

Boll set and cotton yield were determined by randomly selecting flowers from the middle row of each plot. A total of 50 flowers were tagged, on the day of application and the day following application, for each of the two sprays, in all treatments. Bolls were hand harvested for each plot at maturity (approximately 75 days after flowering) and were retained separately according to their replicate plot, their treatment and the date their flowers were tagged. They were placed into paper bags, then debarred, ginned and evaluated for quantity parameters. After ginning, the lint from each plot was mixed by hand to gain a representative sample. Samples (30 g) of cotton lint were then taken from the mixed lint, on the basis of one sample from each of the plots. Each sample was subjected to four standard cotton lint quality measurements: length, uniformity, strength and micronaire [35].

B. Meteorological Conditions during the Investigation Period

As temperature is one the major climatic factors influencing honey bee activity and/or cotton plant physiology, these data were collected at the field site from 31 January to 5 February 2006 using a data logger (Tinytag® -Hastings, Port Macquarie, NSW), logging at 30 min intervals.

C. Statistical Analysis

Data for mean bee floral visitation, yield quantity and quality were compared between treatments using mixed model analysis of variance (ANOVA) SPSS® for Windows™ Version 14 [36], with two fixed factors: treatment (QMP) and time of assessment, and a random factor, block. Prior to analysis, each variable was visually tested for normality using P-P plot and Levene's test was used to test the assumption of equality of error variance [37]. When the assumption of homogeneity of variance was met Ryan's Q test was used to separate treatment means if data met the assumption of equality of variance, and Dunnett's T3 test was used if assumption of equality of variance was not met after appropriate transformation of data. The relationship between the number of bees visiting flowers and the number of bolls set was explored using Pearson correlation [38]. In all cases, significance was accepted at the 0.05 level.

III. RESULTS AND DISCUSSION

The application of QMP at either 50 QEQ or 500 QEQ/ha did not significantly ($F_{2,12} = 0.484$; $p = 0.628$) affect honey bee visitation of cotton flowers compared to the water only control. The mean flower visitation rate to treated plots, combining the two time applications, was 0.57, 0.49, and 0.45

bees/100 flowers on 50 QEQ, 500 QEQ and control plots, respectively (Table I).

TABLE I
EFFECT OF TWO APPLICATIONS OF DIFFERENT DOSES OF FRUIT BOOST® ON THE FORAGING ACTIVITY OF HONEY BEE (MEAN NUMBER OF BEES PER 100 FLOWERS RECORDED, AT 12.00 AND 14.00 H, N=3) ON COTTON FLOWERS. ONLY DAY OF FIRST APPLICATION DIFFERED SIGNIFICANTLY THAN OTHER DAYS ($P = 0.05$). FRUIT BOOST® DID NOT INCREASE BEE VISIT TO COTTON FLOWERS. DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES

Measurement	Treatment			Mean \pm s.e. (across all plots)
	50 QEQ	500 QEQ	Control	
Application 1				
Day 1	0.85	0.91	1.05	0.92 \pm 0.08 ^a
Day 2	0.82	0.20	0.20	0.53 \pm 0.15 ^b
Application 1				
Day 1	0.5	0.42	0.42	0.42 \pm 0.07 ^b
Day 2	0.10	0.23	0.42	0.16 \pm 0.07 ^b
Mean \pm s.e.	0.57 \pm 0.12 ^a	0.49 \pm 0.13 ^a	0.45 \pm 0.13 ^a	

The mean flower visitation rate in the treated plots for both applications was significantly higher ($F_{3,12} = 9.382$; $p = 0.002$) on the day of application (0.92 and 0.42 bees/100 flowers) than the day after application (0.53 and 0.16 bees/100 flowers, respectively) (Table I). There was no interaction between treatments and date of application ($F_{6,12} = 0.171$; $p = 0.383$); hence, there were no significant differences in mean total or daily bee activity ($F_{2,12} = 0.484$; $p = 0.628$) between any of the QMP or control treatments.

The total number of cotton flowers observed in the experiment was 22,052 and a total of 109 bees were recorded visiting these flowers. This is a visitation rate of 0.49 bees per 100 flowers. The number of bolls set (and % boll set) from 300 tagged flowers following the first spray application, was 107 (35.6%), 155 (51.6%), and 166 (55.3%) in the 50 QEQ, 500 QEQ and control plots, respectively, with a mean boll set across all treatments of 47.5% (Table II). Flowers exposed to the second application had lower boll set of 76 (25.3%), 78 (26%), and 67 (22.3%) in the 50 QEQ, 500 QEQ and control plots, respectively, with mean fruit set across all treatments of 24.5% (Table II).

TABLE II
EFFECT OF TWO APPLICATIONS OF DIFFERENT DOSES OF FRUIT BOOST® ON THE INFLORAL HONEY BEE VISIT (POLLINATING BEES PER 100 FLOWERS RECORDED, AT 12.00 AND 14.00 H ON DAY OF APPLICATION AND THE FOLLOWING DAY, N=3) ON THE BOLL SET OF 300 TAGGED FLOWERS PER APPLICATION (%). THERE WAS NO SIGNIFICANT INFLUENCE BY FRUIT BOOST® ON BEE VISITATION RATE OR BOLL SET. THERE WAS NO CORRELATION BETWEEN BEE VISITATION AND BOLL SET. ($P = 0.05$)

Measurement	Treatment			Average*
	50 QEQ	500 QEQ	Control	
Bee visits				
Application 1	0.24	0.07	0.18	0.17
Application 2	0.08	0.08	0.07	0.08
Boll set				
Application 1	107 (35.6%)	155 (51.6%)	166 (55.3%)	(142.6) 47.5%
Application 2	76 (25.3%)	78 (26.0%)	67 (22.3%)	(73.6) 24.5%

*Mean for all treatments at the same application time

Total percentage boll set, based on assessments of 600 tagged flowers per treatment, was 30.5%, 38.8% and 38.8% in the 50 QEQ, 500 QEQ and control treatments, respectively. No significant differences were found between treatments in relation to bee visitation rate ($F_{2,12} = 0.484$; $p = 0.628$), or boll set ($F_{2,6} = 0.585$; $p = 0.586$). There was also no significant relationship between bee visitation rate and number of bolls set ($r = 0.306$; $p = 0.217$).

There were no significant differences between any other parameters measured, including total mass of bolls ($F_{2,6} = 0.518$; $p = 0.620$), mean boll weight ($F_{2,6} = 0.656$; $p = 0.553$), number of seeds per sample ($F_{2,6} = 0.481$; $p = 0.640$), mean weight of 100 seeds ($F_{2,6} = 0.501$; $p = 0.629$), number of seeds per boll ($F_{2,6} = 0.069$; $p = 0.934$), mass of lint ($F_{2,6} = 0.544$; $p = 0.607$), and weight of lint per boll ($F_{2,6} = 0.403$; $p = 0.685$) (Table 3). Furthermore, a comparison of lint quality data showed that there were no significant differences between treatments with respect to lint length ($F_{2,6} = 0.173$; $p = 0.845$), uniformity ($F_{2,6} = 1.33$; $p = 0.330$), strength ($F_{2,6} = 1.31$; $p = 0.336$) and micronaire ($F_{2,6} = 0.838$; $p = 0.478$) (Table III).

TABLE III

EFFECT OF TWO APPLICATIONS OF DIFFERENT DOSES OF FRUIT BOOST[®] ON COTTON YIELD AND LINT QUALITY. ALL DATA ARE MEANS \pm S.E. THERE WERE NO SIGNIFICANT DIFFERENCES ($p = 0.05$) BETWEEN TREATMENTS FOR ALL QUANTITY AND QUALITY PARAMETERS

Measurement	Treatment		
	50 QEQ	500 QEQ	Control
Mass of bolls (g) / plot	327 \pm 62	432 \pm 108	399 \pm 36
Boll weight (g)	5.3 \pm 0.1	5.4 \pm 0.2	5.1 \pm 0.2
Weight of 100 seed (g) /plot	11.3 \pm 0.2	11.5 \pm 0.3	10.9 \pm 0.3
Number of seeds / boll	28.4 \pm 1.1	28.5 \pm 0.9	27.9 \pm 1.2
Weight of lint (g) / boll	2.13 \pm 0.07	2.18 \pm 0.08	2.07 \pm 0.10
Length (mm)	1.18 \pm 0.01	1.17 \pm 0.03	1.6 \pm 0.00
Uniformity (%)	83.2 \pm 0.15	85.4 \pm 0.7	84.2 \pm 1.4
Strength (g / tex)	28.8 \pm 0.3	28.9 \pm 1.0	27.5 \pm 0.1
Micronaire	4.90 \pm 0.15	5.10 \pm 0.11	4.93 \pm 0.06

The temperature exceeded 40°C on all days of the investigation period (Fig. 1), but reached this temperature by as early as late morning on 2 and 4 February 2006.

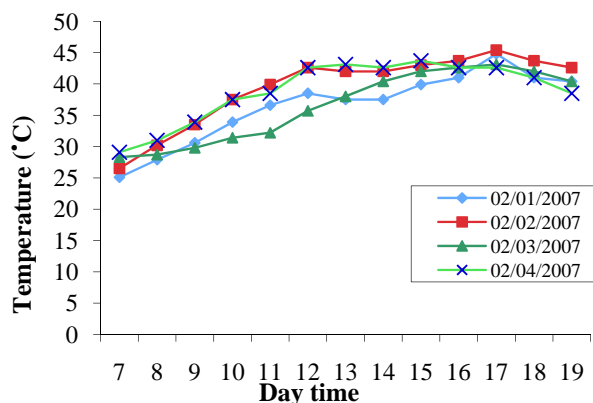


Fig. 1 Diurnal temperatures (°C) from 7.00 to 19.00 h, at the study site during the 4 d experimental period, February 2006

IV. DISCUSSION

The study showed that the bee attractant (Fruit Boost[®]) tested in this study did not attract honey bee foragers to cotton flowers, either on the day of its application or the subsequent day. As a result, there was no increase in fruit set, yield, or lint quality compared with the water-only control, nor for any other parameter associated with successful cross-pollination.

Fruit Boost[®] has been used successfully in pollination as a management tool to stimulate bee foraging behavior [39], and to increase fruit set, yield and fruit size in a range of horticultural crops [31]-[34], [40]. However, there are no published data on its use in field crops, except that of Shashidar and Manjunath, who reported increased attraction of *Apis dorsata* F., *Apis cerana* F. and *A. mellifera* to treated sunflower inflorescences with an associated 25% increase in yield [41].

Observations of insect visitation to cotton flowers in the current study showed that *A. mellifera* were the only pollinating visitors to flowers. However, the majority of honey bee foragers (77.7%) preferred to collect nectar from outside the flowers rather than foraging within flowers and should, therefore, be regarded as non-pollinating bees. This behavior in cotton has also been reported by El-Sarrag et al. [5]. The overall bee floral visitation level was 0.49%; but the "pollinating bee" visitation rate did not exceed 0.11 bees per 100 flowers, which is much lower than the reported minimum level for effective pollination of cotton (viz., 0.5%) [42]. This may, in part, explain the low percentage of bolls set (Table II). Even if QMP were able to attract honey bees to cotton crops, this observed foraging behaviour may still not result in higher in-floral visitation.

The only other invertebrate associated with the cotton flowers during the investigation period was the pollen beetle, *Carpophilus aterrimus* Macleay, but there is no published information about its possible role in cotton pollination [4].

It is possible that the lack of honey bee attraction to cotton treated with QMP recorded in our investigation may be a result of unsuitable doses being applied (viz., 50 and 500 QEQ). Doses of QMP required to optimize bee flower

foraging activity seem to be crop dependent. Reports of successful use of QMP in horticultural crops appear to be for doses between 100 and 1000 QEQ/ha [31], [32]. However, in some other crops, similar rates have been ineffective; e.g., kiwi fruit at 100 QEQ [43], sweet cherry at 100 and 500 QEQ [34], cranberry and blueberry at 1000 QEQ [32], blueberry at 500 QEQ and cranberry at 100 QEQ [39]. Furthermore, the application of QMP at 1000 QEQ has been reported to reduce honey bee activity in kiwifruit [43]. Further studies are required to determine whether there is an appropriate dose of QMP in cotton, or if it is unattractive at all doses.

Meteorological conditions during the investigation period may have also impacted on honey bee foraging activity, and the activity of the QMP. Numerous researchers have reported the effects of high temperature on honey bee pollinating activity [44], [45], flight activity and nectar gathering activity [46] and pollen gathering activity [47]. High diurnal temperatures ($> 40^{\circ}\text{C}$), during the four days following the first application of QMP were not conducive to bee activity. Even in the control plots, honey bee numbers decreased sharply on 2 and 4 February, probably as a result of high morning temperatures (42.6°C). Fig. 1 shows that, except for 1 February, temperatures increased gradually to reach 40°C at 11.00, and then were between 40°C to 45°C during 12.00 to 19.00 h. The high temperatures may have also affected performance of QMP in the field, as a result of volatilization of its components. There is a dearth of information on the effect of high temperatures on performance of QMP.

One explanation for the significantly lower bee visitation rate following the second application is the higher number of flowers at this time. QMP was applied on 1 February and repeated again on 3 February 2006, at peak flowering. Although more cotton flowers were present at the time of the second application, subsequent honey bee visitation was lower, and this was accompanied by a lower boll set than occurred after the first application. This could have been because, even if the number of bees in the field was constant, the increased number of flowers would have resulted in a lower calculated visitation rate (recorded as the number of bees/100 flowers).

Although QMP applied as Fruit Boost® did not attract honey bees to flowers of treated cotton plants, the results obtained in the study may not be adequate to conclude that QMP is ineffective as a pollination enhancement chemical in cotton. Nevertheless, based on the results reported here and given the normal weather conditions which prevail during cotton flowering in Australia, it appears that farmers may be better investing financial resources to rent additional colonies of honey bees for cotton pollination rather than investing in a commercial honey bee attractant.

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