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# An evaluation of mini-nucleus honeybee hives for pollination of honeydew melons in enclosures

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SUMMARY

Recent declines of honeybee populations create deficiencies in agricultural pollination, and motivate the search for alternatives to traditional honeybee colonies. Mini-nucleus colonies (Mininucs), small honeybee hives containing a few hundred workers, are easier and cheaper to set up, maintain and transport than regular-size colonies. We tested whether Mininucs can provide effective agricultural pollination in enclosures. We compared the efficiency of Mininucs vs. traditional Langstroth colonies in pollinating honeydew melons in tunnel net-houses. Flower visit frequencies were higher in nethouses with regular hives than in nethouses that contained Mininucs. Fruit mass and density were not affected by colony type, however. We suggest that Mininucs can effectively pollinate melons in enclosed spaces. Their ease of handling and non-aggressive behavior further increases their appeal for agricultural pollination.

KEYWORDS: **Apis mellifera / honeydew melon / mininucleus colony / pollination**

## INTRODUCTION

Pollinators appear to be in short supply in many agricultural systems (Allen-Wardell et al., 1998; Kevan, 1999; Kremen et al, 2002). Honeybees have become particularly vulnerable to environmental stresses (Kevan, 1999; Committee on the Status of Pollinators in North America, 2007). The use of wild or domesticated non-*Apis* bees for agricultural pollination may compensate for the decline in honeybee populations in several orchard and field crops (Kremen et al., 2004; Bosch & Kemp, 2002, 2005). The steady increases in vegetables growing under cover or in enclosures enhance the needs for pollinating insects indoors (Castilla, 2002; Cook & Calvin, 2005). While *Bombus terretris* efficiently pollinates some greenhouse crops, most non-*Apis* pollinators do not answer needs for crop pollination in enclosures. This motivates the search for additional solutions for agricultural pollination in greenhouses and nethouses.

In the present study we explore the possibility of using honeybees for crop pollination in enclosures, but to do so more economically than in current practice. Rather than exploiting regular honeybee colonies, we test the feasibility of setting up much smaller colonies for pollination purposes only. Potential advantages of this approach are: (a) It requires using only a small portion of the population of a regular hive to populate a small and independent pollination unit. This can reduce the cost and the risk involved in setting up regular-size hives, because the work force of a single large colony (20,000-40,000 workers) can be divided among several small pollination units. (b) The pollination efficiency of regular-sized honeybee colonies is low under certain conditions. This is especially true for crops under cover, where regular ten-frame Langstroth hives frequently suffer from disorientation and losses of foragers (e.g. McGregor, 1976; Free, 1993). Cucurbits grown under cover pose additional problems, since the number of flowers and reward quantity cannot support the needs of a regular hive with tens of thousands of bees. Consequently, only a tiny percentage of the hives' foraging force works within the enclosure (Dag & Eisikowitch, 1996). Colonies of several hundred bumblebees were shown to provide efficient pollination in melon greenhouses (Fisher, 1989), suggesting that a rather small number of honeybees may also suffice for melon pollination in enclosures. The use of small honeybee colonies in melon greenhouses may provide better matching between the foraging needs of the colony and the available food supply. (c) The technology for creating small honeybee colonies has already been developed for the purposes of queen rearing. Queen rearing involves mass production of mated queens for use or sale to other beekeepers. Small mating hives (Mininucs) are used to maintain young queens economically, requiring only a small number of workers, until the queens are proven and ready to be transferred to full colonies.

The aim of the present study was to compare the efficiency of Mininucs vs. regular-sized hives in pollinating honeydew melons in a nethouse. Our study follows earlier attempts to use small honeybee colonies for pollination in almond orchards, plant breeding and seed production in cages (Thorp et al., 1973, Erickson et al., 1975, Ellis et al., 1981, Cox et al., 1996).

MATERIALS AND METHODS

2.1 Mininuc colonies

The Mininuc is a top-bar hive made of high-density polystyrene. The sides slope inwards vertically to prevent the bees from sticking the combs to the hive wall. Entrances for foraging bees are situated at the front of the hive and underneath it. The hive contains four combs, separated by a division board to allow for a feeder. A polythene sheet is adhered across the top of the hive to prevent sticking of combs to the roof (Horr, 1999). For the purposes of the present study, we used four SBK mating hives (Bienen-Voigt & Warnholz Ltd., Ellerau, Germany, http://www.warnholz.de/). The colonies were populated by a queen and ca. 400 workers, and the queen was left in the colony for the whole duration of the study. All colonies contained four populated frames throughout the study.

2.2 Net-house pollination experiment

The experiment was conducted between May 11 and June 8, 2004 at Revadim in central Israel. Four L45.5×W9.0×H0.7 m tunnel-shaped net-houses of unwoven cloth were erected to cover existing rows of commercial honeydew melons within an open melon field. Two net-houses contained melons of cultivar 60402, while the other two net-houses contained an experimental, yet-unnamed, melon variety. Melons produce male and hermaphrodite flowers, but hermaphrodites do not self-pollinate spontaneously, creating a need for insect pollination (Orr, 1985, Delaplane & Mayer, 2000). We recorded the numbers of male and hermaphrodite melon flowers in plots of known area on three days during the course of the experiment, in each of the net-houses and in the open field. Net-houses 1-4 were sampled 9, 8, 12 and 7 times for melon flowers, respectively, while the open field was sampled 12 times. We calculated flower densities and sex ratios for each sampled location.

On May 13, we introduced two Mininuc colonies into a net-house containing the 60402 cultivar, and two other Mininucs into a net-house with the 'experimental' cultivar. On May 11, we introduced one regular ten-frame Langstroth beehive into each of the remaining net-houses (Fig. 1). The colonies were removed from the net-houses between June 4 and June 8. Each Mininuc contained ca. 400 workers, and each Langstroth hive contained ca. 25,000 workers. Buckets with water were introduced into the net-houses to allow the bees access to a water source. The bees received no sugar or pollen supplementation during the experiment. We estimated the percentage of open and sealed honey-storing cells, pollen-storing cells, open and sealed brood in the Mininuc colonies two weeks and four weeks after introduction. We recorded these data separately for each side of each frame in the hives. This procedure yielded 32 records of frame condition (4 frames × 2 sides × 4 colonies) on each observation. We weighed the Mininucs three times, at weekly intervals, during the course of the experiment. The Langstroth beehives were inspected qualitatively before introduction to ensure they met the pollination requirements. Their condition was checked again qualitatively at the end of pollination period. We recorded the number of bee visits to patches of known numbers of melon flowers (a patch contained on average 28.13±23.53 (SD) flowers) in observation periods of 3-5 minutes during peak bee activity. Mean temperature in the nethouses during our observation sessions was 22.5 C (range 13.5-32.5 C), and mean humidity was 70% (range 28%-83%). We conducted several such counts of visits in randomly selected parts of each net-house twice a week. This resulted in 29-34 counts in each of the net-houses. Based on these counts, we calculated the mean number of bee visits per flower per minute in each net-house. We sampled crop yield by counting and weighing all melons in three random $5\times0.6$ meter plots in each net-house, one week after pollinator removal.

The area of the open field that surrounded the net-houses was 5 hectares. It was pollinated by twenty regular-sized honeybee colonies (ca. 25,000 workers in each), following standard practice (McGregor, 1976, Delaplane & Mayer, 2000). We set up six control plots in the open field (Fig. 1), and recorded bee visitation rates (based on 41 five-minute counts) and crop yield in these plots, using the same protocol as in the enclosures. This control tested for the effects of confinement within the net-house on pollination performance.

Data of flower densities, visit frequencies, and melon weight were checked for normality and subsequently analyzed using parametric methods. Counts of fruit numbers were based on only three replicates for each combination of cultivar and hive type. We therefore used the Kruskal-Wallis test to compare their means. Repeated records of hive weight were compared using the Wilcoxon matched-pairs signed-ranks test, since the data are based on a small number of replicates (4 Mininucs). Repeated records of comb condition within the Mininucs were based on 32 paired data points, and were analyzed with paired t-tests.

## RESULTS

The Mininucs' weight did not change significantly during the experiment (Wilcoxon matched-pairs signed-ranks test, $n=4$, $P<0.38$, Fig. 2). The estimated open brood and pollen storage areas in the combs significantly increased during the experiment (paired t-tests, $t_{31}$=3.49, p=0.001 for open brood, $t_{31}$=2.29, p=0.03 for pollen). The cover percentage of sealed brood and open honey cells significantly decreased between the two sampling dates (paired t-tests, $t_{31}$=2.78, p=0.008 for sealed brood, $t_{31}$=5.25, p<0.001 for open honey). Storage area of sealed honey did not differ significantly between sampling dates ($t_{31}$=1.98, p=0.06; Fig. 3). The Langstroth beehives, on the other hand, deteriorated significantly during their stay in the net-house and had to be combined with other hives after the end of the experiment.

The overall mean density of melon flowers was 30.62±1.11 /m, and the mean sex ratio (proportion of male flowers) was 0.94±0.01. Both variables were significantly affected by sampling date (ANOVA, $F_{4, 88}$=11.40, p<0.001 for flower density, $F_{4, 88}$=11.40, p<0.001 for sex ratio) and by melon cultivar (ANOVA, $F_{1, 46}$=15.81, p<0.001 for flower density, $F_{1, 46}$=9.82, p=0.003 for sex ratio). However, flower density and sex ratio did not significantly vary between plots pollinated by Mininucs and regular colonies (ANOVA, $F_{1, 46}$=3.42, p=0.07 for density, $F_{1, 46}$=0.08, p=0.77 for sex ratio).

Flower visitation rates were significantly higher in net-houses pollinated by regular colonies than in net-houses containing Mininucs ($F_{1, 136}$=22.02, p<0.001). Flower visitation rates in net-houses resembled those recorded in the open field ($F_{1,123}$=0.92, p=0.34), and were not significantly affected by melon cultivar ($F_{1, 177}$=1.28, p=0.26) (Fig. 4).

Mean fruit weight in the 'experimental' cultivar was lower than in the '60402' cultivar (ANOVA, $F_{1, 546}$=217.47, p<0.001), and fruit number was higher (Mann-Whitney U-test, n1=n2=9, Z=-3.58, p<0.001, Fig. 5). We therefore tested for the effect of hive type on fruit set and weight in each cultivar separately. Fruit weight was significantly affected by hive type for both cultivars (ANOVA, $F_{2,218}=4.40$, $p=0.01$ for cultivar '60402', $F_{2,423}=13.14$, $p<0.0001$ for the 'experimental' cultivar). Tukey's post-hoc tests revealed that this effect was due to the lower weight of melons in the open field, as compared to both net-houses, in both cultivars. Fruit weight within the net-houses was not significantly affected by hive type ($F_{1,156}=1.88$, $p=0.17$ for cultivar '60402', $F_{1,263}=0.03$, $p=0.86$ for the 'experimental' cultivar). Fruit densities did not differ between net-houses and open field for both varieties (Kruskal-Wallis tests, $H_2=3.09$, $p=0.21$ for cultivar '60402', $H_2=5.60$, $p=0.06$ for 'experimental' cultivar).

## DISCUSSION

Our experiment shows that two Mininuc colonies (containing together fewer than 1000 workers) were as effective as a 25,000-worker regular hive in pollinating a commercial crop of honeydew melon. Although Mininuc workers visited fewer flowers per minute than workers of regular hives, there was no difference in fruit set and fruit mass produced by both types of hives (fruit quality was not assessed). The mean flower visitation rate in the Mininuc-pollinated net-houses was 0.1visits/minute. This visit rate is equivalent to dozens of visits during a flower's period of receptivity, far in excess of the estimated number of visits required to ensure good pollination (McGregor, 1976, Dag, Pers. Comm.).

Each regular hive in the net-houses pollinated an area of 0.0309 hectares, while the area per hive in the open field was 0.25 hectares. Despite the almost tenfold difference in bee density, we recorded similar worker activity levels and crop yields in both types of plots. This similarity suggests that bee density in the net-houses pollinated by regular hives may have been excessive relative to the number of available flowers, causing low foraging activity. Mininucs, on the other hand, have lower foraging needs and may have been limited to a lesser extent by the number of melon flowers in the net-house. Crops yields showed a tradeoff between density and weight – melons of the 'Experimental' cultivar were less dense, but had higher mass, than melons of the '60402' cultivar. However, yields were not affected by the type of pollinating unit. This finding suggests that additional factors, such as plant resources allocated for fruit development, limited fruit size and quantity in our experiment (McGregor, 1976).

Small units of honeybees (package bees) are routinely used by beekeepers in several countries to establish new colonies and replace winter losses (Anonymous, 2005). They are shipped in parcels of 2-3 pounds, and contain 3000-5000 bees per pound. Package bees (also named Disposable Pollination Units, or DPUs) were compared to regular colonies with a similar number of workers as pollinators in almond orchards. Bees from DPUs collected more nectar, but less pollen, than workers of regular colonies. DPU flight activity in the orchards was lower, but more uniform, than the activity of regular colonies, and was more strongly depressed by low temperatures (Thorpe et al., 1973, Erickson et al., 1975). These foraging patterns reduced the pollination efficiency of DPUs in almonds, but did not diminish the efficiency of Mininucs on melons in the present study. Possibly, the small number of flowers available for pollination in the enclosures, combined with the favorable weather conditions, allowed the Mininuc workers to provide good pollination service. To our knowledge, the only previous evaluation of pollination of greenhouse crops by package bees was performed on cucumbers (Krieg, 1987). We suggest that the use of

Mininucs or package bees may be extended to the commercial pollination of additional food crops in enclosures.

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RUNNING HEAD: Evaluating Mininucs for pollination

## FIGURE LEGENDS

Fig. 1: Arrangement of plots in the net-house experiment. One ten-frame Langstroth hives were placed in tunnels 1 and 2. Two Mininucs were placed in each of tunnels 3 and 4. The tunnels were erected in a 5-hectare field of honeydew melons. Twenty Langstroth hives were placed in the open field. Bee activity and yield were recorded in six control plots in the field. The northern half of the field was planted with the 60402 melon cultival, while the southern part was planted with an "experimental", yet-unnamed cultivar.

Fig. 2: Mean (±SE) mass of the Mininucs (n=4 colonies), weighed at weekly intervals during the course of the field experiment.

Fig. 3: Mean (±SE) percentage of the Mininuc combs cells occupied by honey, pollen and brood during (May 25) and immediately after (June 8) the field experiment. Data are based on visual inspection of both sides of four combs in each of the four Mininuc colonies in the experiment.

Fig. 4: Mean (±SE) rates of flower visits in the field experiment. Net-houses 1 and 2 and the open field plots were pollinated by regular-sized colonies, while net-houses 3 and 4 were pollinated by Mininucs. Flower visitation rates were sampled twice a week in randomly determined locations of the net-houses.

Fig. 5: Mean (±SE) weight (top) and fruit density (bottom) for the different pollination units in the field experiment. Data for the two melon cultivars ("experimental" and "60402") are shown separately. "Nucleus" and "Regular" refer to the type of pollination unit inside the net-houses, while "Open field" refers melons outside of the net-houses pollinated by regular honeybee hives.

Fig. 1

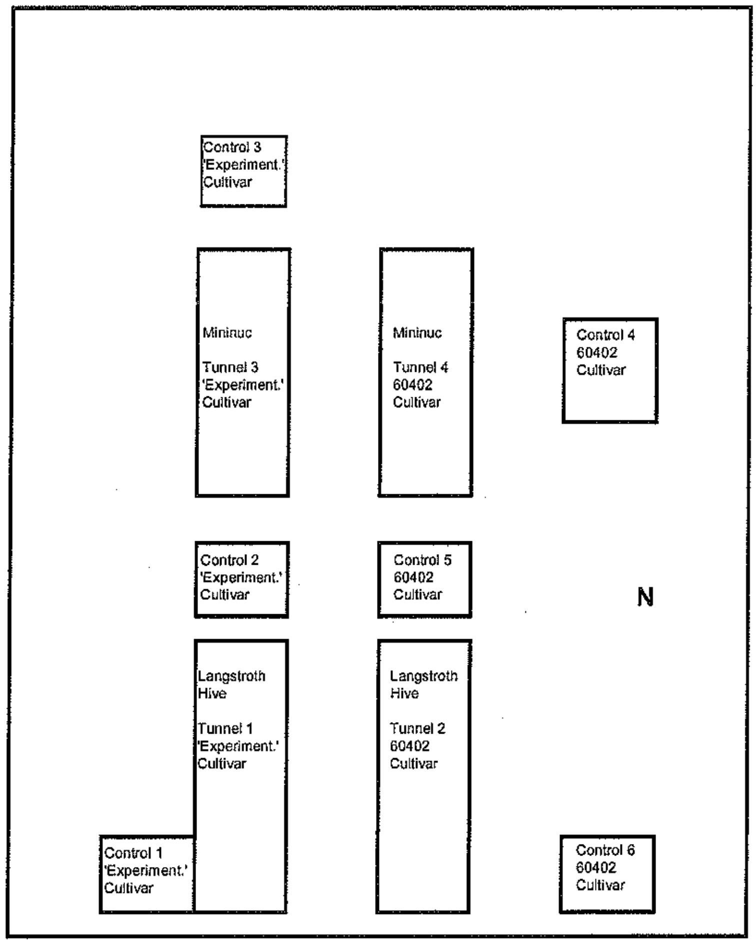

Control 3 'Experiment.' Cultivar
Mininuc
Tunnel 3 'Experiment.' Cultivar
Mininuc
Tunnel 4 60402 Cultivar
Control 4 60402 Cultivar
Control 2 'Experiment.' Cultivar
Control 5 60402 Cultivar
N
Langstroth Hive
Tunnel 1 'Experiment.' Cultivar
Langstroth Hive
Tunnel 2 60402 Cultivar
Control 1 'Experiment.' Cultivar
Control 6 60402 Cultivar

Fig. 2

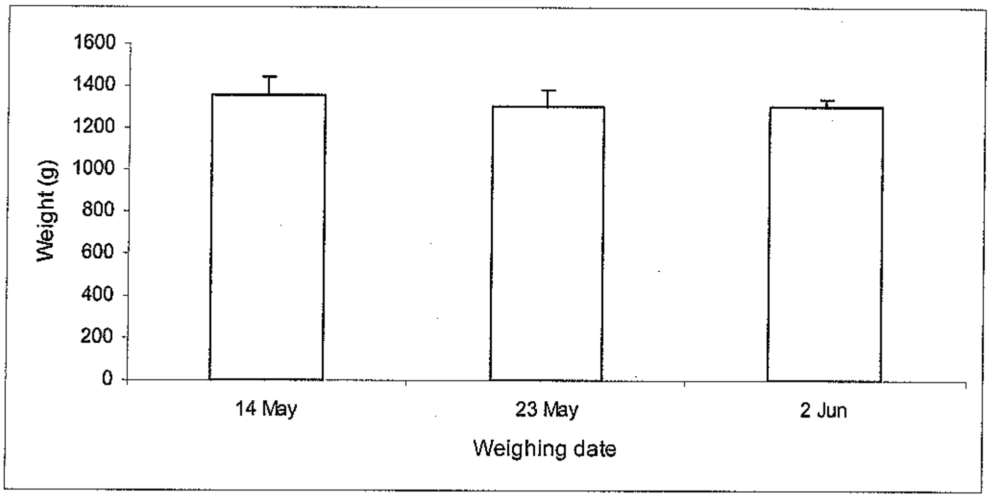
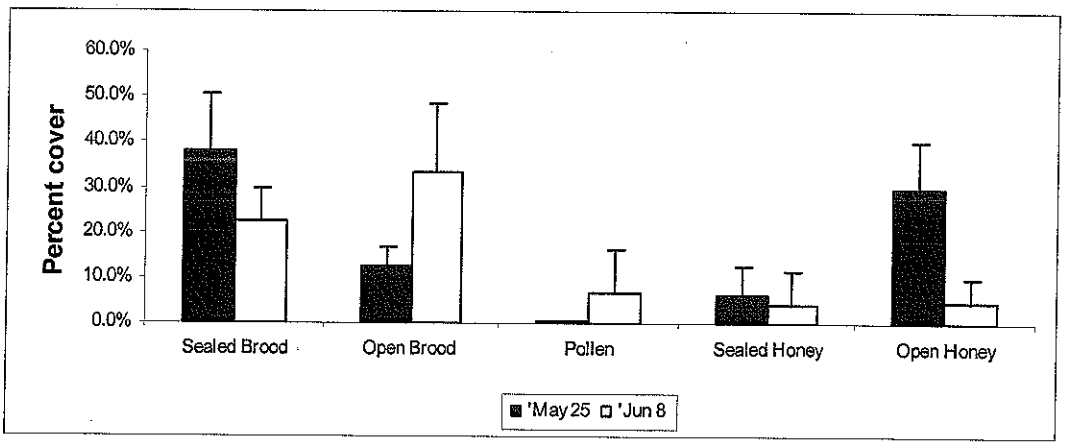
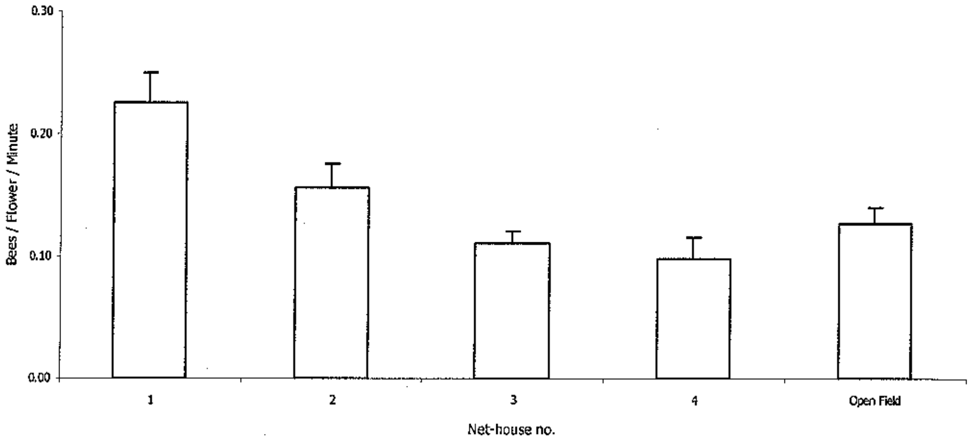

1600
1400
1200
1000
800
600
400
200
0
Weight (g)
14 May
23 May
2 Jun
Weighing date

Fig. 3



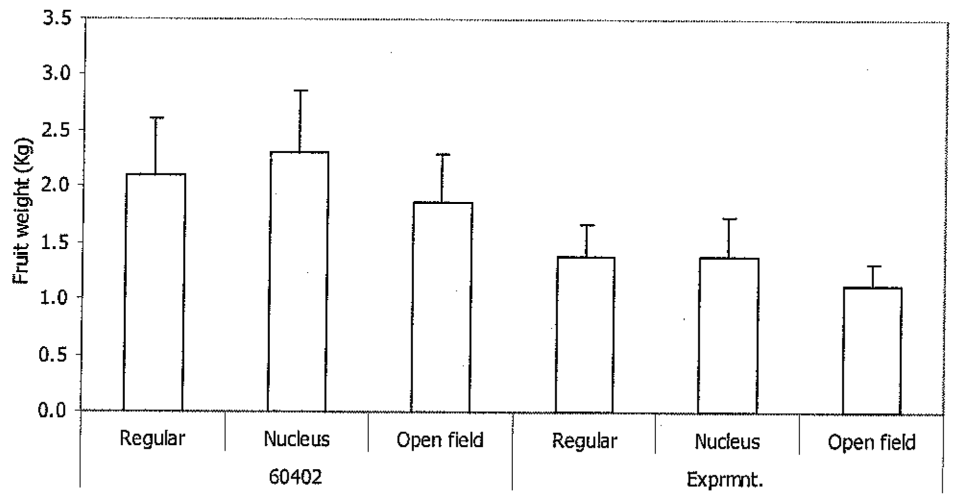
Percent cover
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Sealed Brood
Open Brood
Pollen
Sealed Honey
Open Honey
'May 25
'Jun 8

Fig. 4

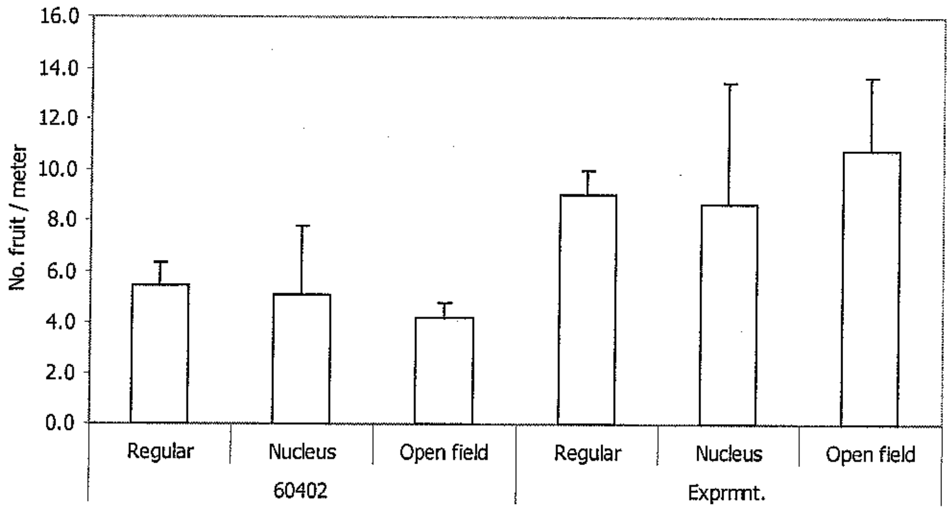


0.30
0.20
0.10
0.00
Bees / Flower / Minute
1
2
3
4
Open Field
Net-house no.

Fig. 5



3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Fruit weight (Kg)
Regular
Nucleus
Open field
Regular
Nucleus
Open field
60402
Exprmnt.

16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
No. fruit / meter
Regular
Nucleus
Open field
Regular
Nucleus
Open field
60402
Exprmnt.