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POLLINATION OF GREENHOUSE TOMATOES BY THE MEXICAN BUMBLEBEE *BOMBUS EPHIPPIATUS* (HYMENOPTERA: APIDAE)

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Abstract—The Mexican native bumblebee *Bombus ephippiatus* Say was evaluated as a potential pollinator of greenhouse tomatoes (*Solanum lycopersicon* L.). The experiments were performed at San Andrés Cholula, Puebla, Mexico, from June to December 2004 in two 1 000 m^2 greenhouses planted with tomatoes of the cultivar Mallory (Hazera ®). For the experiments, we used two colonies of *Bombus ephippiatus*, reared in the laboratory from queens captured in the field. Four treatments were applied to 20 study plants: pollination by bumble bees, manual pollination, pollination by mechanical vibration and no pollination (bagged flowers, no vibration). We measured percentage of flowers visited by bumble bees, number of seeds per fruit, maturing time, sugar content, fruit weight and fruit shape. All available flowers were visited by bumblebees, as measured by the degree of anther cone bruising. The number of seeds per fruit was higher for bumble bee-pollinated plants as compared with plants pollinated mechanically or not pollinated and was not significantly different between hand-pollinated and bumble bee-pollinated plants. Maturation time was significantly longer and sugar content, fresh weight and seed count were significantly higher for bumblebee pollinated flowers than for flowers pollinated manually or with no supplemental pollination, but did not differ with flowers pollinated mechanically.

Keywords: Bombus ephippiatus, Mexican bumble bees, tomato pollination,greenhouses

INTRODUCTION

Greenhouse tomatoes, *Solanum lycopersicon* L., require supplemental pollination for fruit set (McGregor 1976, Review by Picken 1984, Free 1993) and were usually pollinated by mechanical vibration (manual pollination), which was labour intensive and thus expensive. In Europe, laboratory- or mass-reared colonies of *Bombus terrestris* L. have been in tomato greenhouses since 1987 and have subsequently replaced manual pollination (Ravenstijn and Nederpel 1988, Ravenstijn 1989, Heemert et al. 1990). Pollination by *B. terrestris* resulted in significantly heavier fruit (Banda and Paxton 1991, Ravestijn and Sande 1991) when compared with manual pollination, although fruit were of similar weight in another study (Kevan et al. 1991).

In North America, Agriculture Canada, the United States Department of Agriculture, and the Mexican SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) restrict the importation of European bumble bee species. To provide for the greenhouse tomato market, Mexico has been importing *B. impatiens* from the United States and Canada since 1995. Between 2005 and 2009 more than 128 000 queens or small colonies of *B. impatiens* were imported into Mexico by two commercial companies that sell bumble bee colonies in the country (Campuzano-Hernández 2010). In an assessment report presented recently (Medina-Valdez 2010), importation of *B. impatiens* was regarded by the Mexican animal health authority as a potential risk to native Mexican bumble bees because, due to the possibility of accidental release of the species, it could become a competitor for pollen and nectar (Inari et al.2005, Ishii et al. 2008, Ings et al. 2006) and transmit diseases to the native species, as has already happened in some other countries (Otterstatter and Thomson 2008).

One of the strongest recommendations of the same report is "to promote the study and use of native bumble bee species as greenhouse pollinators". Research and production of *Bombus ephippiatus* Say colonies at the laboratory level has been carried out since 2001 and, more recently, at the commercial rearing scale by greenhouse tomato producers in west Mexico (Cuadriello pers. com.). Since *B. impatiens* and *B. ephippiatus* are very closely related (Cameron et al. 2007), there is the additional risk of interbreeding, which could have a negative impact on the number of *B. ephippiatus* queens (Goka 1998).

Bombus ephippiatus occurs naturally from Northwest Mexico to West Panama, and is more abundant in association with pine-oak or cloud forests, above 800 masl (Ayala 2009). The abundance, wide distribution of the species and the fact that that we observed it buzz-pollinating tomato and potato flowers cultivated in the open, makes it a promising candidate to replace imports of the non-native *B. impatiens.*

Because no information exists on the effectiveness of *B. ephippiatus* as a greenhouse tomato pollinator, our objective

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was to evaluate this Mexican bumblebee as a pollinator of greenhouse tomatoes.

MATERIALS AND METHODS

The experiments were conducted in two 1 000 m^2 greenhouses located in San Andrés Cholula, Puebla, Mexico. The greenhouses were rectangular (78 x 12.8 m), covered with plastic, with liquid-feed systems and ambient lighting. Daily temperatures were maintained between 20 and 25°C. Density of "Mallory"® (Hazera) was three plants per square meter. Trusses were pruned to 6-7 flowers per truss and plants were maintained according to standard commercial practices. Plants were 2 months old in late June at the start of the experiment. Fruit was harvested during November and December.

Twenty test plants were selected at random in each greenhouse. To minimize the effect of inter plant variation, trusses 3, 4, and 5 were used on each of the test plants in the first greenhouse, to apply the first three treatments. The mechanical pollination treatment was applied to the plants in the second greenhouse.

Pollination treatments

Four pollination treatments were applied to the 20 study plants (n = 20):

- Pollination by *B. ephippiatus*. Two colonies of *B. ephippiatus* were reared under controlled conditions (Gretenkord 1996) at the Laboratory of Entomology, Universidad de las Américas Puebla. The colonies were started from queens captured in the field at the Volcán de Colima, Jalisco, Mexico in January 2004 and were introduced to the first greenhouse on 24 June 2004, when they had between 70 and 90 workers. Because tomato flowers do not produce nectar (Free 1993), the colonies were supplied with syrup (sugar mixture according to Kammerer 1994) until September 8, 2004, when the colonies were returned to the laboratory. Bumble bee foraging activity was assessed on 27 June and 7 July at 1300 hours by counting incoming and exiting bumble bees. Intensity of foraging activity was 12 bees per 5 min per colony (mean of 2 colonies) of which 0.75 bees were incoming pollen foragers. At midday on 30 June, the average bumble bee colony population estimate was 85 workers (range, 70-90). This colony population estimate excludes foragers working on the crop. The majority of bumble bee foraging occurred between 1000 and 1500 hours. Although this level of pollen foraging could be regarded as low, it is comparable to the level recorded in a similar study (Dogterom *et al.* 1998). Additionally, visitation by bumble bees was measured by the degree of anther cone bruising (Morandin et al. 2001), and indicated that a 100% of the flowers displayed bruising levels higher than 2, which can be considered as efficiently pollinated. One truss per plant was left uncovered and no manipulation was done on it.

- Manual Pollination. Upon anthesis of the flowers of the truss picked for this treatment, the anther cone of each flower was cut open and pollen was transferred to the stigma of the same flower, by using a fine brush. Once pollen transfer was performed, the truss was covered with a 500-μ white Nytex ® bag to prevent visitation by bumblebees. Once fruit set was confirmed, the fruit was uncovered to minimize the effect of bagging. Manual pollination was completed between 1000 and 1200 hours 3 times per week.

- No supplemental pollination. The truss chosen for this treatment was covered with a 500-μ white Nytex ® bag before anthesis and uncovered after fruit set was confirmed.

- Mechanical pollination was performed by vibrating the training wire associated with the test plants by hitting it lightly with a wooden rod. This system of mechanical vibration is traditionally used by Mexican tomato growers.

The effects of pollination by *B. ephippiatus* were determined by measuring its impact on fruit ripening time, fresh and dry weight, sugar contents, fruit roundness, and seed count of the fruits produced by the test plants.

All tomatoes were harvested at the same ripeness, based on visual assessment of colour. The tomatoes were considered ripe when they had a uniform orange-red colour. Ripening time was calculated by counting the days between fruit set and harvest date.

Tomatoes were weighed fresh and then dried for 48 h in an electric oven at 60°C. Both weights were measured to 0.01 g using an electronic scale (Ohaus CT200, Pine Brook, NJ). Sugar percentage was measured by extracting a sample of juice using a new Terumo ® disposable 5 ml syringe for each fruit sampled, The percentage of sugars and other dissolved contents in the juice was measured to one decimal place using a hand-held 0.0 ~ 90.0% refractometer (ATAGO, HSR500, Itabashi, Tokyo, Japan). Seed count was performed manually by rehydrating dry fruits and separating the seeds from the flesh. A roundness index was calculated by measuring the maximum and minimum diameters of the fruit (Morandin et al. 2001)

Statistical analyses

The data were first analyzed by MANOVA (multivariate ANOVA, Statistics, StatSoft, 1999), with roundness, weight, sugars, number of seeds, minimum diameter, the difference in diameter between the minimum and maximum diameter, and days until ripe as the response variables. MANOVA was followed by univariate ANOVA and Tukey's pairwise comparisons.

RESULTS

Multivariate ANOVA of the four pollination treatments (n = 20) showed a difference among pollination treatments with respect to roundness, weight, sugars, number of seeds, and days until ripe (F = 0.239; df = 18,11854; P = 0.001).

Univariate ANOVA showed that there was a difference in tomato fresh weight (F = 168.29; df = 3, 4196; P = 0.0001), dry weight (F = 112.17; df = 3, 4196; P = 0.0001), ripening time (F = 160.67; df = 3, 4196; P = 0.0001), percentage of sugars (F = 472.80; df = 3,4196; P = 0.0001), and number of seeds (F = 1734.26; df = 3, 4196; P = 0.0001), with respect to pollination treatments (Table 1). There was no difference among pollination

TABLE 1. Comparison of five measures of tomato quality recorded from four pollination treatment groups.

Treatment (n=20)	Ripening Time (Days)	Fresh weight (g)	% sugars	Number of seeds/fruit	Roundness
Pollination by bumble	55.45 ± 18.7a	62.60±23.7a	4.97 ± 1.8a	201.00 ± 80.5a	0.81 ± 0.06a
Mechanical Pollination	49.76 ± 18.6a	60.84 ± 22.9a	4.93 ± 1.9a	159.32 ± 31.1b	0.81 ± 0.03a
Manual Pollination	49.71 ± 19.6 a	59.36±22.2b	5.79 ± 3.1b	153.28 ± 25.3b	0.80 ± 0.05a
No supplemental Pollination	46.50 ± 17.3b	57.72±21.5b	4.70 ± 1.7c	139.03 ± 60.1c	0.88 ± 0.07a

Average ± S. E. of the response variables. Means followed by the same letter in any given column are not significantly different from one another (Tukey's HSD, $P < 0.05$)

treatments with respect to tomato roundness ($F = 2.16$; df = 3, 4196; $P = 0.09$).

Flowers pollinated by *B. ephippiatus* produced larger fruit than manually pollinated flowers (Table 1) as evidenced by significant increases ($P < 0.001$) in fruit weight and seed count. Mean fruit weight for bumble bee pollination was 5.46 % higher than for mechanical pollination.

DISCUSSION

Our results indicate that *B. ephippiatus* is a commercially and practical alternative to the use of imported bumble bees for pollination of greenhouse tomatoes in Mexico.

Pollination of tomato flowers by *B. ephippiatus* provides a greater yield of tomatoes (variety Mallory) than does manual-pollination or no pollination under greenhouse conditions. Fruit weight, percentage of sugars, and seed count were higher for bumble bee pollinated flowers than for non-bumble bee-pollinated flowers (manual and no pollination), although fruit quality, as indicated by roundness indices, did not significantly differ between treatments. Larger fruit size resulting from bumble bee pollination of flowers was reported in other studies (Sande 1989; Banda and Paxton 1991; Ravestijn and Sande 1991; Kevan et al. 1991; Dogterom et al. 1998). Dogterom et al. (1998), Banda and Paxton (1991), and Ravestijn and Sande (1991) reported that bumble bees produced heavier fruit than when manual pollination was used, although Kevan et al. (1991) found no significant difference in a similar study. Our results also suggest that perhaps pollination quality is different when pollination is completed by bumble bees versus manual pollination. Manual pollination is conducted according to a schedule of 3 times per week, whereas bumble bees may visit flowers at an optimal time (for fertilization) and perhaps visit flowers more than once. Thus, although differences between regression equations are significantly different, these differences did not appear to constitute practical differences between treatments.

The effect of bagging plants was not addressed in this study. It is possible that bagging decreased the amount of light that reached the developing fruit, thus inhibiting fruit development. However, the length of time that the top part of each tomato plant remained inside the bag was kept to a minimum by continually moving the bags when flowers were set.

The level of flower bruising can be used to monitor flower visitation by bumble bees (Morandin et al. 2001) and could be used to indicate when additional bumble bee colonies are required in the greenhouse. In this study we monitored flower visitation by bumble bees and found that 100% of the flowers showed a level of bruising (higher than 1) that guaranteed the transferral of enough pollen grains to set fruit of commercial value, according to Morandin et al. (2001).

Seed count may be the most accurate method for determining levels of pollination because fruit weight, but not seed count (Picken 1984), is influenced by environmental conditions such as plant resources. Manual pollination significantly increased seed count over the no-pollination treatment. A further increase in seeds resulted from bumble bee pollination, indicating that bumble bees are better pollinators than the manual pollination technique.

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