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Efecto de la polinización de la fresa por *Apis mellifera* L. y *Chrysoperla carnea* S. sobre la calidad de los frutos
Effect of pollination of strawberry by *Apis mellifera* L. and *Chrysoperla carnea* S. on quality of the fruits

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Resumen

Se evaluó la contribución de *Apis mellifera* L. y *Chrysoperla carnea* S. como polinizadores del cultivo de la fresa, utilizando flores primarias de la variedad Festival en un experimento realizado en Irapuato, Guanajuato, México. Los tratamientos utilizados fueron: autogamia (SF), polinización abierta (OP), *Apis mellifera* (AM) y *Chrysoperla carnea* (CC). La tasa de polinización mostró que hubo diferencias significativas en el tratamiento AM (85.20 ± 2.41) en comparación con SF (41.51 ± 3.92), OP (77.98 ± 2.11) y CC (48.46 ± 2.97). Igualmente se encontraron diferencias significativas en el grado de malformación en AM (16.78 ± 1.20) en comparación con el SF (52.53 ± 1.54), OP (23.34 ± 1.03) y CC (47.88 ± 2.02). El total de antocianinas, peso, diámetro y sólidos totales solubles de los frutos fueron significativamente mayores en AM comparados con los tratamientos SF, OP y CC. El número de óvulos fertilizados se correlacionó positivamente con el peso de la fruta. Se considera a *Chrysoperla carnea* como deficiente polinizador, pero en contraparte se concluye que la utilización de *Apis mellifera* puede ser una alternativa para incrementar la producción y calidad de la fruta en los cultivos de la fresa establecidos en Irapuato, Guanajuato, México.

Palabras clave: Polinización, *Apis mellifera*, *Chrysoperla carnea*, fresa

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Abstract

The contribution of *Apis mellifera* L. and *Chrysoperla carnea* S. as strawberry pollinators were evaluated using primary flowers of the Festival cultivar from Irapuato, Guanajuato, Mexico. The treatments were: self-fertilizing (SF), open pollination (OP), *Apis mellifera* (AM) and *Chrysoperla carnea* (CC). The pollination rate per treatment showed that there were significant differences in AM (85.20 ± 2.41) compared with SF (41.51 ± 3.92), OP (77.98 ± 2.11) and CC (48.46 ± 2.97). There were significant differences for misshaped rate in AM (16.78 ± 1.20) compared with SF (52.53 ± 1.54), OP (23.34 ± 1.03) and CC (47.88 ± 2.02). The total anthocyanin, weight, diameter and total solid soluble of the fruits were significant higher in AM compared with SF, OP and CC treatments. The number of ovules fertilized was positively correlated with the weight of the fruit. *Chrysoperla carnea* is considered as poor pollinator, but the use of *Apis mellifera* could be an alternative to increment production and quality of fruit of strawberries cultivated in Irapuato, Gto., México.

Keywords: Pollination, *Apis mellifera*, *Chrysoperla carnea*, strawberry

Introduction

Strawberry flowers are hermaphrodite and self-fertile, have different fruiting potentials depending on number of stigmas per flower; primary flowers bear about 350 stigmas, secondary 260 and tertiary 180 (McGregor, 1976). The presence of auxin produced by achenes (fertilized ovules) is essential for the expansion of the receptacle during strawberry fruit development, on other hand receptacle zones containing non-fertilized ovules will not develop, originating a misshapen and small strawberry (Nitsch, 1950). The auxin concentration in the achenes of strawberry fruit may modulate its rate of ripening (Given *et al*., 1988) and also regulates changes in levels of transcripts related to content of sugars, organic acids and phenolic compounds important for strawberry maturation (Aharoni and O'Connell, 2002; Aaby *et al*., 2005), therefore a higher pollination rate may help to increment the fruit quality.

The most pollination rates are done by insects and by combined action of gravity, the wind rarely surpasses 60% (Pion *et al*., 1980). Several reports have compared the pollination effectiveness of insects in strawberry crops. Dimou *et al*. (2008) estimated the pollination effectiveness as an increment of the total fruit production. Lopez-Medina *et al*. (2006) determined how *Bombus terrestris* Cresson and wind can reduce the number of misshaped fruits. Paydas *et al*. (2000) showed the effect of bumble and honey bees on shape and some pomological characteristics of the fruits. Kakutani *et al*. (1993) compared the pollination effectiveness of honey bee and stingless bee as a function of the number of bee visits to a flower.

Honey bees (*Apis mellifera* L.) are recognized as the main pollinator for many crops (Selim and Keith, 2003; Sabara *et al*., 2004; Kasina *et al*., 2009; Mahmut and Yavaksuz, 2010). Chagnon *et al*. (1989; 1993) found a relationship between the number of fertilized ovules (pollination rates) and weight, also between the total length of visits for pollination and the increase of strawberry production pollinated by *A. mellifera*.

Albano *et al*. (2009) based their analysis on the pollination effectiveness of a flower of strawberry as a function of pollination rates. They indicated that the flowers visited by *A. mellifera* had 84% of effectiveness in contrast with unpollination flowers with only 53%. Kakutani *et al*. (1993) found that the rate of deformed strawberry pollinated by honey bees was lower than unpollination. Young-Duck *et al*. (2001) showed that *A. mellifera* has positive effects on weight and percentage of malformed fruits.

Even with the information available, where the benefits of honey bees as pollinators of strawberry are demonstrated, these insects are not used by growers from Irapuato, Guanajuato an important region of crops from Mexico. Research like this, developed for local climate conditions and strawberry varieties, can demonstrate the effectiveness of pollination on strawbwrry crops.

Lacewing (*Chrysoperla carnea* S.) is an efficient predators with a high prey consumption and excellent search capacity; lacewing is the most commonly used species in biological control programs from North America and Europe (Bond, 1980; Tauber *et al*., 2000). *C. carnea is* a natural and permanent component in the agroecosystem of strawberry crops, where it is possible to get free-pesticides production with appropriate strategies of releases in the field, also; it can be used for controlling aphids and others insect-pests with bland body (Rondon and Cantliffe, 2004; 2005). Lacewing has the advantage to be more tolerant to pesticides when it has been compared to honey bees considered highly sensitive (Shour and Crowder, 1980; Iannacone and Lamas, 2002). The adults of *C. carnea* are not only predaceous, also they feed on pollen and nectar of flowers (Hagen *et al*., 1970; Principi and Canard, 1984), whereas larvae, feed on floral nectaries when their prey populations decline (Limburg and Rosenheim, 2001); for these reasons, Green and Bell (2007) evaluated the pollination effectiveness in *Trifolium* between *C. carnea*, *A. mellifera* and *B. occidentals* to compare the seed weight. The pollination effectiveness in strawberry crops of *C. carnea* has not been documented, so in this research was proposed the possibility that adults of *C. carnea* could participate in the pollination of strawberry, with two ecological functions pollinators and predators.

In the present study, we evaluated the effect of pollination of strawberries by *A. mellifera* and *C. carnea* on the quality of strawberry fruit.

Methods

Area study and field work

The study was conducted at the División de Ciencias de la Vida (DCV), Guanajuato University from Irapuato, Guanajuato, Mexico located at 20°44'22" N and 101°20'10" W. The data was collected from June to August 2010 on strawberry cultivar variety Festival. During second week of February 2010, two hundred and fifty strawberry plants were planted at a distance of 25 cm from each other, in order to get cropping protection were made procedures of Integrated Pest

Management, the fertilization was 50 N + 40 K + 40 P. Sections to each treatment were designed. The treatments used in this study were self-fertilizing (SF), open pollination (OP), *Apis mellifera* (AM) and *Chrysoperla carnea* (CC). In the first section, seventy strawberry plants were placed in a pollination cage (4 x 3 x 3 m) covered with anti-aphid mesh and before blooming began one nucleus of beehives was introduced, the flowers visited by *A. mellifera* were marked with linen cloth red, a nucleus of beehives was obtained of apiary in DCV. In the second section, fifty strawberry plants were placed in a pollination cage (2 x 3 x 1.5 m) covered with anti-aphid mesh and before blooming began forty *C. carnea* adults were introduced; the density of lacewing was determined according to the releases made on commercial farmland, searching if the densities used for biological control have an effect on the pollination of the strawberry flowers; the flowers visited by *C. carnea* were marked with linen cloth black; *C. carnea* was obtained from the Laboratory of Entomology of DCV. In section where self-fertilizing was used, the flowers were isolated with a cotton bag (3 x 5 cm) before blooming began and opened when they had senesced to allow fruit developing (Cauich *et al*., 2006). The flowers that received unrestricted visits by mixed pollinators were marked with linen cloth blue. In this study only primary flowers were used (Albano *et al*., 2009).

Pollination rate

In thirty pieces of fruit from primary flowers (>300 achenes) for each treatment the pollinated and nonpollinated achenes were counted. The Pollination Rate (PR) was calculated as the proportion between numbers of fertilized achenes divided by the total number of achenes per strawberry according to Chagnon *et al*. (1989; 1993) and Albano *et al*. (2009).

Total anthocyanin, total soluble solids and pH determinations

Total anthocyanin was analyzed on twenty fruits for each treatment. According to Lopez da Silva *et al*. (2007), the anthocyanin was extracted with MeOH containing 0.1% HCl. Total anthocyanin content of the strawberry extracts was measured using the pH differential method (Cheng and Breen, 1991). Results are expressed in milligrams of pelargonidin 3-glucoside per 100 g of fresh weight (Zheng *et al*., 2007). Total soluble solids (TSS) were determined at 20 °C on an Atago® refractometer and the pH was measured with a pH meter Idipsa® on forty and thirty pieces of fruit for each treatment respectively.

Misshaped rate, weight, polar and equatorial diameters of fruits

Fruits were harvested when they turned red as is required for market. Fruits produced on twenty flowers selected at random in each treatment were collected and classified as well-shaped or misshaped. Misshaped rate was calculated as the proportion between number of misshaped fruits divided by all fruits harvested. There were three replicates for each treatment. The weight of fruit was recorded weighing fifty pieces of fruit produced on flowers randomly selected for each treatment also polar and equatorial diameters of each fruit was measured with a vernier.

Data analysis

For each treatment the data of pollination rate, weight, TSS, polar and equatorial diameters the analysis was made by non-parametric Kruskall-Wallis test due to non-normality data. Misshaped rate, pH and total anthocyanin of fruit produced per treatment were compared using one-way ANOVA (F parametric test). Significant differences among means were detected using Tukey test. To determine the relationships between the fruit weight and fertilized ovules Pearson's correlation and regression analysis were used for each treatment. The significance level of 5 % was applyed. SAS software (SAS, 1995) was used for all statistical analyses.

Results

Pollination rate

Numbers of fruit evaluated were 30 from bagged flowers, 30 from flowers with unrestricted visits by mixed pollinators, 30 from flowers visited by *A. mellifera* and 30 from flowers visited by *C. carnea*. PR differed significantly between all treatments ($P<0.0001$). AM showed a PR significantly higher than SF, OP and CC treatments (Fig. 1). SF showed a PR of 42 % significantly lower than others treatments. The PR of OP treatment was higher than SF and CC; the fruit achieved a PR of 78 %. The treatment with insects reveals significant differences between them. CC treatment was significantly lower than AM (Fig. 1); the strawberry achieved a PR of 48 and 86 % respectively.

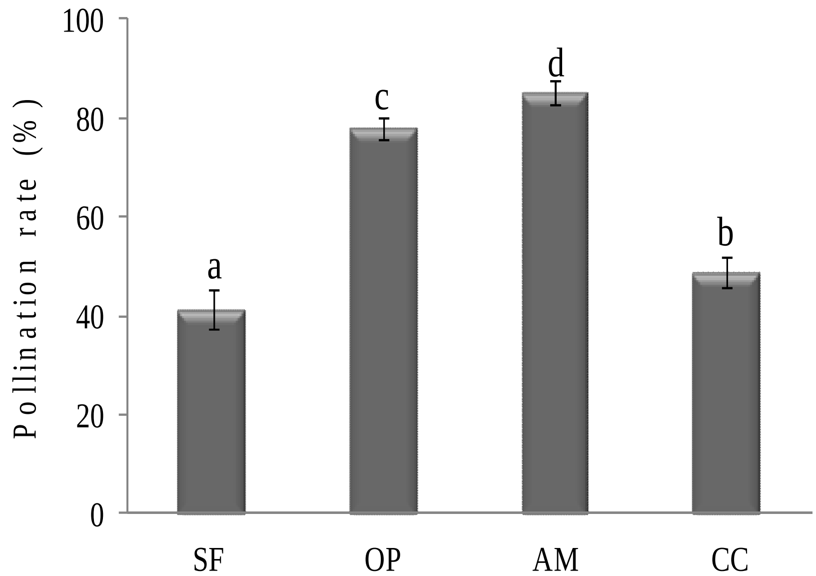

Figure 1. Comparison of pollination rate between four treatments.
(Bars with different letters indicate significant differences at $p<0.05$).

Total anthocyanin

Total anthocyanin differed between all treatments ($P<0.0001$). SF, OP, AM and CC obtained 17.26, 19.20, 22.21 and 17.93 mg/100g of total anthocyanin content respectively. The CC and SF treatment did not revel significant differences between them (Fig. 2). The total anthocyanin content obtained was significantly more in fruits pollinated by *A. mellifera* and mixed pollinators compared with fruits pollinated by *C. carnea* and self-fertilizing.

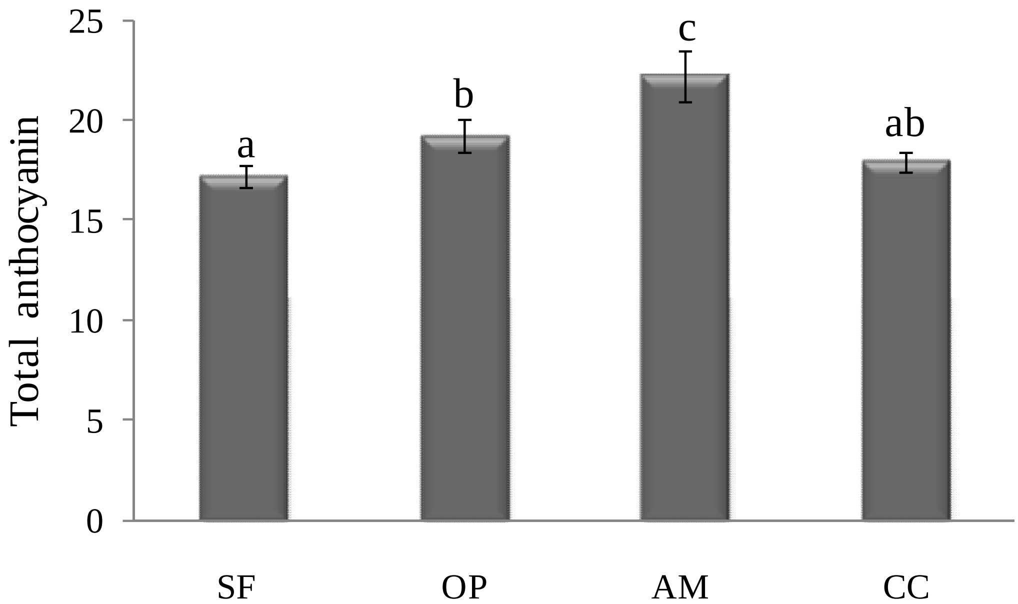

Figure 2. Total anthocyanin obtained from four treatments. Results are expressed in milligrams of pelargonidin 3-glucoside per 100 g of fresh weight. (Bars with different letters indicate significant differences at p<0.05).

Quality fruit

The number of fertilized ovules was positively correlated with the fruits weight, the regression analysis, showed for each case the correlation coefficient r, line of best fit and determination coefficient, AM treatment (r=0.97, y=0.0413x+4.8262 and R^2=0.94) showed a fruit weight higher than SF, OP and CC treatments. In contrast CC (r=0.95, y=0.0376x+5.0877 and R^2=0.91) obtained a fruit weight lower than OP (r=0.99, y=0.0414x+4.4924 and R^2=0.98) but similar than SF (r=0.96, y=0.0338x+4.8771 and R^2=0.98) (Fig. 3). There were statistical differences in the polar diameter (P<0.0001), equatorial diameter (P<0.0001) and TSS (P<0.0016) (Table 1). For pH (P<0.5176) all treatments did not revel significant differences. There were statistical differences for the misshaped rate (P<0.0000), the treatment with *A. mellifera* had significantly better values than the others (Fig. 4).

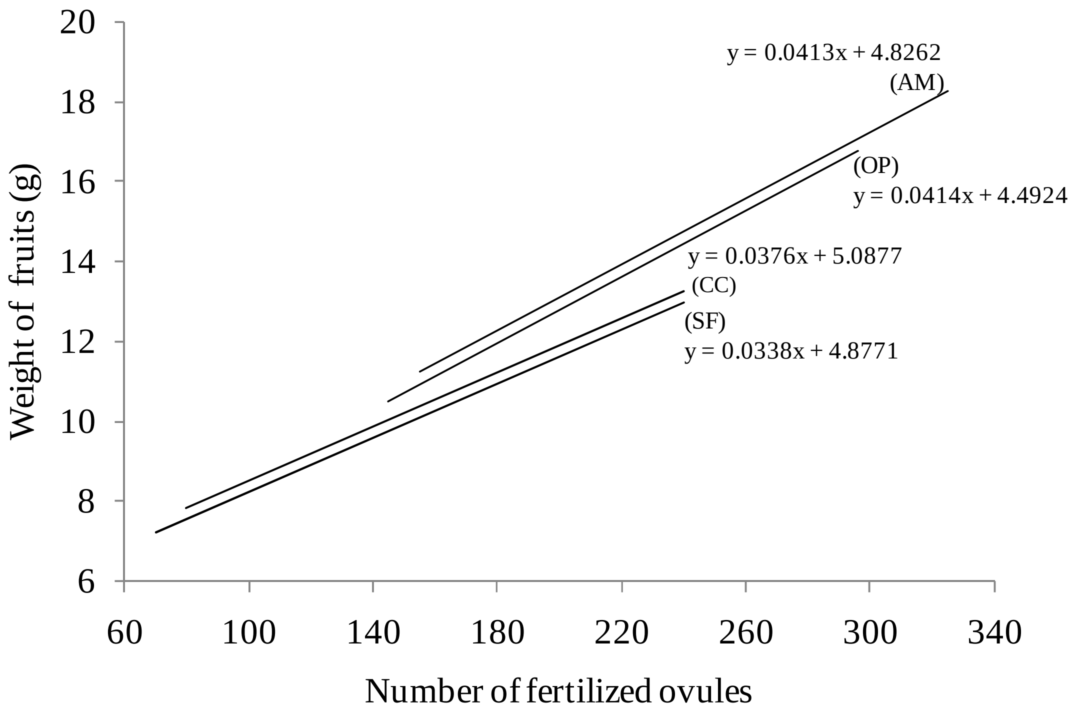

Figure 3. Regression of weigth as function of fertilized ovules for each treatment.

Table 1. Diameters, pH and TSS obtained using four pollination methods (means ± SE).

Treatment	Polar diameter (cm)	Equatorial diameter (cm)	pH	TSS
SF	2.53 ± 0.23 a	2.36 ± 0.29 a	3.54 ± 0.04 a	6.80 ± 0.78 a
OP	3.34 ± 0.23 c	3.06 ± 0.39 c	3.55 ± 0.08 a	7.85 ± 0.66 b
AM	3.60 ± 0.24 d	3.25 ± 0.27 d	3.49 ± 0.07 a	8.20 ± 0.91 b
CC	2.99 ± 0.20 b	2.70 ± 0.27 b	3.55 ± 0.03 a	7.10 ± 0.78 a

Different letters in the same column indicate significant differences at $p<0.05$.

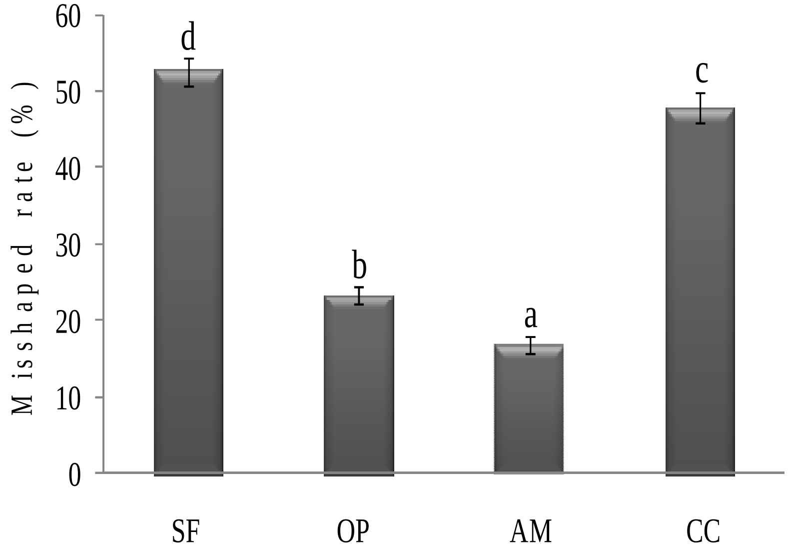

Figure 4. Misshaped rate obtained in four treatments.
(Bars with different letters indicate significant differences at p<0.05).

Discussion

This study is the first to provide information about effect of strawberry fruit pollinated by *C. carnea* and *A. mellifera* on total content of anthocyanin, pH and total solids soluble. In our study we found a strong contribution of *A. mellifera* and open pollination on PR (Fig. 1), in agreement with Chagnon *et al*. (1989; 1993) and Albano *et al*. (2009). It revealed that *A. mellifera* is an efficient pollinator for strawberry crops according to other studies (Svensson, 1991; Paydas *et al*., 2000; Young-Duck *et al*., 2001; Slaa *et al*., 2006; Kasina *et al*., 2009). Self-fertilizing treatment showed low rate pollination due to wind and gravity are not sufficient to promote an appropriate flower pollination (Albano *et al*., 2009). *C. carnea* was not an effective pollinator compared to *A. mellifera*, it is probably to body structure, flight activity and a few hair in the body. Hagen *et al*. (1970), Principi and Canard (1984) and Tian-Ye *et al.* (2006) reported that *C. carnea* may feed on pollen and flower nectar, but to be used as a biological control tool in a strawberry crop 150 adults/ha are necessary (Salas-Araiza and Salazar, 2005), therefore we considered *C. carnea* as deficient pollinator to increase the PR (Green and Bell, 2007).

Zheng *et al*. (2007) determined a good total anthocyanin content of 20 mg/100g of pelargonidin 3-glucoside per 100 g of fresh weight, we found a total anthocyanin content similar where strawberry flowers were pollinated by *A. mellifera* and open pollination, in contrast to self-fertilizing and *C. carnea* (Fig. 2). Given *et al*. (1988) demonstrated that auxin produced by achenes may modulate the accumulation of anthocyanin; therefore a high PR helps to ripen at fruits due to a high concentration of auxin from fertilized ovules (achenes) (Nitsch, 1950; Given *et al*., 1988; Aharoni and O'Connell, 2002; Aaby *et al*., 2005).

The values of fruit quality obtained using *A. mellifera* were heavier, longer and more homogeneous compared to the other three treatments (Table 1); similar results were obtained by Svensson (1991), Paydas *et al*. (2000) and Young-Duck *et al*. (2001). There were few misshapen fruits using honey bees indicating that *A. mellifera* had a positive effect on production of strawberry fruit contrasting to other treatments (Fig. 4). In our studies *C. carnea* did not show a positive effect on values of quality compared to *A. mellifera.*

Stingless bees have been studied like pollinators in strawberry crops due to easy management and docile in greenhouse and open field conditions, but the commercial pollination with stingless bees have been difficult to develop, due to problems as domestication, colony reproduction and mass rearing (Slaa *et al*., 2006), in contrast with *Bombus impatiens* and *A. mellifera* whose have been established successfully as commercial pollinators, additionnally the cost for using *A. mellifera* is cheaper than *B. impatiens*. Paydas *et al*. (2000) found that *A. mellifera* and *B. impatiens* have similar effects on strawberry fruits quality, so it is possible to suggest that *A. mellifera* could be an alternative for incrementing the production and quality of strawberry fruits from Irapuato, Guanajuato, Mexico. However, more research is needed in order to know the management of these species on strawberry crops under greenhouse and open field conditions.

By other hand, *C. carnea* improved slightly the pollination rate, total anthocyanin, weight and diameter of fruits compared to self-fertilizing treatment, but it does not compare to excellent pollination action of honey bees. Its use on strawberry crops should remain at biological control; however, when it would be used for this purpose, an additional ecological function could be the pollination, with the advantage to be tolerant at pesticides compared to honey bees and to be exempt from phenomenon called "colony collapse disorder of honey bees".

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