PHILIPP AGRIC SCIENTIST
Vol. 105 No. 1, 85-91
March 2022 ISSN 0031-7454

Effect of Workers Density on Insect Pest Incidence and Colony Development of Bumblebees, *Bombus terrestris* (L.) (Hymenoptera: Apidae)

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Received: April 19, 2019/ Revised: August 12, 2021/ Accepted: December 30, 2021

Insect pollination improves essential nutrients and qualities of pollinating plants. Approximately 90% of flowering plants and 35% of crops require insect pollination. Bees play a vital role in pollination in natural and agricultural systems. Managed bees have extensively been used for pollination. Unfortunately, the native bee population has been waning globally. Commercial bee-rearing experiences different rearing problems i.e. food, environment, and spatial conditions within colonies. In this research, the impact of different densities of workers within colonies of *Bombus terrestris* L. was observed on colony development and pest incidence. Different worker densities i.e. 60-70, 80-90,110-120 and 140-150, respectively, were maintained under controlled laboratory conditions. The results indicated that emergence of workers was higher in less dense colonies (60-70 workers) as compared with highly dense colonies (140-150 workers). Maximum drone emergence was observed in colonies having 80-90 workers as compared with highly denser colonies. The numbers of young queens were the highest in denser colonies. Mortality percentage of bumblebees and insect pest incidence were also higher in denser colonies. Therefore, the colony with 80-90 workers can be used as an indicator for bumblebee-rearing on a commercial scale in the laboratory.

Keywords: worker density, pest incidence, bumble-bee

INTRODUCTION

Bombus terrestris L. are reliable and efficient pollinators of greenhouse crops as they are able to produce good quality fruit due to their buzzing behavior, pollination speed, low temperature, and sunlight resistance (Paydas et al. 1998; Ahmad et al. 2015). These eusocial bumblebee species are prolific and important pollinators of plants in temperate regions. True social bumblebees have three castes within a colony, namely the queen, the workers, and the drones. The queen holds the entire colony together by initiating egg-laying, producing honey and pollen pots, brood-feeding, and foraging until the emergence of the first batch of workers. Meanwhile, workers have a crucial function in the survival of the colony through brood-feeding by pollen and nectar preparation or foraging, making pollen pots, honey pots, and egg cups, as well as colony defense. Drones perform their function for reproduction only (Alford 1978; Beheshti et al. 2013; Sladen 2014). Some species have been managed commercially for the pollination of high quality fruit and glasshouse crops (Klein et al. 2007; Newbold et al. 2015). Hence, the demand for healthy production of bumblebee colonies has been growing (Pettis et al. 2012). Although rearing at a commercial scale started in the 1980s, problems in rearing, particularly those regarding quality and quantity, still exist (Hughes 1996; Imran et al. 2017).

The size of bumblebee colonies vary with respect to growth rate throughout the season by producing variable amounts of sexual. Colony growth rate may depend on factors such as suitable local habitats, food supply (Goulson et al. 2002), and space availability within the colony. In larger bumblebee colony sizes, the cost of attaining dominance and reproduction might be greater due to energy requirements and fertility signaling (Amsalem and Hefetz 2011). In colonies of social insects, the queen pheromone plays a vital role in the maintenance of the division of labor. When the intensity of queen pheromone vanishes, workers start reproduction (Holman 2014). Large colonies or more workers within the colony may lead to minimal access to individual workers. Moreover, C25 (*n*-pentacosane) was recently discovered to be a queen pheromone affecting small as well as large workers (Holman 2014).

Different sizes of colonies can explain the variation among group individuals as well as nest complexity within the same species. Nest size has been closely related to the structure of kin within social insects. This gives a complete description for the complexity of the colony and set of traits representing sociality level, particularly morphological differences within colony individuals, communication system, workers polymorphism, reproductive division (Hamilton 1964), as well as division of labor (Simpson 2012; Kramer and Schaible 2013). Among social insects, the size of the group has a profound influence on the physiology and behavior of individuals that comprise the group. The size of the colony defines the division of labor which leads to benefits such as increasing the efficiency of physiological processes and patterns for resource allocation for individuals as a response to extrinsic mortality within the colony (Bourke 1999; Muench et al. 2008; Kramer and Schaible 2013). As the size of colony increases, the quality of individuals diverges along with their lifespan by the modification of resource allocation between different casts, which defines the fitness of the colony (Kramer and Schaible 2013). Smaller colonies have greater reproductive potential for workers — the consequence of which is higher suppression ability by the foundation queen (Michener 1974). Colonies that are too small or too large face various problems — smaller colonies experience heating challenge of their broods, while larger colonies face overheating and inadequate ventilation due to overcrowding (Weidenmuller et al. 2002).

Different insect pest species attack bee colonies depending on the density within the colony (Oyerinde and Ande 2009). *Bombus terrestris* L. colonies are susceptible to infestation of *Plodia interpunctella* in controlled environmental conditions (Kwon et al. 2003). *Aphomia sociella* L. has also been reported as a predator (Alford 1978) of *Bombus* spp. with a greater infestation rate within colonies in gardens than farmlands. Heavy infestations within colonies lead to the maximum destruction of bumblebee pupae along with associated frass and webs of moth larvae. A lot of bumblebee colony biomass contains larvae of the wax moth *A. sociella* L. Highly dense colonies with more individuals may cause food scarcity which ultimately affect production, as in garden nests with heavier loads of resources as compared to farmland nests, which may have a higher population (Goulson et al. 2002). The density of workers and their activity level within the colony also affect disease transmission. Spatial separation within colonies may delay the spread of diseases and the severity of the outbreak (Piea et al. 2003). The main objectives of the present research were: (i) to evaluate the effects of different densities of workers on the reproduction of different castes within bumblebee's colonies., (ii) to study the insect pest incidence and mortality of sexuals within colonies in laboratory conditions, and (iii) to determine the optimum number of workers within a colony, which should show good development with minimum insect pest incidence and mortality of sexuals.

MATERIALS AND METHODS

Experimental Insects

Colonies of European bumblebee (*Bombus terrestris* L.) with foundation queen having approximately 30-40 workers of even-age were imported from Koppert Biological Systems, Netherlands and reared in controlled laboratory conditions (25± 2°C and RH 60 ± 10%) in the Non-Apis Bee Laboratory, Department of Entomology, PirMehr Ali Shah, Arid Agriculture University Rawalpindi, Pakistan. Each bumblebee nest was labeled for the clear identification of the different treatments during handling. In the rearing room, total darkness with red light was maintained throughout the duration of the observation. Red light bulbs were hung at a distance of eight feet from the floor and one meter distance was maintained between the bulbs to minimize disturbance and flying attempts. An ample amount of food with frozen pollens collected from honeybee nests and sugar solution (50%) was provided to each hive as a diet for rearing and to avoid the starvation of individuals.

Experimental Setup

All the colonies were maintained in standard rearing boxes (24.0 × 27.0 × 18.0 cm^3) used for the rearing of bumblebees at the commercial level. Four treatments with different worker densities of 60-70, 80-90, 110-120, and 140 -150 were kept within colonies. Desired numbers of workers were attained and labeled with white paint (enamel) on the thorax of all colonies. This number was maintained within the colonies by removing new emerging workers, males, and gynes on a daily basis. All newly emerged castes were kept in separate boxes (24.0 x 27.0 x 18.0 cm^3) with recorded counts. Each treatment with four different densities mentioned were replicated five times with the proper labeling of boxes. New emerging gynes were clearly identified through their large body size as compared to drones and workers. Drones and workers were identified visually through their round (without sting) and pointed (stringed) abdomen. Different parameters of colony health were observed on a daily basis such as total number of workers, emergence of drones and young queens (gynes), emergence of first drone and queen , longevity [foundation stage (above 50 workers) up to the death of the mother queen] of the colonies for each treatment, identification and number of insect pests i.e. *Plodia interpunctella* (Hubner), (Pyralidae: Lepidoptera), *Achroia grisella* (Fabricius) (Pyralidae: Lepidoptera), and *Drosophilla suzukii* (Matsumura) (Drosophilidae: Diptera) attacking the colonies and mortality of bumblebee individuals within each colony at different densities.

Data Analysis

The data of various life history parameters were examined using General Linear Model (GLM) and were subjected to one-way analysis of variance (ANOVA). Means were compared by using Tukey HSD *post-hoc* test at 5% probability level using CoStat statistics software (version 6.45). Graphs were prepared through Microcal origin 6.1software. CRD experimental design was used in this experiment.

RESULTS AND DISCUSSION

Total Number of Worker Emergence

The mean total number of worker emergence ($F = 210.9$, $df = 3$, $p < 0.01$) was significantly different among treatments. The minimum number of worker emergence (168.8 ± 3.1) was observed in colonies having a workers density of 140-150 while the maximum number of worker emergence (306.6 ± 5.0) was observed in colonies having a workers density of 60-70 (Fig. 1).

Total Number of Drone Emergence

The maximum number of drone emergence (236.6 ± 9.1) was observed in colonies having 80-90 workers. The minimum number of drone emergence (58.2 ± 2.8) was noted in colonies having 140-150 workers. A highly significant difference ($F = 52.5$, $df = 3$, $p < 0.01$) was noted between the maximum and minimum numbers of drone emergence (Fig. 1).

Total Number of Young Queen Emergence

Total gyne emergence was ($F = 4.17$, $df = 3$, $p > 0.01$) non-significant among different treatments. The highest numbers of gynes (114.6 ± 5.2) were noted in denser colonies while minimum numbers of queens (62 ± 5.1) were produced in the colonies having fewer workers (Fig. 1).

First Drone Emergence Time

The colonies with worker density of 80-90 had early emergence of drones. The first drone emergence (21.6 ± 2.3) at worker density of 80-90 was ($F = 17.0$ $df = 3$, $p < 0.01$) significantly different from the colonies having 60-70 workers. Maximum elongated (30.2 ± 1.2) period of drone emergence was observed in the colonies having 140-150 workers (Fig. 2).

First Young Queen Emergence Day

First young queen emergence (31.6 ± 1.8) was early in highly denser colonies (140-150) while delayed emergence was noted in the colonies with a small number of workers (60-70). First young emergence was ($F = 0.86$, $df = 3$, $p > 0.01$) highly non-significant among all the treatments (Fig. 2).

Colony Developmental Period

Developmental period was longer (66.4±1.6 days) in colonies having worker density of 80-90 ($F = 25.2$, $df = 3$, $p < 0.01$). Developmental period was shorter (55.4±1.4 days) in the colonies having fewer workers i.e. 60-70 workers (Fig. 2).

Percentage Mortality

Percentage mortality was significantly ($F = 43.4$, $df = 3$, $p < 0.01$) different among all the treatments. Maximum percentage mortality (28 ± 0.6) was observed in colonies having high density of workers i.e. 140-150 and minimum percentage mortality (13.6 ± 3) was noted in colonies having fewer workers i.e. 60-70 (Fig. 3).

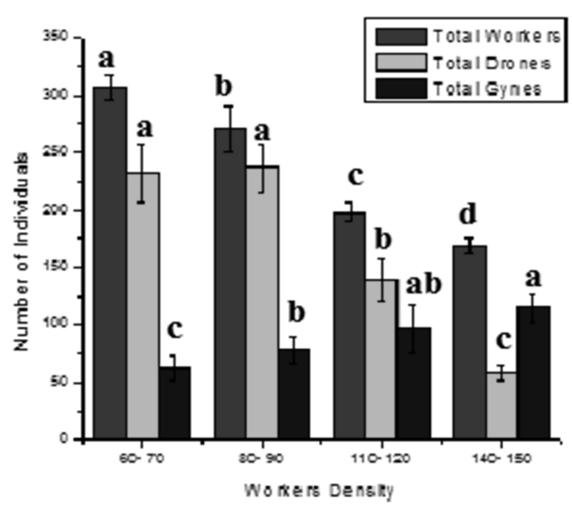

Fig. 1. Total production of workers, drones and queens (Mean ± SD) of *Bombus terrestris* L.

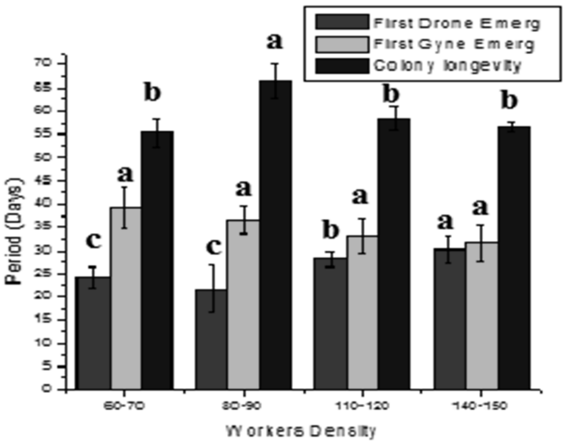

Fig. 2. Production of drones and queen including colony longevity (Mean ± SD) of *Bombus terrestris* L.

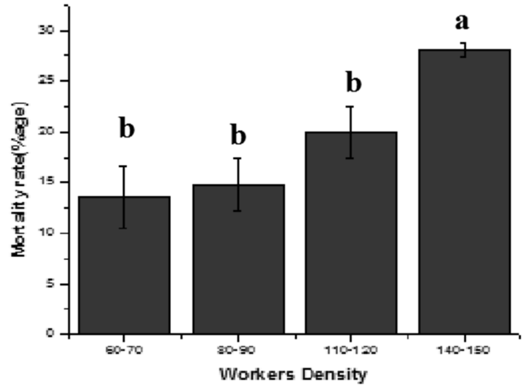

Fig. 3. Percent mortality (Mean ± SD) of *Bombus terrestris* L. workers.

Incidence of Insect Pest

Significant results (F = 11.5, df = 3, $p < 0.01$) were observed for the pest incidence in different densities. The maximum (12.6 ± 2.1) insect pest incidence was observed in colonies having more number of workers while minimum (1.4 ± 0.4) infestation of insect pests was noted in colonies having less number of workers (Figure 4).

The study of density-based colony development is important to improve the colony health and productivity in commercial rearing of bumble bees. Large-sized colonies can increase the range of foraging and defense. Distance by the forager during each travel increases with less predation risks (Jorgensen and Porter 1982; Kramer and Schaible 2013). Achieving dominance regarding density in larger groups has been greater than smaller groups. Hence, group size or density has a profound effect on the physiology and behavior of individuals, especially in social insects (Michener 1974; Amsalem and Hefetz 2011). Based on the results, the total number of emerged workers was greater in colonies having 60-70 workers while minimum emergence was noted in denser colonies. These results are different from the findings of Amsalem and Hefetz (2011) who reported that in larger colonies, production by workers gradually increase due to the inability of the queen to inhibit the reproduction of the workers. The results for the production of workers are in accordance with the findings of Amin et al. (2011) who observed that larger colonies have less colony growth than smaller colonies. Similar results were also reported by Beekman and Van Stratum (1998). An average number of 122 workers has been reported in *Bombus terrestris* L. colonies (Cnaani et al. 2002). Based on the results, the average number of workers production was 168.8 in denser colonies, which might be due to the stress conditions within colonies.

The production of drones at different densities are also in accordance with the findings of Amin et al. (2011), who reported that smaller colonies have a greater number of drones. Fewer workers fail to rear the female offspring which requires more energy for development, while in larger or denser colonies, fewer drones production had been observed. Results show that a similar trend was observed. This could be attributed to the queen's ability to inhibit the production of workers. Denser colonies produce fewer drones but more gynes, which is in agreement with previous studies (Lopez-Vaamonde et al. 2003; Muller and Schmid-Hempel 1992).

Drone and queen production depend on the density of workers within the colony (Roseler and Roseler 1974) wherein the queen's emergence depends on drone brood

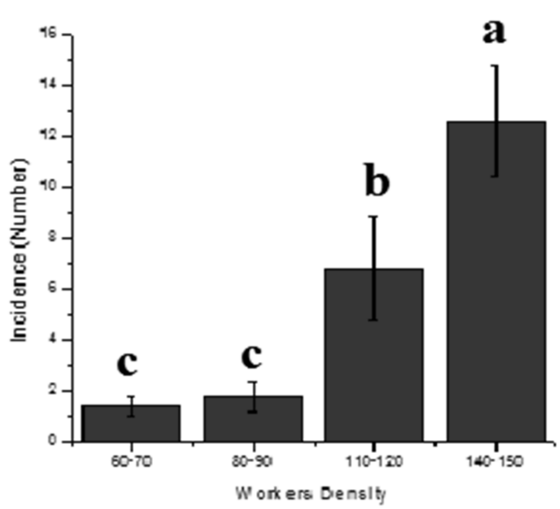

Fig. 4. Insect pest incidence (Mean ± SD) within *Bombus terrestris* L. colonies

(Roseler 1970). In this study, the first drone emerged in colonies with 80-90 workers. Emergence of the young queen was observed early in dense colonies, which is similar to the findings of Pomeroy and Plowright (1982) and Amin et al. (2011). This could be due to the ability of the workers to produce a new queen from the workers' population while the current queen provides pheromones to suppress the workers' egg-laying capacity (Lopez-Vaamonde et al. 2003), which results in the early production of young queens by the present queen in dense colonies due to competition. These results on queen production are also similar to the findings of Pomeroy and Plowright (1982).

Longevity of colony depends on the size of the colony or workers density, including insect pest incidence which results in the mortality of individuals within the colony. Results showed that prolonged time for colony development was observed at 80-90 workers density as compared with other treatments. In less dense colonies (60-70 workers), the minimum duration of colony was observed as compared with the colonies having 80-90 workers; in denser colonies (i.e. with 110-120 and 140-150 workers), the minimum duration of colony was also observed minimum, which might be due to the incidence of insect pests within the colony as well as the mortality of sexual (drones and emerging queens). Similar results were observed in the findings of Amin et al. (2011) who reported that colony longevity depends on the colony size and queen diapause. Based on the findings, the queens have a similar diapause duration; hence, colony size is an important factor in minimizing the insect pest incidence and mortality, which result in the good health of the colony. In non-stressful colonies, workers take good care of the brood through proper maintenance of the entire colony without competition or aggression behavior. This behavior by workers lead to the mortality of the sexual. Results showed that the insect pest incidence increased as colony density increased. This leads to the mortality of sexual, thus reducing the longevity of the colony. The conopid fly provides 10-15% mortality every week in *Bombus lucorum* L. colonies (Schmid-Hempel and Heeb 1991). Different wax moth species like *Plodia interpunctella* Hubner (Kwon et al. 2003; Chauhan et al. 2014; Imran et al. 2016), and *Aphomia sociella* L. (Goulson et al. 2002; Goulson et al. 2017) have also been reported to infest bumblebee colonies, while *Achroia grisella* Fabricius infested honey bee colonies. Based on the studies, *A. grisella* was also found in the bumblebee colonies. Further studies are also recommended for the pathogenic incidence in similar densities.

CONCLUSION

Colonies with worker densities of 80-90 may be used as an indicator for rearing *B. terrestris* in controlled laboratory conditions at the commercial level. Production of all the castes i.e. queens, drones, and workers were satisfactory. Colony longevity was also greater in worker densities of 80-90 as compared with other colonies. Hence, pest incidence and mortality of sexuals were also minimized in these colonies.

ACKNOWLEDGEMENT

The authors are thankful to all who participated in the data collection. The authors are also indebted to the reviewers who improved the manuscript.

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