Effect of temperature on the foraging activity of *Bombus terrestris* L. (Hymenoptera: Apidae) on greenhouse hot pepper (*Capsicum annuum* L.)

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Abstract

The bumble bee *Bombus terrestris* L. is an effective pollinator in the cultivation of greenhouse hot pepper *Capsicum annuum* L. Data indicates that colony traffic and foraging activity was highest at 25.7°C in greenhouse, whereas at 32.7°C, the foraging activity and colony traffic decreased 69.7 and 40.0%, respectively. By increasing the number of larvae and workers, the colony traffic and foraging activity also increased, respectively. Moderate temperature in the morning probably facilitates the overall activity of bees in a greenhouse. The data indicates that bumble bee pollination increased the fruit mass and number of seeds by 27.2 and 47.8%, respectively, compared to that of the control. These significant results substantiate the effectiveness of bumble bees in the pollination of pepper grown in greenhouses.

Key words: *Bombus terrestris* L.; pollination; *Capsicum annuum* L.; temperature; foraging activity

INTRODUCTION

Pollination is the transfer of pollen grains from an anther to a stigma (Stern, 1994) and is mostly brought about by insects or wind. Progressive farmers were using honey bees in a greenhouse for pollination, but this trend has decreased because of a lower availability of the food source for colony development. The constraint is expected to become increasingly important as honey bee numbers decline (Williams et al., 1991) and wild bee communities are eroded (Westrich, 1996). Pollination research can highlight the need for pollination management of certain crops with unstable yield, and can help growers to select appropriate pollinating species and management options and to utilize their resources effectively. In the field, pollination usually occurs by wind agitation or cross-pollination by occasional entomophilous insects (McGregor, 1976). To meet the urban demands for vegetables and fruits, greenhouse cultivation is spreading rapidly among farmers in Korea. One of the major problems in the greenhouse's fruit and vegetable production is insufficient pollination due to confined conditions and limited wind, resulting in low production. Low pollen supply sometimes limits

the seed production directly or affects the progeny vigor by reducing the selectivity among the gametes before and during fertilization (Snow, 1986; Bertin, 1990). Pollinators are absolutely necessary for fruit set and seed formation of some crops (McGregor, 1976; Crane and Walker, 1984; Rasmussen, 1985). To obtain pollen, the insect pollinators must grip and vibrate the wing muscles. In this way the pollen grains are dislodged and fall on to the insects, an activity termed 'buzz' pollination (Buchmann, 1983). Bumble bees and certain solitary bees (*Peponapis* sp., *Xylocopa* sp., *Habropoda laboriosa*) are capable of doing this and this makes them valuable pollinators of buzz pollinating crops such as blueberry, eggplant, seed potato, hot/sweet pepper, and tomatoes (Plowright and Laverty, 1984; Cane and Payne, 1990, 1993); most bee species, including honey bees *Apis mellifera* L., do not possess this ability. Bumble bees provide a very good alternative for this task (Banda and Paxton, 1991; Kevan et al., 1991; Ravestijn and Sande, 1991; Shipp et al., 1994; Lee et al., 1998). Import of more than 20,000 bumble bee colonies per year in Korea shows the increasing interest of growers for insect pollination of crops, especially tomato. There is little information available about pepper

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pollination by bumble bees. Jarlan et al. (1997) reported that pepper flowers visited by Syrphid flies produced larger seed sets than those non-visited flowers. Variability in the amount of seed per pepper fruit (Marcelis and Baan Hofman-Eijer, 1995) may be related to a great extent to the variability in the amount of pollen reaching the stigma. In many species, including pepper, fruit size and fruit set have been reported to be positively correlated with seed number (Rylski, 1973; Varga and Bruinsma, 1976; Stephenson, 1981; Picken, 1984; Stephenson et al., 1988; De Ruijter et al., 1991; Shipp et al., 1994). Fruit set in the flowers was 50–70% more for flowers visited by bumble bees compared to non-visited flowers (Morandin et al., 2001). He also found that levels of pollination was positively correlated with bee activity, and 2,000 bee trips per hectare per day were more than adequate to insure sufficient pollination.

The objectives of this study were to determine (1) the pollination effectiveness of *Bombus terrestris* L. for greenhouse hot pepper production based on the number of seeds produced per fruit, weight and size of fruit and weight of seeds, and (2) bumble bee pollination activity at different times of the day, in relation to temperature, humidity and light intensity in greenhouse environment.

MATERIALS AND METHODS

Seedling of the hot pepper (Chile), *Capsicum annuum* L. were transplanted to a greenhouse in the last week of April 2001. Appropriate growth nutrients were provided to achieve optimal growth of the plants. There was a total of 185 plants in every plot. After two months all plants were covered with a mosquito net to restrict the movement of bumble bees to other plots in the same greenhouse.

Bumble bees, *B. terrestris* (Hymenoptera: Apidae), were reared in the laboratory from the post-hibernating queens in the Department of Agricultural Biology, Kyungpook National University, Korea. After the 2nd brood started, colonies were shifted in to the Koppert Pvt. Ltd. bumble bee boxes. Since the nectar available on pepper plants was not sufficient for colony development, on extra 1.5 l of sugar solution (50% w/v) was provided at the bottom of every colony as an energy source. Bees can suck sugar solution easily through a filter connected with a sugar solution tank at the base of

the colony. Three colonies with approximately 40 workers were introduced into the greenhouse in three separate replications. Frozen fresh pollen was also provided daily outside the colony to meet the colony requirements, because plot size was very small and pollen resources were insufficient for good colony development.

The data for colony traffic and foraging activity were recorded from the last week of June to the second week of July. Data of foraging activity was also recorded by counting the total numbers of workers actively foraging or flying in a plot during one hour in the morning, noon and afternoon between 8:00–9:00, 13:00–14:00 and 16:00–17:00 hours, respectively. During this one-hour the numbers of bees foraging and flying in the plots were counted every 10 min (seven times/h). The plots were covered with mosquito net to facilitate counting of the foraging bees inside the plots. During this one-hour the total number of bees entering and leaving (colony traffic) the box was also calculated. The mean number of bees counted is presented. Three other plots covered with mosquito net with 150 pepper plants in each plot were also maintained in the same greenhouse as a control. In control plots no bumble bee colonies were introduced. Data on the numbers of workers, pupae, larvae, and eggs of the colony were also recorded before the initiation and at the end of trials. Eggcups, larvae clumps, pupae and workers were counted in a room illuminated with red lights. The eggs covered with the wax were called eggcups and only these eggcups were counted. The 1st and 2nd instars larvae covered in wax were very small and were counted as a clump. The larvae covered with wax with a hole for feeding were counted separately. It was easy to observe and count the numbers because bumble bees are red blind. After recording the data, colonies were placed in greenhouse for pollination. All bumble bee colonies were placed one meter above ground at one end of the plots.

Flower pollination data were collected on alternate days by counting the total number of fresh or non-pollinated flowers from five randomly selected plants in the morning. Similarly the bee pollination data for pollinated flowers in every bumble bee plot were recorded from the same plants in the evening bumble bee plot. The bumble bee-pollinated flowers were tagged to allow differentiation between

bee pollinated and non-pollinated flowers at the next data collection. The pepper flowers with brown spots on the filaments with anthers bare of pollen indicate that a bumble bee has visited the flower. Enough pollen can be seen visually on the anthers of pepper flowers which the bees have not visited. The mean percentages of total flowers pollinated by bumble bees and those not pollinated were calculated to assess the success of pollination. The data for temperature, humidity, and light intensity were also recorded at every observation time.

The number of seeds produced by *B. terrestris* pollination was used as an indicator of pepper to measure pollination level. Hot peppers were harvested from plants pollinated with and without bumble bee when their color become red in order to obtain yield data. Twenty fruits from each plot were randomly selected to record the fruit mass (g), fruit length (cm), number of seeds per fruit, and mass of 20 seeds per fruit. The data regarding mass of fruit, number of seeds per fruit, mass of 20 seeds per fruit and length of fruit were examined by a multivariate test.

Data were analyzed for correlation by multivariate ANOVA with temperature, humidity, light intensity, workers foraging activity and colony traffic. MANOVA was followed by LSD for all pair comparisons using Tukey's test. Yield and colony development data were analyzed by analysis of variance (ANOVA) using SAS statistical software package (SAS, 1988) and the conclusion of LSD for all pair comparisons using Tukey's *t* test.

RESULTS

Foraging activity and colony traffic (exiting and entering) of bumble bees show that activity decreased significantly from morning to afternoon with the increase in temperature (Fig. 1). There was strong correlation between colony traffic, foraging activity and average temperature of the greenhouse.

The average colony traffic of *B. terrestris* in the morning, noon and afternoon was 39.2, 23.5, and 14.4 bees/colony ($F=5.89$, $df=2$, 29) respectively with foraging activity being 10.1, 3.1, and 2.10 bees/unit time ($F=8.69$, $df=2$, 29) respectively, and average temperature of 25.8°C, 32.3°C and 30.4°C ($F=17.27$, $df=2$, 29) respectively. The data for foraging activity and colony traffic (Table 1) in the last week of June show that 2.3 bumble bees were actively foraging the pepper flowers with 11.5 bees exiting and entering the colony per hour, (Fig. 2), and pollinating 95.3% of flowers. At that time there was an average of 42.0, 47.7, 43.7 and 7.3 workers, pupae, larvae and egg cups present, respectively, in the colony. These numbers increased significantly (Fig. 2) up to the second week of July with an average of 100, 43, 70 and 6 workers ($F=10.42$, $df=1$, 5, $p<0.01$), pupae, larvae ($F=6.57$, $df=1$, 5, $p<0.025$) and egg cups, respectively. There were 12.0 bees ($F=5.02$, $df=2$, 29) found busy in the foraging flowers per unit time, with 51.8 bees ($F=32.31$, $df=2$, 29) in colony traffic per hour, pollinating 100% of flowers (Table 1).

The data for humidity and light intensity were recorded daily. Data suggest that there was insignificant difference in light intensity ($F=0.72$,

Fig. 1. Effect of average temperature on colony traffic per hour and foraging activity per unit time of day in hot pepper greenhouse ($p<0.05$).

Table 1. Average foraging activity and colony traffic and colony condition in greenhouse

Date	Foraging activity/ unit time/plot	Successful pollination (%)	Colony traffic/ day/plot	Colony condition	
				Workers	Larvae
Last week of June	2.3	95.3	115	42.0	43.7
Second week of July	12.0	100	520	100.0	70.0
Increase (%)	80.7	4.8	77.9	58.0	26.3

Fig. 2. Average foraging activity and colony traffic per unit time and colony traffic per hour from the last week of June to the second week of July. Days of data recording are shown from the last week of June to the second week of July.

Table 2. Comparison of pepper yield between pollinated with bee and without bee (control)

Treatments	Control	Bumble bee pollinated	Increase (%)	p value
Number of seeds per fruit	81.6	156.3	47.8	$p < 0.01$
Length of fruit (cm)	15.6	16.2	3.8	$p > 0.05$
Width of fruit (cm)	4.4	4.5	2.2	$p > 0.05$
Mass per fruit (g)	8.0	11.0	27.2	$p < 0.01$
Mass per 20 seeds (g)	0.11	0.09	-14.0	$p > 0.05$

df=2, 29) and humidity ($F=0.187$, df=2, 29) in the morning (7:00–8:00 hours), noon (11:00–12:00 hours) and afternoon (15:00–16:00 hours). Temperature was $25.8 \pm 1.8^\circ\text{C}$ and $32.3 \pm 2.7^\circ\text{C}$ and light intensity was $10,630.5 \pm 6,703.2$ and $19,649.05 \pm 15,230.1$ lx in the morning and afternoon, respectively.

Fruit yields in Table 2 show a noteworthy difference in yield for both the control (without bumble bee) and with the bumble bee-pollinated plants. Yield data of pepper pollinated with bumble bee suggest 156.3 seeds/fruit, which was significantly higher than without bee-pollinated pepper (81.6 number of seeds/fruit; $F=98.78$, df=1, 5, $p < 0.01$). Data for mass per fruit show 11.0 g for bee-pollinated pepper, which was statistically higher than without bee (8.0 g mass per fruit; $F=134.5$, df=1, 5, $p < 0.01$). The data for length of fruit and mass of 20 seeds show insignificant differences between bumble bee-pollinated and non-bumble bee-pollinated fruits. Fruit length and width of bumble bee-pollinated plots was not considerably different from that without bees (15.6 cm length and 4.4 cm width of fruit, $p > 0.05$). Mass data of 20 seeds per

fruit resulted in 0.11 g non-bumble bee-pollinated fruits compared to bee-pollinated fruits (0.09 g mass of 20 seeds per fruit; NS, $p < 0.05$).

DISCUSSION

Both bumble bee colony traffic and foraging activity peaked at 7:00–8:00 hours in July 2001, which is in contrast to Meisels and Chiasson (1997). They observed greater activity of *B. impatiens* during 11:00–13:00 hours (June to August). This difference can be attributed to different species of bumble bees being studied under different environmental conditions and also helpful for effective pollination by *B. terrestris*. It could also be linked to the fact that temperature in the present study was fluctuating in the greenhouse from morning to afternoon. These conditions were deliberately provided to observe the bee activity. Daily temperature has a direct effect on the activity of bumble bees. The results of this study are in agreement with Heinrich (1975), who reported that in condition of 25°C air temperature, the thoracic temperature can be regulated during free flight at 35 to 40°C . The present study data clearly show higher bee activity at 25°C in the morning. Data also suggest that *B. terrestris* can forage well even in the low light intensity ($10,630 \pm 6,703$ lx) of the morning. An increase in light intensity during the period from morning to noon did not cause an increase in the workers activity. The data (Fig. 1) suggest that temperature is also an important parameter for bumble bee foraging activity in a greenhouse. These results suggest that with a rise in temperature up to an average value of $32.3 \pm 2.7^\circ\text{C}$, during noon, the foraging activity (10.1 to 3.1) and colony traffic (39.2 to 23.5) decreased significantly by 69.7 and 40.0%, respectively, with respect to the activity in the morning. The foraging activity and colony traffic decreased because of high temperature at noon, and the decreased activity after noon might be the completion of food requirements of the colony. Other factors like colony stores, amount of available pollen, amount of brood and fanning behaviour of bees for ventilation of the colony during high temperatures may also have effects on the colony traffic and foraging activity of bumble bees.

An increasing trend of foraging activity and colony traffic with respect to the amount of work-

ers and larvae from the first to the last day of data were also observed (Fig. 2). During the last week of June (Table 1), there were an average 42 (workers) and 43.7 (larvae) bees with the colony traffic 115 bees per plot per day. This traffic increased 520 bees per plot per day as workers and the larval population increased by an average 100 and 70 bees, respectively. Data suggest that an 80% increase (115 to 520) in colony traffic/day was due to a 58 and 26.3% increase in the population of adult worker bees (42–100) and larvae (43.7–70.0), respectively. The increasing number of individuals indicates the adaptability of *B. terrestris* in fluctuating environmental conditions, which resulted in better pollination of pepper in the greenhouse. During the last week of June, there was a total of 185 plants per plot, with an average 9.5 flowers per plant. At that time bee traffic averaged 12,368.9 bees per acre (115 bees traffic per day per plot). An average activity of 2,000 bee trips per hectare per day is more than adequate to insure the sufficient pollination (Morandin et al., 2001). However, this study shows that there was too much colony traffic compared to a report by Morandin et al. (2001). In the pepper plots pollen requirement was not sufficient for good colony development. To satisfy the colony requirement extra fresh pollen was provided outside the colony.

The data for fruit yields (Table 2) indicate that bumble bees enhanced the number of seeds and mass of fruits significantly compared to control. These results are in agreement with Meisels and Chaisson (1997). They observed a significant increase in the number of seeds/fruit of the pepper as compared to control by using *B. impatiens* throughout the growing season. While there was a little increase in the size of fruit in bumble bee-pollinated pepper compared with control, this increase was statistically insignificant ($p > 0.05$). The hot pepper is a self-pollinated crop and fruit development in control plots was because of wind pollination or by some human disturbance. In this case only few pollen grains can transfer to the stigma and make fruit. On the other hand, in bumble bee pollinated flowers, much pollen is transferred to the stigma and that increases its mass as a high number of seeds/fruit. Data revealed that as the number of seeds per fruit increased, the mass of fruit enhanced proportionally. These results were in agreement with Rylski (1986), Kaftanoglu and Abak

(1995). Abak et al. (1997) also support these results, as they reported 52 and 19% increase in the number of seeds per fruit and mass of fruit, respectively, during March comparing bumble bee-pollinated fruit and that devoid of bumble bees. In the present study, 0.62 and 0.20 cm increase in the length and width of fruit were observed, respectively, which were statistically insignificant between pollination with and without bees. These results are in contrast to Abak et al. (1997), who concluded a 0.33 cm decrease in the size of fruit pollinated by bumble bees. These results substantiate that bumble bee pollinated plants gave higher productivity compared to that of the control. This reveals the potential for bumble bees to be utilized in greenhouses for hot pepper pollination.

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