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Original Article

THE EFFECTS OF POLLEN PROTEIN CONTENT ON COLONY DEVELOPMENT OF THE BUMBLEBEE, *BOMBUS TERRESTRIS* L.

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A b s t r a c t

The effects of pollen protein content on the colony development of *Bombus terrestris* were investigated by feeding queens and queenright colonies with four different pollen diets. We used three kinds of commercially available pure pollen (*Cistus* spp. 11.9%, *Papaver somniferum* 21.4%, and *Sinapis arvensis* 21.8% crude protein). We also used a mixture which was made up of equal weights of these pure pollens (18.4 % crude protein). All queens and colonies were fed with sugar syrup and pollen diets *ad libitum* (28 ± 1 °C, 65 ± 5% RH). Until there were 50 workers reached, colonies fed with the *Cistus* pollen diet (167.4 ± 28.9 g) consumed significantly more pollen than colonies fed with the *Papaver* pollen diet (140.7 ± 15.7 g), the mixed pollen diet (136.2 ± 20.1 g) or colonies fed with the *Sinapis* pollen diet (132.4 ± 22.6 g). The date when there were 50 workers reached was approximately one week later in the colonies fed with the *Cistus*, and colonies fed with the *Papaver* diet than in the colonies fed with the *Sinapis* diet, and for colonies fed with the mixed pollen diets. Considering 8 tested criteria, the best performances were observed using the *Sinapis*, and using the mixed pollen diets. The lowest performances were observed using the *Cistus* pollen diet. Results showed that pollen sources play an important role in commercial bumblebee rearing. Results also showed that the polyfloral pollen diets are more suitable for mass rearing of bumblebees than the unifloral pollen diets.

Keywords: *Bombus terrestris*, nutrition, pollen consumption, pollen quality, protein.

INTRODUCTION

The buff-tailed bumblebee (*Bombus terrestris* L.) is an important pollinator of wild flora as well as agricultural crops. This bumblebee is increasingly used as an effective commercial pollinator in greenhouse crops, mainly in tomatoes, all over the world (Velthuis and van Doorn, 2006). Even though production techniques have improved over the last 25 years and over one million commercially produced colonies are used all over the world, there are still serious problems in breeding, feeding as well as in pest and disease control in the commercial rearing process (Kwon, 2008). *Bombus terrestris* colonies vary tremendously with regard to the number of workers, males, and queens produced (Beekman and van Stratum, 2000). Significant differences are also observed in the colony initiation, colony production ratio, the switch point, and the competition point. These characteristics are vital criteria in the mass rearing of *B. terrestris* and depend on several factors such as the food quality, climate in the rearing room, and the larva/worker ratio (Gurel et al., 2012).

Nectar and pollen are essential food sources for bees. In extensive commercial rearing, bumblebee colonies are normally fed with sugar syrup and fresh or fresh frozen pollen collected from pollen traps at honey bee hives (Ribeiro et al., 1996; Velthuis and van Doorn, 2006). Pollen is the sole source of protein in the diet and also provides all of the nutritional requirements for growth and development of the larvae, young adults, and colony (Heinrich, 1979). Pollen is collected from a wide variety of plants. The chemical composition and nutritional value of pollen vary greatly depending on the botanical origin. Pollen may also be affected by factors such as drying, ageing, or storage conditions (Ribeiro et al., 1996). The protein content of pollen can also vary widely, ranging from 2.5 to 61% and is generally accepted as a reliable measure of its nutritional value (Roulston et al., 2000). Similarly, the crude protein content of pollen collected by the honey bee typically ranges from 7.5 to 35% (Schmidt and Buchmann, 2003). On the other hand, recent studies have shown that the total amino acid and polypeptide contents of hand-collected pollen provides more reliable information on the quality of a plant as a pollen resource (Vanderplanck et al., 2014a, b). Previous studies also demonstrated that bumblebees generally could select pollen of significantly higher protein content and more essential amino acids than honeybees. Bumblebees seem to focus on quality instead of quantity (Mapalad et al., 2008; Leonhardt and Bluthgen, 2012).

The influence of pollen quality on reproduction, growth, and development traits has been studied most extensively in honeybees (Herbert, 2003). After the start of commercial rearing, the role of pollen in the bumblebee colonies has been investigated in detail. Regali and Rasmont (1995) created an original method using queenless micro-colonies of *B. terrestris* workers to compare the nutritional quality of 2 mixtures of pollens (low protein content 13% and high protein content 22%) and found that more male progeny were produced by queenless colonies when fed high protein pollen content. Riberio et al. (1996) found that queens reared on dried-frozen pollen were smaller, had lower biomass, higher mortality, and produced smaller colonies than queens reared on fresh-frozen pollen. Genissel et al. (2002) compared the effects of three unifloral pollens (*Prunus, Salix*, and *Taraxacum*) and a commercial pollen blend. In their study, they found that among the unifloral pollens, pollen quality did not influence egg production, but did influence egg laying delay, and larval growth. Tasei and Aupinel (2008a) used queenless micro-colonies and investigated 10 parameters to compare the effects of the nutritive value of 6 pure pollen and 9 commercial pollen mixes on bumblebee larvae. They found that among 10 tested parameters, the most sensitive features to the different pollens were the mean weight of the larvae and the rate of discarded larvae. Similarly, Vanderplanck et al. (2014b) found that *B. terrestris* colonies fed on *Sorbus aucuparia* and *Cytisus scoparius* pollen, produced larger larvae and fed less on nectar, compared to the other diets (*Salix caprea*, *Calluna vulgaris*, and *Cistus* sp. diets).

The nutritive value of pollen blends purchased by bumblebee producers from beekeepers is a key factor for successful mass rearing (Tasei and Aupinel, 2008b). It is estimated that more than 200 tons of honeybee-collected pollen are used annually by the commercial bumblebee breeding industry (Velthuis and van Doorn, 2006). In Turkey, approximately 200 000 *B. terrestris* colonies are reared commercially and 50 tons of honeybee-collected pollen are used annually. In year-round rearing of bumblebees, rapid colony initiation and successful colony production are the major criteria for being able to reduce the production costs. As mentioned above, previous studies showed that pollen quality has a considerable influence on *B. terrestris* at the individual and colony level. However, there has been limited research into the effects of commercial pollen diets on the economic criteria in *B. terrestris* mass rearing. In our study, we used three kinds of commercially available pure pollen (*Cistus* spp., *Papaver somniferum*, and *Sinapis arvensis*) and a mixture which contained equal weights of these pure pollens. The aim of this study was to compare the effects of the four different pollen diets on the several colony characteristics related to the mass rearing of the bumblebees. The following effects were compared: total number of workers, males, and queens, time to emergence of first worker, the time it took to reach 50 workers, the number of workers in the first brood, and pollen consumption.

MATERIAL AND METHODS

A total of 60 hibernated *B. terrestris* queens were separately placed in standard nest boxes and randomly divided into four groups. All queens and colonies were fed the same sugar syrups *ad libutum* (approximately 1:1 water: sugar, w/w) and reared under standard laboratory conditions in a climate controlled room (28 ± 1°C, 65 ± 5% RH). To stimulate egg laying, two newly emerged callow bumblebee workers were added to each queen. These callow workers were changed every week until the first worker emerged. When workers of the first brood emerged, the colonies were transferred to larger plastic boxes. To assess the nutritive value of the pollens, three different unifloral pollen diets (*Cistus* spp. - *Cistus creticus* and *Cistus salviifolius, Papaver somniferum* and *Sinapis arvensis*), and one polyfloral pollen diet, were supplied *ad libitum* to queens and queenright colonies. The polyfloral pollen diet was obtained by mixing equal weights of the three unifloral pollens (33.3% of each according to their weight). We decided to compare them because they are available commercially and are common pure pollens used by bumblebee producers in Turkey. Pollen samples collected from honeybee colonies were purchased from beekeepers in the Mediterranean and inland regions of Turkey and stored at - 20°C.

Pollen samples were hand-sorted by colour and appearance which resulted in 100% purity, confirmed by a palynological test. The total nitrogen content of pollen samples was determined by the conventional Kjeldahl method. To determine total crude protein, the nitrogen values were multiplied by a conversion factor of 5.6 (Rabie et al., 1983; Campos et al., 2008). Pollen was moulded into a paste with honey to make pollen lumps. Pollen lumps of different sizes (1 g, 5 g, 10 g, 20 g, 50 g, and 100 g) were prepared depending on the age of the colony. To determine the weekly pollen consumption of each colony, the pollen diets were prepared and replaced every week. Pollen consumption was measured by weighing the amount provided and the amount left of the unconsumed pollen every week. Colony development was tracked by daily observation. The date of egg laying after queens were placed in nest boxes (colony initiation), the date of reaching 50 workers, the number of workers in the first brood, the pollen consumption, the total number of workers, males, and queens produced by each colony, and the percentage of queen production in a colony were recorded by periodical observations. During the observations, dead bees were removed from colonies and noted to determine the total number of workers, males, and queens produced in each colony. The percentage of queens produced by colonies was calculated as follows: the number of queens that produced a progeny queen/the number of queens that produced a colony x 100. In this study, the queens that produced fewer than ten workers were not considered to be colony producers. Data on the ten colonies of each group were analysed. The nutrition experiment was completed in 15 weeks. Data on the parameters: total number of workers, males, and queens, did not meet the assumptions for parametric statistics and did not conform to a normal distribution. Therefore, we used the non-parametric, Kruskal-Wallis test and median and quartiles as the central estimators. When the data showed a normal distribution we used the mean ± standard deviation. We also carried out ANOVA tests using the statistical program Minitab 10.5. The significance of differences between the means was measured using Duncan's multiple range test.

RESULTS

The number of workers in the first brood did not vary significantly among the four pollen diets (ANOVA, $F = 1.04$, $df = 3$, $P = 0.39$). On average, 6.9 ± 3.8 workers were produced in the first brood fed the mixed pollen diet followed by *Sinapis arvensis* (6.3 ± 2.9), *Cistus spp*. (4.9 ± 2.6), and *Papaver somniferum* (4.8 ± 3.4) pollen diets (Tab. 1). The date of egg laying after queens were placed in nest boxes (colony initiation) was also similar, and varied from 11.3 ± 3.4 to 12.5 ± 3.2 days (ANOVA, $F = 0.26$, $df = 3$, $P = 0.86$). There was no significant difference among the pollen diets in the total number of workers, males, and queens produced by each colony (Kruskal-Wallis test, $H = 0.80$, $df = 3$, $P = 0.85$ and $H = 3.18$, $df = 3$, $P = 0.36$ and $H = 7.79$, $df = 3$, $P = 0.05$, respectively Tab. 2). Percentage of queens produced by colonies, however, varied within a wide

Table 1.

Characteristics of *B. terrestris* colonies fed with different pollen diets

Colony characteristics	**Pollen diets**			
	***Cistus* (n = 10)**	***Papaver* (n = 10)**	***Sinapis* (n = 10)**	**Mixed (n = 10)**
Colony initiation (days)	12.2 ± 2.9	12.5 ± 3.2	12.1 ± 3.3	11.3 ± 3.4
Number of workers in the first brood	4.9 ± 2.6	4.8 ± 3.4	6.3 ± 2.9	6.9 ± 3.8
Time it took to reach 50 workers (days)	65.1_a ± 5.8	63.0_a ± 5.7	57.4_b ± 2.9	56.0_b ± 3.3
Total pollen consumption until reaching 50 workers (g)	167.4_a ± 28.9	140.7_b ± 15.7	132.4_b ± 22.6	136.2_b ± 20.1
Percentage of queens produced by colonies (%)	30	20	60	70

All data show the mean ± standard deviation; n: number of queens or colonies.
Values marked with different letters in each row were significantly different when Duncan's multiple comparison test (P<0.05) was used.

range: from 20% when the *Papaver somniferum* diet was used to 70% when the mixed pollen diet was used. With the use of the mixed pollen diet, and with the use of the *Sinapis arvensis* pollen diet, the colonies reached 50 workers 56.0 ± 3.3 days and 57.4 ± 2.9 days, respectively, after they were placed in the starting boxes. However, it took colonies, on average, 63.0 ± 5.7 days to reach 50 workers when the *Papaver somniferum* pollen diet was used, and 65.1 ± 5.8 days when the *Cistus spp.* pollen diet was used, which were significantly longer times than when the mixed and *Sinapis arvensis* pollen diets were used (ANOVA, F = 8.92, df = 3, P<0.01). Figure 1 shows the cumulative pollen consumptions of the colonies during the 14 weeks. The total pollen consumption needed to reach 50 workers was also significantly different among the four treatments (ANOVA, F = 5.04, df = 3, P<0.01). Until reaching 50 workers, colonies fed the *Cistus spp.* pollen diet (167.4 ± 28.9 g) consumed significantly more pollen than colonies fed the *Papaver somniferum* pollen diet (140.7 ± 15.7 g), or the mixed pollen diet (136.2 ± 20.1 g), or the *Sinapis arvensis* pollen diet (132.4 ± 22.6 g).

DISCUSSION

The most prominent difference we found among the four pollen diets was the period it took the colonies to reach 50 workers. Other important difference among the groups was the total pollen consumption until 50 workers were reached. The date that

Table 2.
Total number of workers, males, and queens produced by *B. terrestris* colonies fed different pollen diets

Colony individuals	Pollen diets							
	Cistus (n = 10)		*Papaver* (n = 10)		*Sinapis* (n = 10)		Mixed (n = 10)	
	Median	Q1 - Q3	Median	Q1 - Q3	Median	Q1 - Q3	Median	Q1 - Q3
Total number of workers	350	302 - 405	287	157 - 424	294	139 - 432	349	183 - 469
Total number of males	5	0 - 82	15	5 - 153	62	9 - 134	24	0 - 66
Total number of queens	0	0 - 5	0	0 - 1	21	0 - 63	8	0 - 70

n: number of colonies

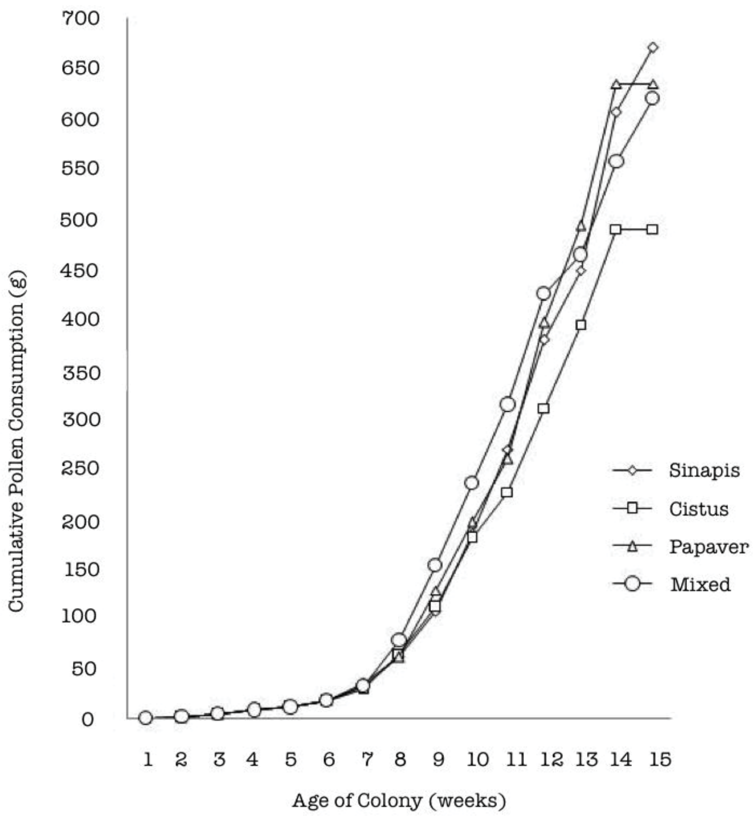

Fig.1. Average weekly cumulative pollen consumptions of *B. terrestris* colonies fed different pollen diets.

50 workers was reached was approximately one week later in the colonies fed the *Cistus* diet and in the colonies fed the *Papaver* diet than in the colonies fed the *Sinapis* diet and in the colonies fed the mixed pollen diets. Colonies fed the *Cistus* pollen diet consumed approximately 16, 19, and 21% more pollen than colonies fed the *Papaver,* or the mixed, or the *Sinapis* pollen diets, respectively, until 50 workers were reached. The colonies used for standard pollination work in a tomato crop are typically selected when they have reached the size of around 50 workers (Velthuis and van Doorn, 2006). Therefore, the date when 50 workers were reached and the total pollen consumption until there were 50 workers, are very important matters to be taken into account for reducing the costs of production in commercial *B. terrestris* rearing.

Pollen diets had no significant effect on the average number of days before queen egg laying (colony initiation) and the rate of queen egg laying. On the other hand, there was considerable variation among the four pollen diets in terms of queen production rates. When the *Papaver* diet was used, 2 out of 10 colonies reared new queens, when the *Cistus* diet was used 3 out of 10 colonies reared new queens, when the *Sinapis* diet was used 6 out of 10 colonies reared new queens, and the mixed diet 7 out of 10 colonies reared new queens. Similarly, the number of new queens that were produced varied within a wide range. However, there was no significant difference among the four pollen diets due to the notable variation within groups. When the 8 tested criteria were taken into account, the best performances were observed with the *Sinapis* diet, and the lowest with the *Cistus* pollen diet. Szczęsna (2006) investigated the protein content and amino acid composition of 16 unifloral honeybee-collected pollens and also reported that *Sinapis arvensis* pollen, characterised by a high content of crude protein and amino acid concentration, is an important source of protein and amino acids for bees and for humans. We found that despite the low protein content, the mixed pollen diet was more suitable as a food source than the *Papaver* diet. Recent studies showed that protein level was not the only component which could be used to determine pollen nutritive value. The mix when compared to a single pollen of a similar protein level, may provide additional nutritive properties due to the presence of other components such as amino acids, polypeptides, vitamins, and lipids (Tasei and Aupinel, 2008a; Alaux et al., 2010; Vanderplanck et al., 2014a).

CONCLUSIONS

Our results have shown that polyfloral pollen diets are more suitable for the mass rearing of bumblebees than unifloral pollen diets. Bumblebee producers should also take into account the pollen protein levels so that the breeding process can be accelerated and the production costs reduced. Further studies to determine the protein quantity and quality and other nutritive components of the pollen blends used by bumblebee producers will be useful for maximising the quality and profitability of artificially reared colonies.

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