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Sphaerophoria rueppelli adults change their foraging behavior after mating but maintain the same preferences to flower traits

Neus Rodríguez-Gasol¹, Jesús Avilla², Simó Alegre¹, Georgina Alins¹

¹ IRTA Fruitcentre, PCiTAL, Park of Gardeny, Fruitcentre Building, 25003 Lleida, Spain

e-mail: neusgasol@gmail.com

² Department of Crop and Forest Science, Agrotecnio, University of Lleida, Avda. Alcalde Rovira Roure 191, 25199 Lleida, Spain

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1. INTRODUCTION

Hoverflies (Diptera: Syrphidae) are an abundant group of beneficial arthropods in agroecosystems. The larvae of about one third of the species feed on soft-bodied Hemiptera, mainly aphids (Hemiptera: Aphididae) (Rojo et al. 2003) and therefore have a high potential for biological control purposes (Hickman and Wratten 1996; Skirvin et al. 2011; Tenhumberg and Poehling 1995; van Rijn et al. 2006; White et al. 1995). Adult hoverflies, in turn, feed on nectar and pollen. Nectar is rich in carbohydrates, which provide the energy required for adult survival, while pollen supplies the proteins and amino acids that allow sexual maturation in both sexes and egg production in females (Gilbert 1981; Haslett 1989a).

Flowers use a variety of cues and rewards to attract visitors, and the efficacy of these traits depends on the spatial scale and the type of pollinator. Hoverflies use floral resources selectively (Gilbert 1981), choosing flowers on the basis of one or more attractive traits (Ambrosino et al. 2006). When foraging, hoverflies rely mainly on visual cues, like the size (Conner and Rush 1996; Sutherland et al. 1999), shape (Gong and Huang 2009) and color (Day et al. 2015; Dinkel and Lunau 2001; Laubertie et al. 2006; Sutherland et al. 1999) of flowers. The latter seems to be crucial for the attraction of hoverflies, as several studies have demonstrated that these insects show a strong preference for yellow (Haslett 1989b; Laubertie et al. 2006; Lunau and Wacht 1994; Sutherland et al. 1999), and that this color can even elicit a proboscis extension response in some species (Dinkel and Lunau 2001; Lunau and Wacht 1994). Nonetheless, other flower traits like odors (Laubertie et al. 2006; Nordstrom et al. 2017; Primante and Dotterl 2010) and accessibility to nectar and pollen can also play an important role in hoverfly preference (Branquart and Hemptinne 2000b; Gilbert 1981; van Rijn and Wäckers 2016). The relative attractiveness of the floral traits differs on the basis of the intrinsic attributes of the visitors, such as species (Haslett 1989b; Lunau et al. 2018), age (Almohamad et al.

2009; Sadeghi and Gilbert 2000; Sutherland et al. 1999) and sex (Sutherland et al. 1999). Sex differences are determined mainly by different nutritional requirements. Males of hoverflies only need an initial amount of pollen to allow spermatogenesis and can later feed mostly on nectar to meet their high energetic demands for sustaining their search for mates. On the other hand, females require higher amounts of pollen and for a longer period in order to achieve ovary maturation and continuous oviposition (Branquart and Hemptinne 2000a; Gilbert 1993; Gilbert 1981; Haslett 1989a). In this regard, hunger can also increase hoverfly attraction to yellow water traps, while satiated individuals are less likely to invest effort in investigating a distant food source (Hickman et al. 2001; Laubertie et al. 2006; Wratten et al. 2003).

Due to the dependence of adult hoverflies on flowers, these insects have been widely used in habitat manipulation schemes to enhance agroecosystem services in farmlands, such as biological control and pollination (Gurr et al. 2017; Haenke et al. 2009; Hogg et al. 2011; Landis et al. 2000; Macleod 1999; Wratten et al. 2012). Notwithstanding, more research is needed to identify the common visual traits that attract hoverflies to flower resources and to better understand the foraging behavior of these insects. In this regard, the objectives of this study were: 1) to assess the attractiveness of three flower traits (shape, number of flowers and color diversity) to hoverflies; and 2) to determine whether mating alters the foraging behavior of these flies.

2. MATERIALS AND METHODS

2.1. Insects

To study hoverfly preferences and behavior, adults of *Sphaerophoria rueppelli* (Wiedemann) (Diptera: Syrphidae) were used. Hoverfly pupae were purchased (Sphaerophoria-System, Biobest, Belgium) and left to develop in a phytoclimatic chamber (Fitoclima, ARALAB 10000 HP) under the following conditions: 25/19 ± 0.5

°C day/night, 60 ± 2 % relative humidity, 16:8 h (L:D) photoperiod, and light intensity of 22,200 lux. The emerged individuals were separated into three categories (adult types from now onwards): virgin males, virgin females and gravid females. In order to obtain virgin individuals, adults between 1 and 24 hours old were placed in two cages: one for males and one for females. Gravid females were obtained by placing males and females in the same cage from emergence to the date of the experiment. After the experiments, virgin and gravid females were examined to check their mating status (Gilbert 1993). Adult hoverflies were fed bee-collected commercial pollen (Apisol, Spain), water and a solution of 1.6% sucrose absorbed on filter paper, all provided *ad libitum*. In addition, a plastic pot (12 cm width x 12 cm length x 20 cm height) with 13 wheat plants infested with aphids was introduced into each cage in order to stimulate mating behavior and oviposition.

2.2. Artificial flowers

White EVA foam (FAIBO, Spain) was used to make the corolla (2 cm diameter) of the artificial flowers, and a 10 cm-long metal wire was used for the stem. The flowers were painted fluorescent yellow, blue or white (3104 Gelb, 3107 Blau, 3108 Weiss, respectively, Sparvar RAL Leuchtfarbspray (Spray-Color GmbH, Merzenich, Germany)).

To evaluate the most attractive flower traits, the following three consecutive experiments were performed (Fig. 1):

- Experiment 1: we made yellow flowers with a diameter of 2 cm and tested the following: a sphere-shaped, a circle-shaped, and a five-petal flower.
- Experiment 2: the shape with the best results in the previous trial was evaluated in bouquets of 1, 3, 6, or 12 artificial flowers.

- Experiment 3: the bouquet with the best results obtained in Experiment 2 was used to assess five combination of colors: 1) all-yellow bouquet, 2) $\frac{1}{2}$ yellow and $\frac{1}{2}$ white bouquet, 3) $\frac{1}{2}$ yellow and $\frac{1}{2}$ blue bouquet, 4) $\frac{1}{2}$ white and $\frac{1}{2}$ blue bouquet, and 5) $\frac{1}{3}$ yellow, $\frac{1}{3}$ white and $\frac{1}{3}$ blue bouquet.

2.3. Experiments

To run the experiments, 15 individuals aged between 7 and 20 days old were exposed to the artificial flowers or bouquets for 60 min per replicate. During this period, the behavior of the hoverflies was recorded using a JVC Everio GZ-MG610 Camcorder at a distance of 50 cm from the flowers or bouquets. These flowers were placed close and along the narrowest side of the cage, spaced 15 cm from each other. The videos were watched, and visits and landings were counted in each replicate. A visit was defined as an individual hovering at less than 3 cm from a flower, and a landing as resting on a flower. A new visit or landing was considered when a hoverfly approached a flower again after previously moving more than 6 cm away from it.

In each replicate, no experienced hoverflies were used and no food or reward was offered during the experiments. Experiments were run in a climatic chamber with the same conditions as those used to maintain the insects. Recordings were performed during all day round, the groups tested were randomized in order to avoid time effects.

2.3.1. Experiment 1

Four replicates for each type of adult were done and the position of the treatments was different in every replicate. Furthermore, all the flower shapes were tested in all the possible positions, thereby avoiding positional preferences. The experimental cage measured 40 cm x 80 cm x 40 cm (width x length x height).

2.3.2. Experiment 2

Replicates and experimental conditions were as in Experiment 1.

2.3.3. Experiment 3

Five replicates for each type of adult were done. In this case, the color of the flower in each bouquet on which insects landed was noted. A larger cage was used in this experiment (50 cm width, 58 cm length and 58 cm height) because of the higher number of artificial flowers tested.

2.3. Data analysis

To study hoverfly preferences to the distinct artificial flowers, we calculated the percentage of visits, landings, and visits + landings and performed a two-way ANOVA (adult type and treatment). In Experiment 3, we also evaluated the color preference for landing. To this end, the percentage of landings per color, regardless of the type of bouquet was calculated. The bouquet which was only yellow (Y) was excluded from this analysis. In this case, neither non-transformed nor transformed data met the ANOVA assumptions, so a two-way analysis was not possible. Since data met the ANOVA assumptions when they were analyzed by adult type, we carried out a one-way ANOVA to test color preferences by type of adult. To analyze behavioral differences between types of adult, the total number of visits and landings was used to perform a two-way ANOVA (adult type and treatment).

When needed, data were arcsin-transformed (in the case of the percentages) or log-transformed (in the case of the sums) to meet the ANOVA assumptions. The HSD-Tukey was used as a post-hoc test after the ANOVA to compare means. Data were analyzed using the JMP statistical software package (Version 13; SAS Institute Inc., Cary, North Carolina).

3. RESULTS

3.1. Selection of the attractive traits of flowers

In Experiment 1, there was no significant interaction between type of adult and treatment (shape of the flower) (Table 1). Flower-shaped flowers were significantly less visited and landed by hoverflies than the circle-shaped and sphere-shaped ones. However, when considering visits and landings together, only the circle-shaped flower was significantly more attractive than the flower-shaped flower (Fig. 2a). Moreover, though no statistical differences were observed between the sphere-shaped flower and the circle-shaped one, the latter was the more approached shape, so it was therefore selected for the next experiments.

In Experiment 2 there was no significant interaction between type of adult and treatment (no. of flowers per bouquet) (Table 1). The number of visits and landings increased as the number of artificial flowers in the bouquet rose (Fig. 2b). Hoverflies visited bouquets with 3 or 12 flowers significantly more than single flowers. In regard to landings, there were significantly more landings on 12-flower bouquets than on 3-flower ones or the single flower. When considering visits and landings together, bouquets of 12 flowers were more attractive than the others (Fig. 2b).

In Experiment 3 there was no significant interaction between type of adult and treatment (composition of the bouquet) (Table 1). The results were similar for all types of approaches: the artificial bouquet that combined yellow and white flowers (YW) received significantly more visits and landings than the rest. The second most visited bouquet comprised only yellow flowers (Y), followed by those with white, blue and yellow flowers (WBY) and then yellow and blue flowers (YB). The least attractive bouquet combined white and blue flowers (WB) (Fig. 2c).

In regard to the color that attracted most landings in Experiment 3, data were analyzed by type of adult. Significant differences were found regarding the percentage of landings on the different colors (Table 2). All adults showed a clear preference for yellow flowers and significantly discriminated between the three colors (Table 2).

3.2. Assessment of mating effects on hoverfly foraging behavior

No significant interaction between type of adult and treatment was found in any of the experiments (Table 3). Significant differences were found between the adult type in Experiments 1, 2 and 3 with regard to movement (Table 3). In Experiments 1 and 2, virgin females made significantly more visits than virgin males and gravid females, while in Experiment 3 virgin females and virgin males moved significantly more than gravid females (Fig. 3). Regarding landings, in Experiment 1 virgin females made significantly more landings than gravid females, while no significant differences were found in the case of virgin males. In Experiment 2 both virgin and gravid females, made significantly more landings than virgin males. No significant differences were found between types of adult in Experiment 3 (Fig. 3).

4. DISCUSSION

To study the attractiveness of distinct flower traits to hoverflies, three consecutive laboratory experiments were run. In Experiment 1 we observed that the hoverflies preferred a circle-shaped flower to a sphere-shaped one, and landed more on the former (Fig. 2a). On the basis of these observations, we conclude that the presence of petals did not have a significant effect on *S. rueppelli* attraction. Golding et al. (1999) found that the number of visits to oilseed rape (*Brassica napus* L. Brassicaceae) by *Episyrphus balteatus* De Geer (Diptera: Syrphidae) was not affected by the presence of petals, thereby suggesting that these parts of the flower are not an attractive cue to this species. On the other hand, floral guides (markings present in the corolla that act as close-range signals to direct pollinators to the floral rewards) have been demonstrated to be very effective in *Eristalis tenax* L. (Diptera: Syrphidae) (Dinkel and Lunau 2001). Petals are often a different color to the corolla and have markings to guide pollinators to the center of the

corolla. In our artificial flowers, there was no color difference between the calyx and the petals, nor were any petal markings present. It is therefore possible that hoverflies were not able to discern the different flower parts and did not detect petals as such but only a flower with an unusual contour, thus resulting in an unattractive shape. Regarding preferences between the circle- and the sphere-shaped flowers, hoverflies took longer to land on the latter (personal observation). When attempting to land on sphere-shaped flowers, they hovered above them, as if doubting how to land, and when they finally landed they continuously walked all over them. To the best of our knowledge, this is the first report of this kind of behavior. We propose that when hoverflies were on the sphere-shaped flower they were not aware of their relative position, as hoverflies always perceived the same shape. In contrast, they landed rapidly on circle-shaped flowers. We therefore assume that the presence of a flat surface helped hoverflies to land and forage. As the circle was the shape that was most approached and also the easiest to construct, it was selected for the following set of experiments.

In Experiment 2, we observed that the number of visits and landings increased with the number of flowers in the bouquet (Fig. 2b). We assume that hoverflies related the higher number of flowers in the bouquet with a greater probability of finding food. In this regard, Conner and Rush (1996) stated that hoverflies prefer to visit wild radish (*Raphanus raphanistrum* L. (Brassicaceae)) inflorescences with a higher number of flowers rather than those with few flowers. In addition, several authors have reported that hoverfly populations and diversity are related to the abundance of flowers (Haenke et al. 2009; Meyer et al. 2009; Power et al. 2016; Sutherland et al. 2001). On the other hand, it could also be concluded that hoverflies are not attracted by the higher number of flowers but by the increased area as a result of it. However, Sutherland et al. (1999) demonstrated that *E. balteatus* hoverflies preferred 2 cm diameter flowers to 7 cm ones. Hence, as our results

are in agreement with the aforementioned authors, we selected a 12-flower bouquet for the next step.

In Experiment 3 we observed that hoverflies showed a preference for bouquets with yellow and white flowers. In contrast, bouquets that combined white and blue were the least attractive, together with yellow and blue bouquets (Fig. 2c). Previous studies have reported that flower patches with a high diversity of flowers are more attractive to hoverflies (Blaauw and Isaacs 2014; Hegland and Boeke 2006). Conversely, Warzecha et al. (2018) found that the abundance of hoverflies was enhanced by the availability of key plant species in a flower mixture rather than by the diversity of species in it. Coinciding with this author, in our case, the approaches did not increase with diversity of colors, as some colors were more attractive than others: combinations with yellow were preferred to others, and blue was less attractive than white (Fig. 2c). Therefore diversity emerges as a plus in the attraction of hoverflies: however, diversity has to be well selected in order to be functional.

With regard to the preferred landing color, all three adult types showed a preference for yellow flowers independently of the number of colors or combinations present in the bouquet (Table 2). Indeed, other studies have demonstrated that hoverflies show a strong preference for yellow (Day et al. 2015; Laubertie et al. 2006; Sutherland et al. 1999). Given that many of these insects feed preferentially on yellow pollen (Lunau 1995; Lunau and Maier 1995), this color might be interpreted as the location of a food resource. Therefore the combination of certain colors appears to encourage the flies to approach more than a single color. However, at a closer range, yellow might elicit a change in hoverfly behavior, enhancing landings and thus foraging for pollen.

When studying hoverfly preferences to the three flowers attributes, the three types of adult behaved similarly (Table 1, Fig. 2 and Table 2). However, they showed differences in movement (number of approaches). Virgin females tended to be the group that showed

the most movement, followed by virgin males, gravid females being those that moved the least (Fig. 3). We did not study the reasons behind this behavior. However, we can hypothesize that virgin males moved more than females with the intention of finding a mate. In several hoverfly species, male mating behavior consists of pouncing on females that are feeding on flowers, and quickly initiating copulation (Maier 1978; Maier and Waldbauer 1979). Our virgin males were observed to throw themselves over other individuals resting on the cage walls or on the artificial flowers. The males attacked in this manner responded by flying away and, as a result, the general activity in the cage was higher. Virgin females did not show such behavior. However, they also moved considerably, in most cases tending to be the group showing the most movement. We attribute the high number of visits made by virgin females to the inspection of the flowers while hovering close to them and rapidly discarding them for landing. Virgin females may have had the capacity to determine whether the artificial flower had nectar or pollen before landing and preferred to invest more energy visiting other unexplored flowers that may have provided better resources.

On the other hand, gravid females were calmer, visiting fewer flowers and landing on at least half the flowers they visited. Furthermore, when landing on an artificial flower, they spent more time on it (personal observation). This might be explained by an increased interest in finding pollen and/or nectar. Male and female hoverflies have different nutritional requirements, females usually requiring higher amounts of pollen to allow continuous oviposition (Branquart and Hemptinne 2000a). In addition, Hickman et al. (1995) and Irvin et al. (1999) found differences between mated and unmated females regarding pollen uptake. Gravid females ingested higher pollen amounts than non-gravid ones, and even the latter ingested more pollen than males. In our case, gravid females were observed to behave differently to virgin individuals.

In our study, gravid females appeared to discriminate less than virgin females and virgin males because they did not discard the flowers before landing, but landed on the few flowers they visited and explored them exhaustively, possibly driven by their increased need for pollen. The loss of discrimination in ageing hoverflies has also been reported not only when foraging (Sutherland et al. 1999) but also when searching for oviposition sites (Almohamad et al. 2009; Chandler 1968; Sadeghi and Gilbert 2000).

Our results are in agreement with Day et al. (2015), who found that under field conditions adult hoverflies laid more eggs on broad bean (*Vicia faba* L. (Fabaceae)) infested with pea aphids (*Acyrtosiphon pisum* Mordvilko (Hemiptera: Aphididae)) when yellow model flowers were present nearby, in spite of these having no reward. These authors proposed that the increased oviposition was a result of the preference of gravid females for yellow. They also concluded that color attraction had a greater effect on enhancing oviposition than food resources. Although we did not study oviposition behavior, we observed that, in addition to a strong preference for rewardless yellow flowers, gravid females were less mobile than virgin individuals and they landed more frequently on the resources that they visited.

The inherent characteristics of species imply differences in their attraction to floral cues such as petal attractiveness (Golding et al. 1999) or color preferences (Sutherland et al. 1999). However, it is important to identify the common traits between species, such as the strong attraction to yellow color, in order to select the more commonly attractive cues. This knowledge can help to improve habitat management practices through the selection of flower species that present the most common attractive cues to hoverflies. In consequence, hoverfly populations will be enhanced, and therefore an increase on biological control and pollination is expected.

In conclusion, we have found that: 1) different flower shapes elicit diverse behaviors in hoverflies: rounded and flat shapes are the most attractive; 2) bouquets are more attractive

than single flowers; 3) more doesn't mean better in regard to flower diversity: combinations with yellow and white are preferred to those that contain blue; 4) mating modifies females' behavior: gravid females make fewer visits than virgin females.

Our findings have given a major insight into hoverfly behavior. This information can be used to improve the design of ecological infrastructures for the promotion of biological control and pollination, and therefore can contribute to increase the environmental sustainability of crops.

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Table 1 Summary of the ANOVA results (F-value and P) of the percentage of the different types of approach in Experiments 1, 2 and 3

Exp.	Type of approach	Treatment*Adult type		Adult type		Treatment	
		F-value	P	F-value	P	F-value	P
1	% Visits	F _{4,27} =1.571	0.211	F _{2,27} =0.000	1.000	F _{2,27} =15.801	0.001
	% Landings	F _{4,27} =1.727	0.173	F _{2,27} =0.000	1.000	F _{2,27} =8.435	0.001
	% Visits + Landings	F _{4,27} =1.537	0.220	F _{2,27} =0.000	1.000	F _{2,27} =7.526	0.003
2	% Visits	F _{6,24} =0.741	0.622	F _{2,24} =0.000	1.000	F _{3,24} =11.768	0.001
	% Landings	F _{6,24} =0.855	0.541	F _{2,24} =0.000	1.000	F _{3,24} =4.717	0.010
	% Visits + Landings	F _{6,24} =0.756	0.611	F _{2,24} =0.000	1.000	F _{3,24} =15.154	0.001
3	% Visits	F _{8,24} =0.487	0.861	F _{2,24} =0.000	1.000	F _{4,24} =15.824	0.001
	% Landings	F _{8,24} =0.411	0.910	F _{2,24} =0.000	1.000	F _{4,24} =78.840	0.001
	% Visits + Landings	F _{8,24} =0.274	0.972	F _{2,24} =0.000	1.000	F _{4,24} =43.244	0.001

Table 2 Percentage ($\pm$ standard error) of landings per color in Experiment 3. Different letters in the same column show significant differences between treatments ($P < 0.05$) according to the HSD-Tukey test

	Virgin females	Gravid females	Virgin males
Yellow	66.13 ± 3.43 a	76.49 ± 1.04 a	68.52 ± 5.62 a
White	26.62 ± 3.00 b	19.64 ± 0.78 b	25.93 ± 5.01 b
Blue	7.25 ± 2.13 c	3.87 ± 0.97 c	5.55 ± 2.25 c
F-value	$F_{2,12}=106.586$	$F_{2,12}=1665.820$	$F_{2,12}=50.180$
P	0.001	0.001	0.001

Table 3 Summary of the ANOVA results (F-value and P) of the number of visits and landings in Experiments 1, 2 and 3

Exp.	Type of approach	Treatment*Adult type		Adult type		Treatment	
		F-value	P	F-value	P	F-value	P
1	Number of visits	F _{4,27} =0.138	0.967	F _{2,27} =18.453	0.001	F _{2,27} =1.709	0.200
	Number of landings	F _{4,27} =0.441	0.778	F _{2,27} =4.167	0.027	F _{2,27} =4.911	0.015
2	Number of visits	F _{6,24} =0.308	0.927	F _{2,24} =6.250	0.007	F _{3,24} =2.022	0.138
	Number of landings	F _{6,24} =0.651	0.689	F _{2,24} =6.999	0.004	F _{3,24} =1.904	0.156
3	Number of visits	F _{8,24} =0.257	0.977	F _{2,24} =30.896	0.001	F _{4,24} =6.025	0.001
	Number of landings	F _{8,24} =0.098	0.999	F _{2,24} =1.340	0.270	F _{4,24} =37.504	0.001

Fig. 1 Schematic diagram of the three consecutive experiments performed. Grey circles represent yellow flowers, black circles represent blue flowers and white circles represent white flowers. In experiment 2: 1= a single flower, 3= bouquet of three flowers, 6= bouquet of six flowers, 12= bouquet of twelve flowers. In experiment 3: Y = all-yellow bouquet, YW = $\frac{1}{2}$ yellow and $\frac{1}{2}$ white bouquet, YB = $\frac{1}{2}$ yellow and $\frac{1}{2}$ blue bouquet, WB = $\frac{1}{2}$ white and $\frac{1}{2}$ blue bouquet, and WBY = $\frac{1}{3}$ yellow, $\frac{1}{3}$ white and $\frac{1}{3}$ blue bouquet.

Fig. 2 Percentage of hoverfly approaches (visits and landings) to the artificial flowers in (a) Experiment 1, (b) Experiment 2 and (c) Experiment 3. Vertical bars show standard error. Different letters within each experiment show significant differences between treatments ($P < 0.05$) according to the HSD-Tukey test. In experiment 2: 1= a single flower, 3= bouquet of three flowers, 6= bouquet of six flowers, 12= bouquet of twelve flowers. In experiment 3: WB = $\frac{1}{2}$ white and $\frac{1}{2}$ blue bouquet, WBY = $\frac{1}{3}$ yellow, $\frac{1}{3}$ white and $\frac{1}{3}$ blue bouquet, Y = all-yellow bouquet, YB = $\frac{1}{2}$ yellow and $\frac{1}{2}$ blue bouquet, and YW = $\frac{1}{2}$ yellow and $\frac{1}{2}$ white bouquet.

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472 **Fig. 3** Number of approaches for the three types of adult in (a) Experiment 1, (b)
 473 Experiment 2 and (c) Experiment 3. Vertical bars show standard error. ns= $P>0.05$.
 474 Different letters within each experiment show significant differences between treatments
 475 ($P<0.05$) according to the HSD-Tukey test.