concentration (ca. $1.5 \times 10^{-5} M$) of myco-laminaran to reduce glyceollin production in soybean cotyledons. This difference was probably due to partial degradation of mycolaminaran, when applied to soybean tissues, since soybean tissues contained β-1,3-endoglucanase capable of degrading the β-1,3-glucan [9,10].

The present study demonstrated the existence of a specific binding site in soybean membranes for an elicitor which was generated under conditions analogous to the natural host-pathogen interaction. In addition to the several properties of the binding, the fact that inhibition of the elicitor binding by mycolaminaran resulted in the inhibition of glyceollin production otherwise induced by elicitor strongly suggested that the observed binding site indeed represents a receptor physiologically important for phytoalexin elicitation. The present study also demonstrated the production by the fungus of a suppressor which inhibited glyceollin production in soybean cotyledons otherwise induced by cell-wall-derived elicitor and disclosed a unique mechanism of the suppressor to compete for elicitor binding to the putative receptor. Although the production of suppressors has been suggested in a few fungi [7,8], the modes of action of suppressors are not well characterized [11]. Future study is necessary to evaluate whether the inhibition of elicitor binding and glyceollin production by mycolaminaran occurs in vivo in fungus-infected soybean tissues and whether the interactions between the elicitor and suppressor determine the disease specificity in this host-pathogen interaction system.

This research was supported in part by Grant-in-Aid (04257103) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

1. Keen, N. T., in: Plant Disease Control, p. 155. New York: Wiley 1981
2. Keen, N. T.: Science 187, 74 (1975)
3. Yoshikawa, M., Matama, M., Masago, H.: Plant Physiol. 67, 1032 (1981)
4. Yoshikawa, M., Takeuchi, Y., in: Molecular Strategies of Pathogens and Host Plants, p. 165. New York: Springer 1991
5. Takeuchi, Y., Yoshikawa, M., Horino, O.: Ann. Phytopathol. Soc. Japan 56, 523 (1990)
6. Yoshikawa, M., Takeuchi, Y., Horino, O.: Physiol. Mol. Plant Pathol. 37, 367 (1990)
7. Shiraishi, T., Oku, H., Yamashita, M., Ouchi, S.: Ann. Phytopathol. Soc. Japan 44, 659 (1978)
8. Doke, N., Garas, N. A., Kuc, J.: Physiol. Plant Pathol. 15, 127 (1979)
9. Yoshikawa, M., Keen, N. T., Wang, M. C.: Plant Physiol. 73, 497 (1983)
10. Keen, N. T., Yoshikawa, M.: ibid. 71, 460 (1983)
11. Shiraishi, T., Araki, M., Yoshioka, H., Ichinose, Y., Yamada, T., Oku, H.: Plant Cell Physiol. 32, 1067 (1991)
12. Yoshida, S., Uemura, M., Niki, T., Sakai, A., Gusta, L. V.: Plant Physiol. 72, 105 (1983)
13. Keen, N. T., Yoshikawa, M., Wang, M. C.: ibid. 71, 466 (1983)

Visual Modulation of a Scent-Marking Activity in the Honeybee *Apis mellifera* L.

M. Giurfa* and J. Núñez

Dpto. de Ciencias Biológicas, FCEyN, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Since the pioneer work of Karl von Frisch, much knowledge has been gained in relation to the different sensory modalities that a bee can exploit to attain a greater efficiency while foraging on a patch of flowers. However, only rare attempts have been made to elucidate the mechanisms whereby a forager selectively employs and/or discards some sensory cues of flowers in its decision-making process. Honeybees, for example, mark exhausted [1] and recently visited flowers with a repellent scent [2]. Since in a foraging bout, bees can visit from 1 to 1400 flowers [3], the possibility that a forager may mark any visited flower could be a rather uneconomical strategy. In this work we raised the question of whether the use of the repellent mark depends of the presence of color signals which could contribute per se to the discrimination between rewarding and unrewarding flowers. We show that the repellent scent-marking activity significantly decreased whenever both kinds of flowers could be visually distinguished, providing thus the first example for a visual modulation of scent-marking activity in honeybees.

We trained honeybee workers, *Apis mellifera ligustica*, from a hive 50 m from the laboratory, to visit and collect 50 % sucrose solution on a food-source simulator (see details in [2]) with 12 identical, numbered flowers. They were spaced 10 cm apart and disposed on a neutral medium-gray background. Six of these flowers received the same flow of sucrose solution (0.34 µl/min) from an automatic microburette of the Scholander type, which was driven by a synchro-motor. Sucrose solution is odorless to honeybees [4]. Bees reached the sucrose solution through a PVC tube on each flower, which acted as a "corolla". The other six flowers yielded no reward. The disposition of rewarding flowers was established following a random number series. To avoid the use of flower position as orientation cue, the patch was sometimes irregularly rotated [2]. Only one bee was allowed to forage on the simulator and was observed over five consecutive visits to the test array. Only bees whose nonvisit times (time spent

* Current adress: Dr. Martin Giurfa,
Institut für Neurobiologie der Freien Universität, Königin-Luise-Straße 28/30, D-14195 Berlin

away from the patch between visits) were less than 5 min were used (mean ± S. E. = 4.02 ± 0.36 min; n = 80). Twenty bees were studied, totaling 100 visits.

The rationale of the experimental design was to record repellent scent-marking behavior when bees are confronted with rewarding and unrewarding flowers differing in color perceptual distances. For this, a series of four shades from a basic yellow (bee-green) pigment was obtained. Figure 1 shows their loci in the color opponent space of the honeybee [5]. The essence of such a representation of color is that the three receptor signals, corresponding to the three photoreceptor types of the honeybee, are assumed to be evaluated in two spectrally opponent neural mechanisms. If the weighting factors of these spectral opponencies are known, as in the case of the honeybee, colors can be represented in a two-dimensional diagram using as axes the two scales assigned to the respective spectrally opponent mechanisms and where the distance between loci is directly related to the perceptual distance between stimuli. Color distance between two stimuli S_1 and S_2 can be calculated as the sum of the absolute differences in the two corresponding excitation values A and B (city-block metric) from Fig. 1 [5]:

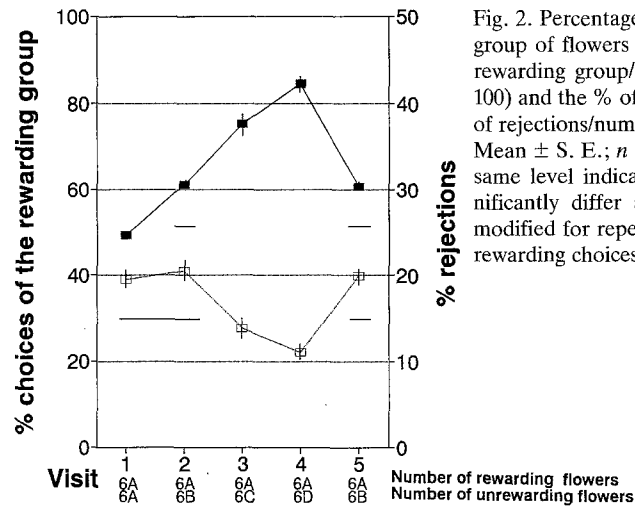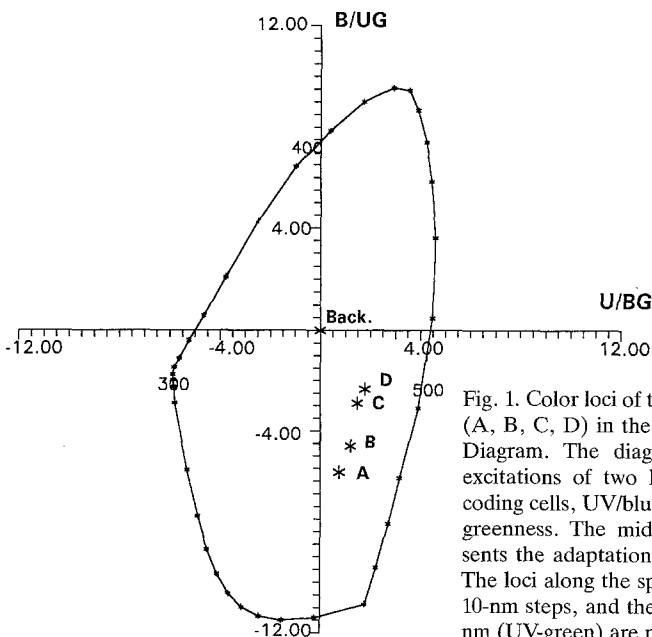$$D(S_1, S_2) = |A_1 - A_2| + |B_1 - B_2| \qquad (1)$$

Fig. 2. Percentage of choices of the rewarding group of flowers (number of landings on the rewarding group/total number of landings × 100) and the % of rejections per visit (number of rejections/number of total landings × 100). Mean ± S. E.; n = 20. Horizontal lines at the same level indicate means which do not significantly differ after a Newman-Keuls test modified for repeated measurements, -■- % rewarding choices, -☐- % rejections

In this case, colors A and D were the most distant in perceptual terms.
For the first visit, both the rewarding and the unrewarding group of flowers presented color A. For the following visits, color A was always assigned to the rewarding group. For the second visit, the unrewarding group presented color B (perceptually closest to color A); for the third visit, color C; for the fourth visit, color D (i.e., the maximal perceptual distance between both colors, A and D, was attained) and finally, for the fifth visit it again presented color B as a control. The spatial distribution of rewarding and unrewarding flowers was inverted from one visit to another.

During each visit, the number of landings on both the rewarding and the unrewarding flowers was recorded. Rejections (landings in which the bee introduced only the antennae into the access tube and immediately flew away) were distinguished. With these data, the percentage of choices of the rewarding group per visit (number of landings on the rewarding group/total number of landings × 100) and the % of rejections per visit (number of rejections/number of total landings × 100) were calculated. The latter is a direct measure of the repellent scent-marking activity [2]. To have a standardized measure, rejections are expressed here as percentage of the number of landings since visits can have different durations and therefore different absolute numbers of landings and rejections.

Since the same individuals were repeatedly tested over a period of time, for repeated measurements ANOVA was employed and comparisons between means were performed with a Newman-Keuls test modified for repeated measurements [6].

Bees performed a mean number of 171.37 ± 3.56 landings per visit (mean ± S. E.; n = 100), staying 24:11 ± 1:24 min on the patch (mean ± S. E.; n = 100). Figure 2 shows that the greater the perceptual difference between the rewarding and the alternative color, the more accurate was the choice of the group of rewarding flowers (fourth visit). This variation was clearly significant (F = 83.05; df: 4,76; n = 20; $P < 0.0001$). When the color information was the same for both groups of flowers, no asymmetries in group choice were

Fig. 1. Color loci of the four color stimuli used (A, B, C, D) in the Color Opponent Coding Diagram. The diagram represents the cell excitations of two kinds of color opponent coding cells, UV/blue-greenness and blue/UV-greenness. The middle point (Back.) represents the adaptation light (gray background). The loci along the spectral line are marked in 10-nm steps, and the mixtures of 300 and 550 nm (UV-green) are marked in 10% steps

detectable (first visit), although one group was not rewarding as explained above. Although bees learned to distinguish color A as the rewarding one, the observed variation was not only the result of improved performance due to learning, as shown by the decrease observed on the fifth visit (A vs. B), where the perceptual distance between colors was again reduced. The choice level attained there did not differ significantly from the levels of the second visit, where the same situation was presented (mean difference = 0.51; C.V. = 5.61; NS).

In the same way, the % of rejections also varied in a significant (F = 28.53; df: 4,76; n = 20; $P<0.0001$) but inverse way: the greater the perceptual difference between the rewarding and the alternative color (and therefore the better the choice of the rewarding one), the lower the % of rejections (fourth visit). This % increased again when the perceptual distance between the colors used decreased in turn (fifth visit). In other words, rejections dramatically decreased whenever the task of discriminating rewarding from unrewarding flowers could be solved visually.

One may argue that the variation in the % of rejections did not result from a visual modulation of the scent-marking activity but only reflects the fact that the unrewarding flowers were less visited by the bees whenever these could be distinguished on the basis of their spectral properties. In other words, the decrease in the % of rejections would only represent the decrease in landings on the unrewarding flowers. This, however, ignores the fact that bees may also mark rewarding flowers [2]. It is, thus, critical

Fig. 4. Correlation between color distances of the stimuli used in foraging visits and percentage of choices of the rewarding group of flowers
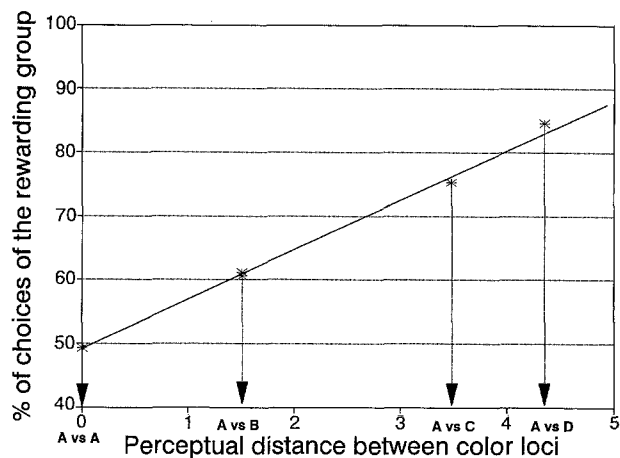
to demonstrate that the postulated modulation occurred in the *rewarding* flowers. Figure 3 depicts the % of rejections of these flowers (number of rejections of rewarding flowers/total number of rejections × 100). Bees marked the rewarding flowers when they could not distinguish them visually but they did not mark them when visual discrimination was possible. The observed variation was highly significant (F = 21.05; df: 4,76; n = 20; $P<0.0001$) and clearly demonstrates that the scent-marking activity here was actually modulated through visual input.

Bees do not have a brightness dimension, at least in all tasks involving color discrimination [5, 7, 8]. Brightness differences can only influence the bees' choice behavior insofar as they are responsible for shifts in color loci (Bezold Brücke Effect; see [8]). Since brightness, as a parameter is clearly ignored by the bees in color choice experiments, one should thus concen-

trate on color distances between the stimuli used as modulating visual input.

In our experimental procedure, color A has been compared to itself and to the alternatives B, C, and D. Figure 4 depicts the correlation between the perceptual distances of the color stimuli used, calculated after Eq. (1), and the % of choices of the rewarding group obtained. The variation in perceptual color distances can account for the variation in choices of the rewarding group since both variables display a highly significant correlation (r = 0.9979, df: 3; $P<0.001$, two-tailed).

We showed thus that the repellent scent-marking strategy can be modulated by the availability of spectral signals providing the necessary and sufficient information to discriminate between rewarding and unrewarding flowers (Figs. 2, 3). This work underlines, therefore, the fact that honeybees are capable of using one or more sensory cues to enhance their foraging efficiency, according to the environmental situation. If one cue is good enough for this aim, then alternative strategies related to other sensory modalities can be totally or partially abandoned. The variation in the choice of the rewarding group could be explained here by the exclusive basis of color discrimination. The bees correctly chose the rewarding group whenever it was possible to distinguish it from the alternative color in perceptual terms.

In a rich, natural flower market, flowers differ not only in their colors, but also in their forms and odors, thus providing conspicuous signals which can be detected and learned by pollinators [9]. The repellent mark, therefore, would be an auxiliary "tool" to attain a greater
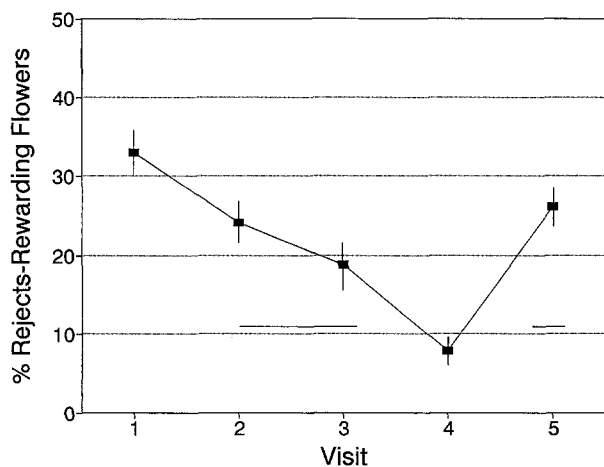

Fig. 3. Percentage of rejections of the rewarding flowers (number of rejections of the rewarding flowers/total number of rejections × 100). Mean ± S. E.; n = 20. Horizontal lines at the same level indicate means which do not significantly differ after a Newman-Keuls test modified for repeated measurements

foraging efficiency and to be employed in situations where no alternative cues are available to discriminate between rewarding and unrewarding, similar morphs from a particular flower species. The use of such a mark may also depend on other factors such as the morphology of the flower, the nectar flow provided, the number of available flowers, and the interflower distance to cite some of the possible elements which may be considered.

1. Núñez, J. A.: Naturwissenschaften 54, 322 (1967)
2. Giurfa, M., Núñez, J. A.: Oecologia 89, 113 (1992); Giurfa, M.: Ins. Soc. 40, 59 (1993)
3. Ribbands, C. R.: J. Anim. Ecol. 18, 47 (1949)
4. Frisch, K. v.: The dance language and orientation of bees. Cambridge: Harvard University Press 1967
5. Backhaus, W.: Vision Res. 31, 1381 (1991)
6. Winer, B. J.: Statistical principles in experimental design. New York: McGraw-Hill 1971
7. Daumer, K.: Z. vergl. Physiol. 38, 413 (1956); Helversen, O. v.: J. Comp. Physiol. 80, 439 (1972); Backhaus, W., Menzel, R., Kreißl, S.: Biol. Cyber. 56, 293 (1987); Giurfa, M.: J. Insect. Physiol. 37, 41 (1991); Chittka, L., Beier, W., Hertel, H., Steinmann, E., Menzel, R.: J. Comp. Physiol. 170, 545 (1992)
8. Backhaus, W.: Vision Res. 32, 1425 (1992)
9. Menzel, R., in: Experimental behavioural ecology, p. 55 (B. Hölldobler, M. Lindauer, eds.). Stuttgart: Fischer 1985; Weiss, M. R.: Nature 354, 227 (1991); Chittka, L., Menzel, R.: J. Comp. Physiol. 171, 171 (1992)

Laboratory Rearing of *Ooencyrtus pityocampae* in Unfertilized and Unlaid Eggs of *Thaumetopoea* Species

G. H. Schmidt and J. Kitt

Lehrgebiet Zoologie-Entomologie, FB. Biologie der Universität, D-30419 Hannover

Thaumetopoea species are forest pests and their caterpillars, which form processions, produce many urticating hairs which are responsible for allergic reactions in man and animals [1]. Control of the species is necessary and is carried out mainly by insecticides or BT preparations. Halperin [2,3] was the first who used *Ooencyrtus pityocampae* Mercet (Insecta: Hymenoptera: Encyrtidae) for biological control of *T. wilkinsoni* Tams in Israel by mass breeding of this parasitoid in eggs of *Bombyx mori*. Battisti et al. [4] tried to rear the same egg parasitoid on artificial substances; it developed until the stage of *matura larva* in artificial eggs with synthetic membranes.

For research work concerning the biology of egg parasitoids, it is necessary to have enough suitable eggs at a definite stage of development. In *Thaumetopoea*

species, which are widespread in Mediterranean countries, this is an important problem because the adults do not copulate under laboratory conditions, and the females do not deposit eggs without copulation. In the field, egg laying takes place mainly at night and it is not easy to find freshly laid egg batches. Thus, the occurrence of parasitization is uncertain.

For our investigation of the mechanism of parasitoid diapause, masses of unlaid eggs were obtained by dissecting freshly emerged females in the laboratory. In springtime during pupation of *T. pityocampa* and *T. wilkinsoni* it is possible to collect pupae in sandy areas at a soil depth of 10–20 cm and store them until September–October when the adults emerge. A prolonged pupal diapause of 1–5(7) years was also observed. Thus,

adults were obtained from one batch of pupae over several years. Each adult female produces up to about 300 ripe eggs which are deposited as one batch after copulation [5,6]. In the absence of copulation, the abdomen of the female was opened and the unlaid eggs were removed. Groups of 50 eggs were exposed for 2 weeks to two females of *O. pityocampae*, an egg parasitoid of both species[7].

To demonstrate the effectivity of the parasitoids two experiments were carried out with 50 eggs and 2 parasitoids each. In both cases we received a high parasitization rate; the hatching rate was calculated to be 53 % in November and 66 % in May (Fig. 1). Under natural conditions in Israel, the highest parasitization rate of one egg batch was found to be 74 % [8]. In unlaid eggs parasitized in May, the parasitoids emerged during two periods of the year (Fig. 2). The first group emerged after some weeks, the second after some months. This corresponds with the appearance of the egg parasitoids under field conditions. No indication could be found for auto- or superparasitism caused by *O. pityocampae* as it is assumed by Battisti [9] for the eclosed parasitoids in September; the second period of eclosion showed no remains of auto- or superparasitoid development [8]. Only one parasitoid