Interaction of visual and olfactory cues in the aggregation behaviour of the haematophagous bug *Triatoma infestans*

**Abstract** We analysed how the assembling behaviour of *Triatoma infestans* is modulated by the convergence of chemical cues released by their faeces and the spectral quality of the light associated with refuges. Second-instar larvae were confronted with refuges associated with a visual stimulus (either blue, green or red lights having the same intensity, or darkness) and a chemical cue (presence or absence of faeces). In this context, faeces constitute a major attractant for bugs. In the absence of faeces, bugs always assembled in dark places. Green light was always rejected despite the presence of faeces, i.e. the assembling behaviour was controlled by a photonegative reaction to this light. In the presence of red light, orientation towards the chemical cue dominated over the bugs’ photonegative reaction to this light. Such a light was avoided in the absence of faeces but not in their presence. Also, negative phototaxis to blue light could be counteracted by the presence of the chemical cue. Thus, a concrete interaction between visual and olfactory cues occurred in these experiments: the bugs’ response changed depending on the specific combination of spectral light and faeces. Finally, bugs responded differentially to blue, red and green lights of the same intensity. They assembled preferentially on red, followed by blue and then by green. Thus, discrimination between lights of different spectral quality is possible, probably through an achromatic mechanism.

**Key words** Triatomines · Assembling behaviour · Phototaxis · Vision · Olfaction

**Introduction**

Insects employ a variety of different sensory modalities to guide themselves, and to identify an appropriate object or condition (Dusenbery 1992). Visual, olfactory and mechanical signals are commonly used by insects for locating food, oviposition sites and mates (Bradbury and Bennett 1974). Co-occurrence of signals of different sensory modalities results in *multimodal convergence*, i.e. in the uptake of information by functionally different sense organs and the resulting interaction at different levels of the sensory pathway (Horn 1985). Such an interaction can occur at the receptor level, in ascending or descending neurones, or even in motoneurones. Usually multimodal convergence elicits specific behavioural reactions. Sometimes animals respond only if a specific pattern of sensory cues takes place, but in other cases some cues are predominant.

Host location in haematophagous insects is an integrated response driven by different sensory cues (Lehane 1991). Multimodal responses in haematophagous insects have a high adaptive value because they improve the chances of host finding (Lehane 1991). These insects use mainly visual, olfactory and thermal signals in their search for food (Bradbury and Bennett 1974; Warnes and Finlayson 1985; Allan et al. 1987; Lehane 1991). This fact has been used in the control of insect vectors: for instance, coloured targets baited with host odours (CO₂) are more efficient in capturing tsetse flies than coloured targets lacking odour (Green 1986, 1993).

*Triatoma infestans* is a haematophagous insect which has a fundamental epidemiological importance in South America as it is the main vector of Chagas disease. Adults and larvae of this bug are domestic in habitat. They exhibit a strong negative reaction to light (Reisenman et al. 1998) and spend daylight hours assembled in shaded places inside wall crevices. The aggregation
behaviour of triatomine insects is mediated mainly by thigmotaxis and by volatiles present in their faeces (Lorenzo Figueiras et al. 1994). In particular, faeces constitute chemical markers for signalling refuges (Lorenzo and Lazzari 1996). Triatomine bugs become active at night when they search predominantly for hosts. To this aim they use different sensory modalities among which olfaction and thermal orientation play an important role (Núñez 1987; Lorenzo Figueiras et al. 1994; Flores and Lazzari 1996; Lorenzo and Lazzari 1996).

The interaction between visual and olfactory cues and their role in the natural behaviour of T. infestans has never been studied. As for many nocturnal insects (Koehler et al. 1987; White et al. 1994; Cutler et al. 1995), visually mediated responses are relevant for T. infestans (Lazzari and Várju 1990; Lazzari et al. 1998; Reisenman et al. 1998). Illumination elicits a strong photonegative reaction in this bug (Reisenman et al. 1998), whilst in general terms, odours activate and orient the insects towards hosts (Núñez 1987) and refuges (Lorenzo and Lazzari 1996).

In this work we investigated how the assembling behaviour of T. infestans is modulated by the convergence of chemical cues present in its faeces and the spectral light quality associated with refuges. Specifically, we asked whether the combination of olfactory and visual stimuli evokes responses that differ from those to individual stimuli. This information is relevant to understand how multimodal convergence operates in the behavioural context of assembling.

Materials and methods

Animals

Second-instar larvae of T. infestans from our laboratory colony were used throughout. Insects were fed weekly on heparinised bovine blood, using an artificial feeder (Núñez and Lazzari 1990). Bugs werestarved for approximately 5–7 days post-ecdysis before experiments.

Procedure

Both the experimental set-up and the procedure used to study the aggregation behaviour of T. infestans have been previously described (Lorenzo Figueiras et al. 1994). A circular arena (diameter 14 cm, height 3 cm) lined with a filter paper as substrate was divided in two or three equal sectors (Fig. 1). On each sector, a filter paper (3 cm × 1.5 cm) folded once was placed 2 cm away from the wall and provided refuge for the bugs. The contribution of both visual and olfactory cues in the assembling behaviour of T. infestans was studied by presenting visual and olfactory cues combined or separated. Visual stimulation was provided by different spectral filters (see below) illuminated from below and covered with the paper substrate. Olfactory stimulation was provided by small pieces of filter paper impregnated with faeces (see below).

In each assay, a group of 9–15 insects contained in a vial was carefully released in the centre of the arena. After 1 h the number of animals in each sector, around or inside refuges, was recorded. To avoid external interferences an acrylic cube without a base covered the whole arena. The acrylic cube was covered in turn by a black cardboard cube. The latter presented a small hole on top to observe the position of the bugs in the arena (Fig. 1a).

Between assays, the materials were carefully cleaned with hexane or ethanol, and the filter papers (substrate, refuges and olfactory stimulation sources) were discarded and replaced by new ones. All experiments were done at 24–26 °C. Each bug was used once. The position of the stimuli and that of the arena were randomly changed between assays.
Olfactory stimuli

Filter papers (each 1 cm × 1.5 cm) impregnated with dry faeces from larvae of *T. infestans* were used as source of chemical stimulation. Faecal droplets were obtained by slight compression of the last section of the abdomen with tweezers. Faeces obtained in this way are effective in inducing aggregation in triatomines (Lorenzo Figueiras et al. 1997). Faecal droplets from different animals were diluted with distilled water to homogenise their distribution on the filter papers. Each paper was impregnated with 50 μl of an 8% (v/v) of this aqueous solution and used 24 h later. At this time faeces were effective in eliciting aggregation behaviour (Lorenzo Figueiras et al. 1994).

Visual stimuli

The experimental arena was illuminated from below. The light source was a white halogen lamp (OSRAM 644055 G4, 5 W/12 V) placed in a central position (Fig. 1a). Coloured stimuli were provided by squared spectral filters (side 5 cm, thickness 1 mm; Schott, Germany). They were placed on the ground of the glass arena, below the substrate of filter paper (see Fig. 1a). Black cardboard squares having the same dimension as the filters were used as “dark zones”. Thus, before reaching the insects, the light passed through the diffusing glass base, the Petri dish, the spectral filters, and finally through the paper substrate. The paper substrate did not modify the spectral composition of the light source (transmittance measurements performed with a Hewlett Packard diode array spectrophotometer HP8452A).

The spectral filters used were BG 12, VG 9 and OG 590 and were blue, green and red to humans, respectively. The choice of these filters allowed to cover the spectral range from 400 nm to 700 nm and beyond (Fig. 2).

The light intensity that reached the insects was measured with a radiometer (SEL 033 sensor module, IL 1400 radiometer; International Light, USA). To this aim, the surface of the sensor was placed upside down within the arena. Measurements were performed on the spectral filters (or the black cardboard) covered by the paper substrate, and on the paper substrate alone (background). The light intensity measured at the coloured stimuli was kept at a constant value of 1.68–2.00 μW cm⁻², by using neutral-density papers. The intensity of the black cardboard square was 0.00 μW cm⁻². The intensity of the white background alone was 21.7–25.4 μW cm⁻². Thus, as the spectral stimuli had a light intensity ten times lower than that of the white background, they acted as contrasting low-light-intensity zones with a defined spectral quality. As bugs prefer low-light-intensity places for assembling, our design ensured that insects actively searched for refuges, and thus uncovered the effect of light of different spectral qualities in their aggregation.

Experiments

Experiment 1: assembling response to olfactory cues

This experiment was performed in order to test that the standard experimental conditions employed (i.e. the amount of faeces, the light intensity, etc.) were adequate to reproduce in a consistent way the assembling behaviour of *T. infestans* as already characterised in previous studies (Lorenzo Figueiras et al. 1994). Three series were conducted in a three-sectored arena (Fig. 1b1):

1. Each sector had a clean filter paper and a refuge [number of animals (n) = 60, number of replicates (k) = 5].
2. Each sector had a filter paper impregnated with faeces and a refuge (n = 48, k = 4).
3. One sector had a filter paper impregnated with faeces and a refuge, whilst each of the two other sectors had a clean paper and a refuge (n = 117, k = 10).

The first and second series allowed the distribution of insects among refuges to be established when these are identical in terms of olfactory information. The third series enabled aggregation as evoked by the olfactory signal to be tested.

Experiment 2: assembling response to visual cues in the presence or absence of olfactory cues

A three-sectored arena was used in this experiment. In each sector we presented a different coloured light (blue, green and red) associated with a refuge (Fig. 1b1i). Two series were performed:

1. Each sector had a clean filter paper (n = 177, k = 12).
2. Each sector had a filter paper impregnated with faeces (n = 178, k = 12).

In both series the three sectors of the arena were equivalent with respect of their olfactory information. Thus, they should be chosen equally if there are no preferences for a given spectral light. Moreover, a comparison of the results from both series will show whether the simultaneous presence of colour and odour changes the assembling pattern obtained for each cue separately.

Experiment 3: assembling response to a combined stimulation of visual and olfactory cues

A two-sectored arena was used in these assays since pairs of stimulation conditions were being tested. In the first to fourth series, a sector with a black cardboard (B) was always presented against a sector with a spectral filter (C), blue, green or red (Fig. 1b1ii). Faeces could be absent or present in one or both sectors (see Tables 1, 2, 3 for values of n and k). Thus, the following experimental series were conducted:

1. Series BC: each sector (black and coloured) had a clean filter paper.
2. Series B⁺C⁻: the black sector had a paper impregnated with faeces (⁺) whilst the coloured sector had a clean filter paper.
3. Series BC⁻⁺: the sector with the coloured stimulus had a paper impregnated with faeces whilst the black sector had a clean filter paper.
4. Series B⁻C⁻⁺: each sector had a paper impregnated with faeces.

![Fig. 2](image-url) Spectral transmittance of the glass filters used as coloured stimuli. Filters were BG 12, VG 9 and OG 590 (Schott, Germany; side 5 cm, thickness 1 mm) and appeared blue, green and red to humans, respectively.
The first series (BC) was performed to investigate whether any of the coloured stimuli was equivalent to darkness for the bugs. The other series (B⁺C, BC⁺ and B⁻C⁺) were performed to study the interaction between visual stimuli of different spectral composition and the olfactory signal.

In the fifth series both sectors presented the same visual stimuli [either blue (L), green (G), red (R) or black (B)] but only one of them presented faeces (see Table 4 for values of n and k). The aim of this series was to determine the role of olfactory cues in the presence of visually identical refuges. Specifically, we asked whether olfactory cues lose their effectiveness in inducing aggregation when combined with a particular spectral light.

Statistical analysis

The distribution of insects in the arena was tested against a random distribution by means of a G-test of goodness of fit (i.e. one-third or one-half of the animals in each sector, depending on the use of the three-sectored or two-sectored arena, respectively; Sokal and Rohlfs 1981). The results of the three-sectored arena were analysed by a subdivided G-test analysis (log-likelihood ratio) to find variations in the number of bugs aggregated in different sectors (Zar 1984).

Results

Experiment 1: assembling response to olfactory cues

Three experimental series were conducted in the three-sectored arena. In the first and second series, the aggregation of bugs was not statistically different from a random distribution (Fig. 3, G-test: first series: \( G = 2.0, df = 2, \) n.s; second series: \( G = 0.2, df = 2, \) n.s.). In the third series, bugs significantly aggregated in the sector of the arena with faeces (Fig. 3, G-test: \( G = 13.1, df = 2, P < 0.001 \)). Therefore, olfactory cues from faeces proved to be effective in eliciting the assembling response of T. infestans under our experimental conditions.

Experiment 2: assembling response to visual cues in the presence or absence of olfactory cues

In this experiment each sector of the arena had a different coloured light and a refuge, in the absence (first series) or presence (second series) of faeces.

Figure 4 shows that the type of spectral light significantly affected the aggregation behaviour of T. infestans. In both series the distribution of bugs among refuges differed significantly from random (first series: \( G = 15.6, df = 2, P < 0.001; \) second series: \( G = 49.5, df = 2, P < 0.001 \)). The addition of faeces modified the aggregation evoked by spectral cues alone (first versus second series: \( G = 13.6, df = 2, P < 0.005 \)). For each light the specific effect of faeces (white versus black bars in Fig. 4) could not be analysed because the response to a single light was always affected by the response to the other two lights. Such effect was specifically studied in the next section (experiment 3) where dual-choice experiments allowed to exclude this interference.

In both series bugs avoided the sector with the green light (G-subdivided analysis, first series: \( G = 15.7, df = 1, P < 0.001; \) second series: \( G = 6.5, df = 1, P < 0.025 \)). In the absence of faeces (first series), bugs

![Fig. 3 Olfactory cues in the assembling response of T. infestans. Results of three experimental series are shown (mean ± SE). The dashed line at 33.33% indicates random choice in an arena with three sectors. In the first series (\( n = 60, k = 5 \)) each refuge had a clean filter paper; in the second series (\( n = 48, k = 4 \)), each refuge had a filter paper impregnated with faeces. In the third series (\( n = 117, k = 10 \)), only one refuge had a filter paper with faeces whilst the other two presented clean papers](image1)

![Fig. 4 Visual cues in the assembling response of T. infestans in presence or absence of olfactory stimulation. Each sector of the three-sectored arena presented a different spectral light (blue, green and red) associated with a refuge. Results of two experimental series are shown (mean ± SE). The dashed line at 33.33% indicates random choice in an arena with three sectors. In the first series, each refuge had a clean filter paper (open bars; \( n = 177, k = 12 \)); in the second series, each refuge had a filter paper impregnated with faeces (striped bars; \( n = 178, k = 12 \)). Means coded by different lower-case (first series) or upper-case (second series) letters differ significantly at the 5% level](image2)
assembled equally on the blue and red sectors (G-subdivided analysis, \( G = 0.3, df = 1 \), n.s.). However, in the presence of faeces, they preferred the red sector (G subdivided analysis, \( G = 43.0, df = 1, P < 0.001 \)), followed by blue and finally by green (G-subdivided analysis, \( G = 6.46, P < 0.05 \)). Thus, bugs always avoided green light and preferred a refuge where both red light and faeces were present.

Experiment 3: assembling response to a combined stimulation of visual and olfactory cues

In this experiment a two-sectored arena was used. One sector had a black cardboard and the other sector had a spectral light. The first series excluded olfactory cues and was aimed to study whether bugs equated any of the spectral lights with darkness (black cardboard). If this were the case, bugs should choose equally both the sector with the spectral light (blue, green or red) and the sector with the black cardboard. This never occurred. For all three colours, bugs significantly preferred the black sector (open bars in Fig. 5a, b, c and first row of Tables 1, 2, 3). Therefore, no spectral light was equivalent to darkness.

In the second series, in which faeces were added only to the dark sector, the preference of bugs was also clear:

![Graphs](image)

**Fig. 5a-c** Visual and olfactory cues in the assembling response of *T. infestans*. One sector of the two-sectored arena presented a spectral light (a blue, b green or c red) and the other sector a black cardboard. Results of four experimental series are shown (mean ± SE). Faeces could be absent in both sectors (first series: open bars), present only in the sector with the black cardboard (second series: black bars), present only in the sector with the spectral light (third series: grey bars), or present in both sectors (fourth series: striped bars). The dashed line at 50% indicates random choice between both sectors of the arena. Asterisks indicate significant differences from a random distribution (*\( p < 0.05 \); **\( p < 0.01 \))

<table>
<thead>
<tr>
<th>Series</th>
<th>G</th>
<th>( P )</th>
<th>( n )</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BL</td>
<td>24</td>
<td>&lt; 0.001</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>B+L</td>
<td>14.4</td>
<td>&lt; 0.001</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>BL+</td>
<td>2.94</td>
<td>n.s.</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>B+L+</td>
<td>19.87</td>
<td>&lt; 0.001</td>
<td>65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series</th>
<th>G</th>
<th>( P )</th>
<th>( n )</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BG</td>
<td>44.4</td>
<td>&lt; 0.001</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>B+G</td>
<td>25</td>
<td>&lt; 0.001</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>BG+</td>
<td>15.3</td>
<td>&lt; 0.001</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>B+G+</td>
<td>23.75</td>
<td>&lt; 0.001</td>
<td>78</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series</th>
<th>G</th>
<th>( P )</th>
<th>( n )</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BR</td>
<td>18.8</td>
<td>&lt; 0.001</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>B+R</td>
<td>18.8</td>
<td>&lt; 0.001</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>BR+</td>
<td>4.7</td>
<td>&lt; 0.05</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>B+R+</td>
<td>1.3</td>
<td>n.s.</td>
<td>80</td>
</tr>
</tbody>
</table>
for all the three spectral lights they assembled in the dark sector (black bars in Fig. 5a, b, c and second row of Tables 1, 2, 3). Such a result is expected from the joint attractiveness of darkness and faeces.

In the third series (grey bars in Fig. 5a, b, c and third row of Tables 1, 2, 3) faeces were only associated with the spectral light, thus contrasting the attractive effect of faeces with that of darkness. For the blue light, the addition of faeces changed the original preference of bugs for the black sector (Fig. 5a and third row of Table 1). In this case, the choice between both sectors was not different from random even if a tendency towards the black sector was observable. In the case of the green light, the addition of faeces did not modify the original preference for the dark sector (Fig. 5b and third row of Table 2). In the case of the red light, the preference for the black sector was reversed when faeces were associated with the light (Fig. 5c and third row of Table 3). Bugs preferred the sector with the red light and faeces to the sector with the black cardboard alone.

Finally, in the fourth series (striped bars in Fig. 5a, b, c and fourth row of Tables 1, 2, 3) faeces were presented in both sectors. For both blue and green lights, bugs aggregated in the dark sector (Fig. 5a, b and fourth row of Tables 1, 2). For the red light, however, bugs distributed randomly between sectors (Fig. 5c and fourth row of Table 3). These results show that preference for darkness predominates over olfactory cues in the case of blue and green lights. When a red light was used, such predominance disappeared and aggregation was guided mainly by olfactory cues.

In the fifth experimental series, both sectors presented the same visual stimuli but only one of them had faeces. Figure 6 and Table 4 show that bugs significantly preferred the sector with faeces to the clean one when both sectors contained a spectral light (blue, green or red).

![Fig. 6 Visual and olfactory cues in the assembling response of T. infestans (mean ± SE). Both sectors of a two-sectored arena displayed the same visual information but only one of them was associated with faeces. The dashed line at 50% indicates random choice between both sectors of the arena. Asterisks indicate significant differences from a random distribution (*P < 0.05; **P < 0.01) (Table 4 G-test analysis of data from experiment 3. Both sectors presented the same visual stimuli (L: blue, G: green, R: red, B: black cardboard) but only one was associated with faeces (+) (n number of insects; k number of replicates, n.s. not significant)

<table>
<thead>
<tr>
<th>Series</th>
<th>G</th>
<th>P</th>
<th>n</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LL+</td>
<td>9.52</td>
<td>~0.01</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>GG+</td>
<td>4.08</td>
<td>~0.05</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>RR+</td>
<td>4.06</td>
<td>~0.05</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>BB+</td>
<td>0.10</td>
<td>n.s.</td>
<td>89</td>
</tr>
</tbody>
</table>

However, insects distributed randomly when both sectors contained a black cardboard. In this case, the visual input (i.e. darkness) predominated over the olfactory one.

**Discussion**

The present work analyses for the first time how sensory cues interact in the assembling behaviour of T. infestans. In particular, we focused on two of the main cues that mediate assembling: chemical cues from faeces (Lorenzo Figueiras et al. 1994), and visual cues (light versus darkness) that elicit a photonegative reaction. As expected from previous results (Lorenzo Figueiras et al. 1994), faeces alone induced assembling (Fig. 3). Also, in the absence of faeces, darkness was always preferred to any spectral light (Fig. 5, white bars). This result was also expected from the bugs’ photonegative reaction (Reisenman et al. 1998). The three spectral lights used induced different levels of aggregation although they had the same intensity (Fig. 4). Thus, blue, green and red were differentially evaluated by the insects. In particular, all experiments were consistent in showing that green light was always rejected by bugs (Figs. 4, 5). Blue and red lights were less rejected than the green one. None of them was equated to darkness (Fig. 5).

The addition of faeces changed the choice pattern of spectral lights: bugs preferred to assemble on the red light followed by the blue and then by the green light (Fig. 4, striped bars). When insects had to choose between two identical lights, one with faeces and the other without faeces, they followed the odour cue (Fig. 6). When a colour light was tested against a black cardboard, the effect of faeces varied depending on the spectral light. Green light was always rejected despite the presence of faeces on it (Fig. 5b). In the case of blue light, faeces counteracted the preference of bugs for darkness such that both were equally chosen (Fig. 5a). Finally, red light with faeces was preferred to darkness (Fig. 5c). Thus, a concrete interaction between visual and olfactory cues was shown in these experiments such that the response of bugs changed depending on the specific combination of spectral light and faeces.

In the following sections we will discuss the response of T. infestans to visual cues and to the combined stimulation of visual and olfactory stimuli. The response to olfactory cues alone as found in this work is already well known (Lorenzo Figueiras et al. 1994; Lorenzo Figueiras and Lazzari 1998, 2000) and thus will not be discussed here.
Response of *T. infestans* to visual cues

Bugs responded differentially to blue, red and green light, thus implying that discrimination among these stimuli is possible. As the light intensity of the coloured stimuli was always kept at an equal constant value, the response of *T. infestans* can be interpreted on the basis of two main hypotheses. First, bugs discriminated between spectral lights on the basis of chromatic mechanisms. Their behaviour would be indeed consistent with a common definition of colour vision, which is the capacity of distinguishing between spectral lights independently of intensity differences (Menzel 1979). Second, bugs discriminated between spectral lights on the basis of achromatic mechanisms; i.e. their response would be colour blind. These mechanisms could be based on either a single receptor signal, or on the summed signals of different receptor types. Responses mediated by a single receptor type are common in insects (Menzel 1979; Menzel and Backhaus 1991). Achromatic responses based on the summed signals of different receptor types have been also found in the phototactic response of bees leaving a food source (Menzel and Greggers 1985).

Subtractive interactions between at least two different photoreceptor types are required for true colour vision (Menzel and Backhaus 1991). Unfortunately, there is no information about photoreceptor types in *T. infestans*, thus making speculative any conclusion about true colour vision. The second possibility, the colour-blind response, is consistent with the nocturnal habits of *T. infestans*. The simplest explanation for the spectral sensitivity of this insect as found in this work is to assume that its photonegative reaction is mediated by a single receptor type, maximally sensitive in the green region of the spectrum. This would explain why bugs always avoided refuges associated with green light and why even the attractive nature of faeces cannot counteract such a primary avoidance. Although such a receptor type is not known in *T. infestans*, in nearly all insects so far studied a photopigment with maximal absorption between 490 nm and 550 nm has been found (Chapman 1998). Assuming the existence of such a theoretical green receptor also explains why red and blue lights yielded a similar level of aggregation (Fig. 4, white bars). The amount of stimulation produced in this photoreceptor by both stimuli would be in principle similar. A receptor type maximally sensitive in the green region (ca. 550 nm) has been shown to mediate several colour-blind responses in some insect species such as those related to motion detection (Lehrer 1994). However, we cannot discard that the discrimination of lights in our experiments was not based on other kinds of achromatic mechanisms.

Response of *T. infestans* to the combined stimulation of visual and olfactory cues

In analysing the interaction between both sensory modalities (olfactory and visual) the question arises of which one is more effective in eliciting the assembling response of bugs around refuges. However, in answering the question one needs to consider that such a response arises from two specific behavioural drives: an attraction mediated by faeces, and a photonegative reaction. The interaction between these specific inputs in this behavioural context proved to be dependent on the specific combination of stimuli used.

When green light was used, insects always assembled in the dark sector despite the presence or absence of faeces. Therefore, we conclude that in this case the assembling behaviour was controlled basically by the bugs’ photonegative reaction to green light. In this condition the response to the olfactory signal was inhibited by the visual input. When red light was used, insects assembled in the sector with faeces, either the sector with the black cardboard or the one with the light. When both sectors contained faeces, bugs distributed at random. These results show that in presence of red light the assembling behaviour was controlled by the chemical cue. Finally, when blue light was used, an intermediate response was found: bugs distributed at random between a black cardboard alone and blue light plus faeces. In this case, the two stimuli (olfactory and visual) were equally able to induce assembling when set in competition. When faeces were only associated with the black cardboard, insects always preferred it to the blue light. Thus, the assembling behaviour was controlled by a hierarchy of sensory inputs in which darkness was ranked highly, followed by faeces and by blue light. Finally, although the differences were not statistically significant, for all the three spectral lights the percentage of bugs assembled in the dark sector was lower in presence than in absence of faeces (first versus second series, Fig. 5). This result could be interpreted as a non-specific effect of faeces or as an increase of activity due to the presence of the chemical attractant (activation).

When both sectors of the arena were presented with the same spectral light but only one of them had faeces (Fig. 6), insects assembled in the sector with the chemical cue. However, when two black cardboard were offered (only one of them presented faeces), insects distributed randomly. These results can be explained by postulating that each spectral light elicits a photonegative reaction and this, in turn, elicits a search for chemical cues as the latter would be the only cue available to identify a potential refuge. When darkness is available, it is sufficient to arrest the bugs on it, independently of the presence of chemical cues.

Ecological significance

The ecological correlate of the assembling response to a combined stimulation of visual and chemical cues is obvious: in an illuminated ambient, it is of fundamental importance to find a protected place. To this aim, a primary reaction is to avoid light. The bugs’ high sensitivity to green light is consistent with this
interpretation. Light reflected from terrestrial environments during the day is characterised by a high content of green wavelengths (Menzel 1979; Dusenbery 1992). Thus, refuges associated with green light would be assimilated to open places where the risk of being predated is high.

Faces act as chemical landmarks for refuges, in particular at dawn, when the ambient light intensity increases (Lorenzo and Lazzari 1996, 1998). When insects look for protected places, they search for chemical cues that guide them towards shelters. Thus, our results stress the biological significance of multimodal responses in triatomines, which would not rely on an unique input to find a resource.

In addition, our results would be relevant for optimising the design of artificial devices aimed to detect Chagas disease vectors in the field. At present, manual collection and cardboard boxes that offer shelter for bugs are the methods employed for monitoring triatomine populations (Gürtler et al. 1999). The use of chemical attractants (i.e. faces) that actively draw insects into such devices could improve their effectiveness. We here show that the spectral properties of an artificial refuge affects the probability that insects use it as a shelter, and modulates the response to a chemical attractant. According to our results, baited refuges should be as dark as possible and should avoid to reflect green wavelengths of light.

Acknowledgements The authors are grateful to the staff members of the Laboratorio de Fisiologia de Insectos (UBA) for many helpful discussions, and also to the referees for their helpful suggestions. This work received support from the University of Buenos Aires, CONICET (Argentina) and the WHO/UNDP/World Bank Special Programme for Research and Training in Tropical Diseases (TDR). This work was also made possible by a DAAD fellowship to CR.

References


Dusenbery (1992) Sensory ecology: how organisms acquire and respond to information. Freeman, New York


