Responses of the blister beetle *Hycleus apicicornis* to visual stimuli

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Abstract. Insect attraction to host plants may be partly mediated by visual stimuli. In the present study, the responses of adult *Hycleus apicicornis* (Guér.) (Coleoptera: Meloidae) to plant models of different colours, different combinations of two colours, or three hues of blue of different shapes are compared. Single-colour models comprised the colours sky blue, bright green, yellow, red, white and black. Sky blue (reflecting light in the 440–500 nm region) is the most attractive, followed by white, which reflects light over a broader range (400–700 nm). On landing on sky blue targets, beetles exhibit feeding behaviour immediately. When different hues of blue (of different shapes) are compared, sky blue is preferred over turquoise, followed by dark blue, indicating that *H. apicicornis* is more attracted to lighter hues of blue than to darker ones. No significant differences are found between the three shapes (circle, square and triangle) tested, suggesting that reflectance associated with colour could be a more important visual cue than shape for host location by *H. apicicornis*. The preference of *H. apicicornis* for sky blue can be exploited in designing an attractive trap for its management.

Key words. Blister beetles, colour targets, shape, spectral reflectance, visual cues.

Introduction

Insects make use of stimuli from their external environment, including visual and olfactory cues, to detect resources appropriate for their different needs, including their preferred hosts and mates (Bernays & Chapman, 1994; Rojas & Wyatt, 1999; Raguso & Willis, 2002; Schmera *et al*., 2004; Campbell & Borden, 2006a). Colour plays a major role in the visual ecology of insects for locating their mates (Allan *et al*., 1987; Brunton & Majerus, 1995; Jiggins *et al*., 2001) and for locating and identifying food resources (Menzel, 1979; Harris & Miller, 1983; Kelber *et al*., 2003b; Zaccardi *et al*., 2006). It is especially important for flower-visiting insects when discriminating between different flowers (Bernays & Chapman, 1994; Balkenius & Kelber, 2004; Hirota & Kato, 2004; Goyret

et al., 2007). Depending on the species, certain cues (visual or olfactory) work at longer range for orientation towards the resource, whereas others are employed at short range for landing or identifying the resources (Raguso, 2001; Ômura & Honda, 2005; Balkenius *et al*., 2006; Fukaya *et al*., 2006; Campbell & Borden, 2006b; Jönsson *et al*., 2007; Kulahci *et al*., 2008). The size, shape and colour of a potential host plant often comprise the three key components allowing an insect to differentiate visually between host and nonhost plants (Prokopy & Owens, 1983).

Colour can play a critical role in the visual attraction of insects irrespective of whether true colour vision or wavelength-specific behaviour is involved (Menzel, 1979). True colour vision is confirmed in a few orders such as Hymenoptera (Chittka & Menzel, 1992; Giurfa *et al*., 1997; Kelber *et al*., 2003b; Giurfa, 2004; Dyer & Chittka, 2004a, b) and Lepidoptera (Weiss, 1997; Kelber *et al*., 2003a, b; Goyret & Raguso, 2006). However, in other groups, such as Coleoptera, the presence or absence of true colour

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vision is not established, although, in some studies, beetles show behaviours associated with colour detection (Hausmann *et al*., 2004; Döring & Skorupski, 2007).

The ability of insects to perceive and detect colours enables them to make use of these as indicators of plant identity (Prokopy *et al*., 1983a; Lunau, 1993) and quality (Döring & Skorupski, 2007). In such cases, colours either mediate long-range orientation of an insect towards its host or landing on its host (Prokopy *et al*., 1983a, b). If the colour that stimulates an insect for host searching is known, it can be employed singly or in combination with other stimuli to manipulate its behaviour in pest management. Traps of certain colours and shapes attractive to specific insect taxa are reported for use in monitoring pest populations (Epsky & Heath, 1998; Blight & Smart, 1999) or in the mass trapping of pests (Hesler & Sutter, 1993).

Hycleus apicicornis (Guér.) (Coleoptera: Meloidae) is one of approximately five *Hycleus* Latreille species in western Kenya that are observed to feed on floral parts of crops such as *Desmodium* spp., sweetpotato, cowpea and okra (L. N. Lebesa, unpublished observations). *Desmodium* spp. intercropped with maize or sorghum represent an important component of 'push–pull' technology in the control of stemborers (Lepidoptera) and *Striga* spp. weeds (Khan *et al*., 2000, 2002, 2008). Production of *Desmodium* spp. seed is affected adversely by adult *Hycleus* spp. that feed on flowers. Consumption of flowers by blister beetles has a negative effect on seed setting (Evans *et al*., 1989; Gahukar, 1991; Lale & Sastawa, 2000). The flowers of different host plants of *H. apicicornis* differ in shape, size and colour, although the majority of their colours are hues of violet, as perceived by the human eye. By contrast to adults, larvae are carnivorous. Females lay their eggs in the soil. Upon hatching, larvae search for and feed on grasshopper eggs (Nikbakhtzadeh, 2004).

Hycleus apicicornis may be making use of visual cues associated with flowers of host plants together with olfactory cues to locate their food resources. In the present study, the behavioural responses of *H. apicicornis* to different colours (and associated reflectance spectra) and shapes are evaluated to determine the role of visual cues in the location of floral food resources by the beetle, and as part of the development of an efficient trapping system.

Materials and methods

Insects

Because *H. apicicornis* larvae are cannibalistic and go into diapause for extended periods, the rearing of blister beetles has proven difficult (Selander, 1986). Therefore, field-collected adults were used in the present experiments. To ensure representation of beetles that had learned to search for flowers of different host plants in different locations, adults were collected from different host species in three districts: on-station plots of silverleaf desmodium (*Desmodium uncinatum*) at ICIPE, Thomas Odhiambo Campus, Mbita (0°25′S, 34°12′E, 1200 m above mean sea level), sweetpotato and beans fields

in Bungoma (0°34′S, 34°30′E, approximately 1700 m above mean sea level), and from desmodium and sweetpotato fields in Kitale (1°28′S, 37°16′E, approximately 1890 m above mean sea level). In addition, adults were also collected on morning glory, *Ipomoea hilderbrantii*, a wild alternate host widely found at Mbita and neighbouring places. The insects were kept in cages in the laboratory and maintained on a mixed diet of differently coloured fresh flowers of different host plant species (silverleaf desmodium, morning glory, sweetpotato *Ipomoea batatas*, Convolvulaceae, okra *Abelmoschus esculentus*, Malvaceae, cowpea *Vigna unguiculata*, Fabaceae and bean *Phaseolus vulgaris*, Fabaceae). A stock of beetles was maintained by regular collection in the field throughout the duration of the experiments. Field-collected insects used in the experiments were maintained in the laboratory for a minimum of 1 week for acclimatisation and were not kept longer than 3 months.

Choice experiments of response to visual stimuli

Three different sets of experiments were conducted using three different types of paper models as colour targets for *H. apicicornis*. A preliminary trial showed that, when beetles were exposed to nonshiny cotton fabric targets and to printed papers, they landed more frequently on the latter, indicating that they preferred the reflective paper surfaces. Therefore, colours for the targets were generated using the red : green : blue (RGB) colour coordinates system and reproduced on 80-GSM printing paper (P.T. Pindo Deli Pulp & Paper Mills, Karawang, Indonesia) using an HP Laserjet 2600 printer (Hewlett-Packard, Palo Alto, California). All three experiments were undertaken in a screen-walled greenhouse (length 12 m, width 7 m, height 8 m; black screen mesh on the side walls and plexi-glass for the back and front walls and roof) (Seyoum *et al*., 2002). Plant models were placed 4 m from the rear side of the screened greenhouse, and approximately 7 m from the front. Targets were rearranged after every ten individuals had been observed, and papers were changed daily.

Adult beetles were separated by sex and starved for 25–26 h before use. Beetles were released at the middle point on the ground 3 m from colour targets. The ground was selected as the release point because beetles were observed to take off more readily after orientating themselves on the ground. Numbers of beetles landing on targets were quantified as described previously by Harris *et al*. (1993). Beetles were recorded as having made a choice when they took off from the ground, landed on a target and exhibited feeding behaviour. To avoid any interference or influence of conspecifics in their choice, one beetle was released at a time and was removed after making a choice. Each beetle was given 5 min to make a choice. If a beetle did not take off from the ground within 3 min, it was excluded from the experiment.

To reduce variation in behaviour, observations for all experiments were made on cloud-free days between 10.00 and 13.30 h, the period during which *H. apicicornis* is most active. Experiments were run for six or seven consecutive days using new sets of beetles every day. Depending on the availability of beetles, 30–70 beetles were used per day. Light intensity

(daylight) averages throughout the study period were in the range 55 983–67 700 lux. Mean temperature and relative humidity (RH) were 35 °C and 66%, respectively. Daily measurements for light, temperature and relative humidity were recorded with a digital light meter (RS 180-7133, RS Components, U.K.) and a dual Tinytag data logger for temperature and RH, TGP 4500 (Gemini data Loggers Ltd, U.K.), respectively.

Spectral reflectance measurements of coloured inks printed on white paper

The reflectance spectra of the coloured inks on white paper and of the white paper on which they had been printed, were measured using a Stellarnet EPP2000C spectroradiometer (Stellarnet Inc., Oldsmar, Florida; calibrated to an operating range of 300–850 nm). The instrument was calibrated against a high-reflectivity white standard (SRS-99-010, Labsphere Inc., North Sutton, New Hampshire). Measurements were taken at a distance of 35 cm below an overhead light source that emitted daylight wavelengths. The sensor of the spectroradiometer was positioned 5 cm away from the printed samples or reflectance standard, and at an angle of 45° to prevent shading. The spectra were recorded in steps of 0.5 nm, and the reflectance of the sample expressed as a percentage of that reflected by the white standard at each wavelength.

Experiment 1: models with single colour and different heights

To determine the attractiveness to beetles of the different colours as perceived by the human eye, six individual colours of the RGB colour system were used as paper targets in plant models [sky blue (0 : 204 : 255), bright green (0 : 255 : 0), yellow (255 : 255 : 0), red (255 : 0 : 0), white (255 : 255 : 255) and black (0 : 0 : 0)].

Blister beetles feed on flowers of different heights. Therefore, two different heights were evaluated to determine the preferred height for the subsequent experiments, and later, for the field trapping experiments. The models were constructed from five 4×4 cm^2 printed papers affixed on a wooden stick. Short plants were simulated by placing the models at 0.5 m and tall plants at 1 m above ground. Adults of *H. apicicornis* feed on flowers of different shapes and sizes, making it difficult to have one flower model representing all. Therefore, paper pieces were arranged to mimic the appearance of flowers of the target host plant, *D. uncinatum*, which have the appearance of small leaflets (Fig. 1, model A).

Twenty-four plant models (12 short and 12 tall) were placed in a straight line at a spacing of 0.3 m. Heights and colours were arranged randomly with the restriction that same heights and/or colours were not placed next to one another.

Experiment 2: models with different colours combined and different heights

To determine whether colour contrasts enhance landing preference, two colours in combination were used in one plant model. Sky blue was the most attractive colour in Experiment 1. Therefore, sky blue and one of the other colours (except black, because it did not attract any beetles) were combined and used together in plant models, as explained in Experiment 1. The model was similar to that in the first experiment, except that blue paper squares were alternated with
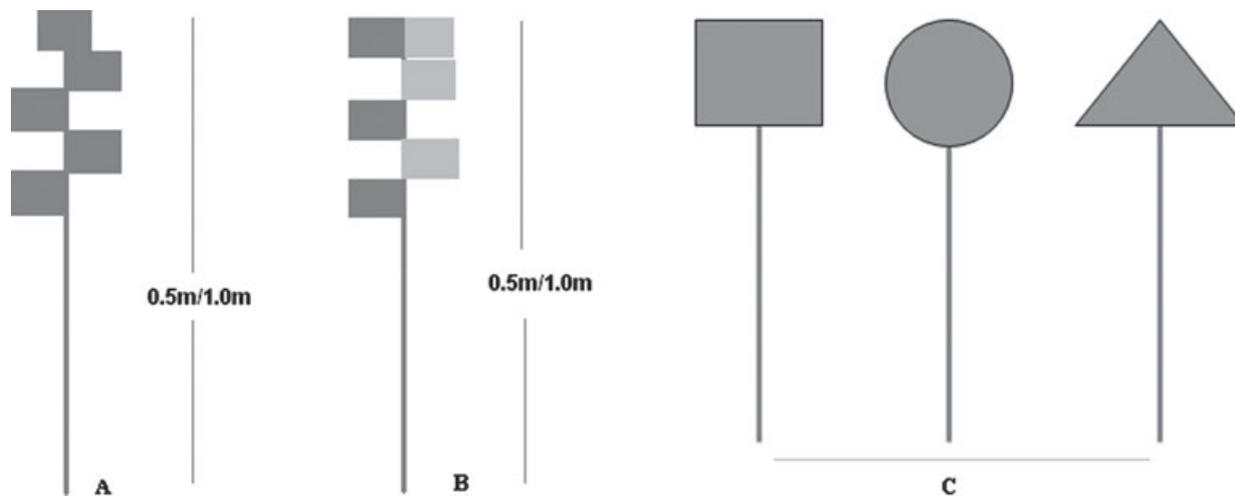

Fig. 1. A schematic drawing illustrating the plant models used as colour targets for adult blister beetles. Model A was used to determine the most attractive colour(s). Each plant model consisted of five (4×4 cm) square pieces of the same colour (Experiment 1). Model B consisted of the most attractive colour (blue), determined in Experiment 1, in combination with one of the colours: green, yellow, red or white. The lighter shade of grey in the figure represents any of the other colours, and dark grey indicates blue. Each plant model consisted of six (4×4 cm) square pieces: three blue and three of one of the other colours (Experiment 2). To identify the most attractive hues of blue (sky, turquoise and dark blue) and shape (triangle, square and circle), plant model C was used (Experiment 3). The height of models tested was 0.5 and 1.0 m for models A and B (to simulate short and tall plants) and 0.5 m for model C.

another colour; thus, six squares instead of five were used. The blue and the other colour were assigned randomly to either side of the model (Fig. 1, model B). As in Experiment 1, models were evaluated at two different heights: 0.5 and 1 m above ground. A sky blue, leaf-like model used in Experiment 1 was included for comparison. Twenty plant models (ten short and ten tall) were placed in a straight line at a spacing of 0.3 m. Heights and colours were arranged randomly with the restriction that same heights and/or colours were not placed next to one another.

Experiment 3: models of shapes and hues of blue

Because adult *H. apicicornis* feed on flowers of different shapes, the responses of adults to shapes and hues of blue (which was the most preferred colour) were evaluated. Three hues, sky blue (0 : 204 : 255), turquoise (0 : 255 : 255) and dark blue (0 : 0 : 128), and three shapes, square (8 × 8 cm), circle (9 cm diameter) and triangle (14 cm base, 9 cm height), were used in combination (Fig. 1, model C). Printed papers with different combinations of hues of blue and shape were affixed on wooden sticks to serve as plant models. A sky blue, leaf-like model used in Experiment 1 was included as control. For this experiment, only short plant models (0.5 m above ground) were used. Twenty models were placed in a straight line at a spacing of 0.3 m. Each of the models was represented twice in the line. Shapes and different hues of blue were arranged randomly with the restriction that similar hues were not placed next to each other.

Statistical analysis

Heterogeneity tests were carried out to determine whether data for different replicates could be pooled for subsequent analyses (Sokal & Rohlf, 1969; Zar, 1984). When data were homogeneous chi-square tests for testing the goodness-of-fit were carried out on pooled data. Where landing responses were found to differ significantly, paired chi-square tests corrected with the Bonferroni adjustment (Norman & Streiner, 1994) were used. Data were analysed with GENSTAT (Payne *et al.*, 2007) and STATISTICA, version 7.0 (StatSoft, Inc.,Tulsa, Oklahoma).

Results

General observations

Before taking off from the point of release, beetles would generally tilt their head upwards and fan their wings just before flying. Sometimes, a beetle would spin around in one position as if surveying the whole background before take-off. Some beetles did not fly directly towards the targets but flew straight to the roof of the screenhouse, especially towards the position of the sunlight, and then would wander around randomly on the roof before flying down to the targets. Upon reaching a

chosen target, a beetle would appear to explore the target, and would start to nibble, as if feeding. Sometimes, a target was approached by a beetle, which it would circle once or several times (when still in flight) before moving away and finally landing and settling on another target. In such a situation, the circled target was not considered to be a choice.

Experiment 1: models with a single colour and different heights

Of the 310 (males and females in equal numbers), beetles released in the presence of blue, green, yellow, red, white and black plant models, 74 (24%) did not make a choice. These were excluded from the subsequent analyses. Of the other 236 beetles, 169 (71.6%) landed on sky blue plant models, followed by white (12.3%), yellow (8.9%), red (4.7%) and green (2.5%). Black targets did not attract any beetles and models of this colour were excluded from subsequent experiments.

There were no significant differences between the numbers of male and female beetles attracted to the targets ($\chi^2 = 1.373$, d.f. = 1, $P = 0.241$) (Fig. 2a), so subsequent analyses were based on data pooled for both sexes. With the exception of green plant models, which attracted six beetles only, short plant models were preferred as landing targets by 67% of beetles that made a choice (Yates corrected $\chi^2 = 25.779$, d.f. = 1, $P < 0.001$); within each colour. Fifty-five percent to 76% of beetles landed on short plant models (Fig. 2b). Therefore, models of different heights were analysed separately.

Numbers of beetles landing on the models of different colours differed significantly for both heights (short plant models: $\chi^2 = 259.401$, d.f. = 4, $P < 0.0001$; tall plant models: $\chi^2 = 134.987$, d.f. = 4, $P < 0.0001$) (Fig. 2c,d). A comparison of the numbers attracted to the short blue, green, yellow, red and white plant models showed that the sky blue models differed from all others and were preferred by 71% of the beetles that landed. White models attracted 14% of beetles, yellow attracted 9%, red attracted 4% and green attracted 2%. The number of beetles landing on white models was not significantly different from yellow but differed from red and green (Fig. 2c). Similarly, for tall plant models, 71% of beetles preferred those that were sky blue (Fig. 2d). However, unlike the models of short plants, there was no significant difference between the number of beetles landing on white (9%), yellow (10%), red (6%) or green (4%) tall plant models (Fig. 2d).

Experiment 2: models with different colours combined and different heights

A total of 290 beetles were tested (males and females in equal numbers). Sixty-one beetles (38 females and 23 males) did not make a choice and were excluded from the analyses. Of the 229 that made a choice for a specific plant model, more beetles (31%) landed on nonmixed sky blue plant models, followed by sky blue-white (26%), sky blue-red (17%), sky blue-green (14%) and sky blue-yellow (12%) models. There were no significant differences between the numbers of male and female beetles making a choice
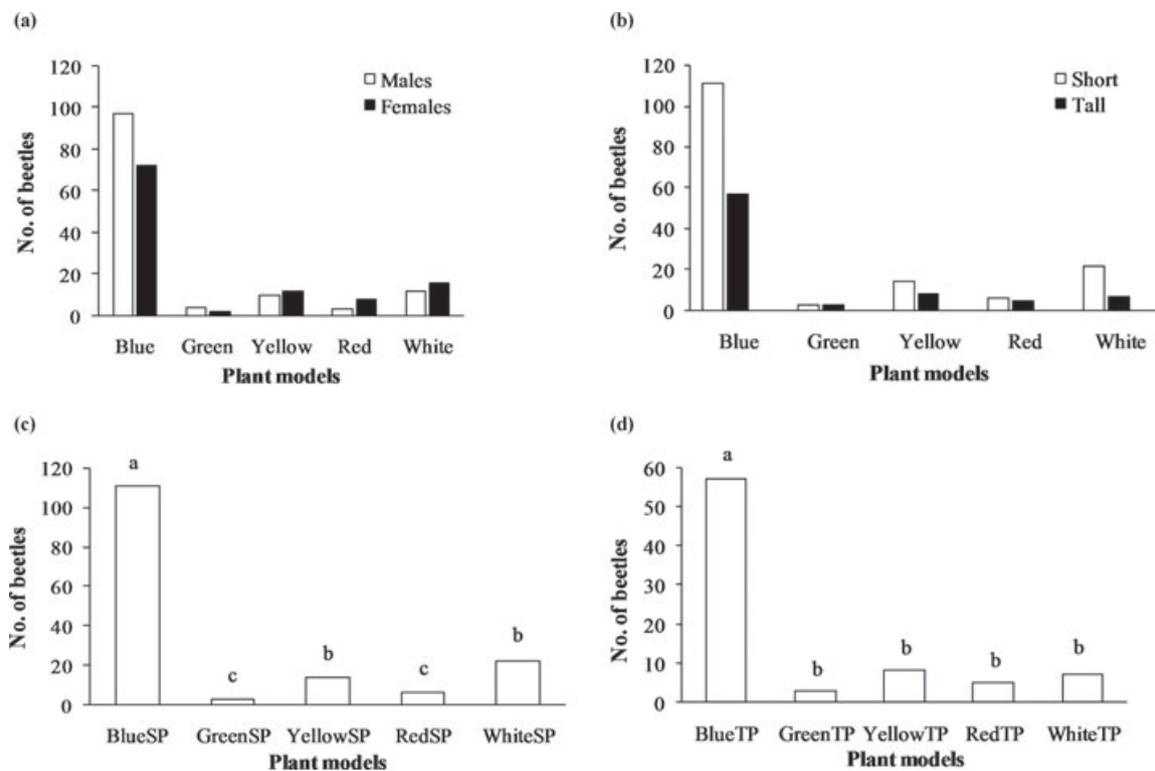
Fig. 2. Responses of adult blister beetles to the coloured plant models: (a) males and females, (b) short and tall plant models (0.5 and 1.0 m high, respectively), (c) short plant models (SP) and (d) tall plant models (TP); blue refers to sky blue and green to bright green. Different letters above bars indicate significant differences ($P < 0.05$) (paired chi-square tests corrected with Bonferroni adjustment).

across all colour combinations ($\chi^2 = 0.983$, d.f. $= 1$, $P = 0.322$) (Fig. 3a), so data for both sexes were pooled. As was observed in Experiment 1, short plant models were significantly more attractive than tall ones ($\chi^2 = 46.328$, d.f. $= 1$, $P < 0.001$) (Fig. 3b) and these were analysed separately. Landing rates on different colours differed significantly for the short plant models ($\chi^2 = 42.680$, d.f. $= 4$, $P < 0.0001$), whereas differences were not significant for the tall models ($\chi^2 = 8.349$, d.f. $= 4$, $P = 0.079$) (Fig. 3c,d). The nonmixed short sky blue plant model, was more attractive than short models in colour combinations (blue–green, blue–yellow, blue–red), except for the blue–white combination. Numbers of beetles landing on all colour combinations did not differ significantly (Fig. 3c).

Experiment 3: models with shapes and hues of blue

Of the 266 beetles released (males and females in equal numbers), 74% landed on one of the targets of different shapes (circle, square and triangle) or hues of blue (sky, turquoise and dark blue) combined. As was observed in the previous two experiments, there was no significant difference between the numbers of males and females landing on these plant models ($\chi^2 = 0.505$, d.f. $= 1$, $P = 0.477$) (Fig. 4a), so data for both sexes were pooled.

The preference of beetles for different plant models differed significantly ($\chi^2 = 99.475$, d.f. $= 9$, $P < 0.0001$). The round

sky blue and square sky blue models attracted 23% of beetles each, and were the most preferred targets, whereas the lowest preference was observed for the square dark blue models, which attracted 2% of the beetles that made a choice (Fig. 4b). The round sky blue and square sky blue models were more attractive to the beetles than the leaf-like sky blue model, which attracted only 10% of beetles.

The response of beetles towards the models was dependent on the two characteristics, shape and colour ($\chi^2 = 20.349$, d.f. $= 6$, $P = 0.02$). Therefore, each type of model was analysed separately. Landing preference of beetles on plant models differed significantly between the three hues of blue ($\chi^2 = 164.419$, d.f. $= 2$, $P < 0.0001$). Sky blue was preferred by 65% of beetles, followed by turquoise blue (23%), and dark blue (12%) was the least preferred (Fig. 4c). When shapes were compared, the choices made by beetles differed significantly ($\chi^2 = 30.121$, d.f. $= 3$, $P < 0.0001$). The highest number of beetles was recorded on the round (34%), square (31%) and triangular (25%) plant models. Significantly fewer beetles (9%) landed on the leaf-like shape (Fig. 4d).

Reflectance spectra of the coloured paper samples

The reflectance of the coloured papers used in experiments was in the range 400–650 nm. The highest percentage reflectance was observed with white paper, the reflectance of which was in the range 41–98%, with double-peak
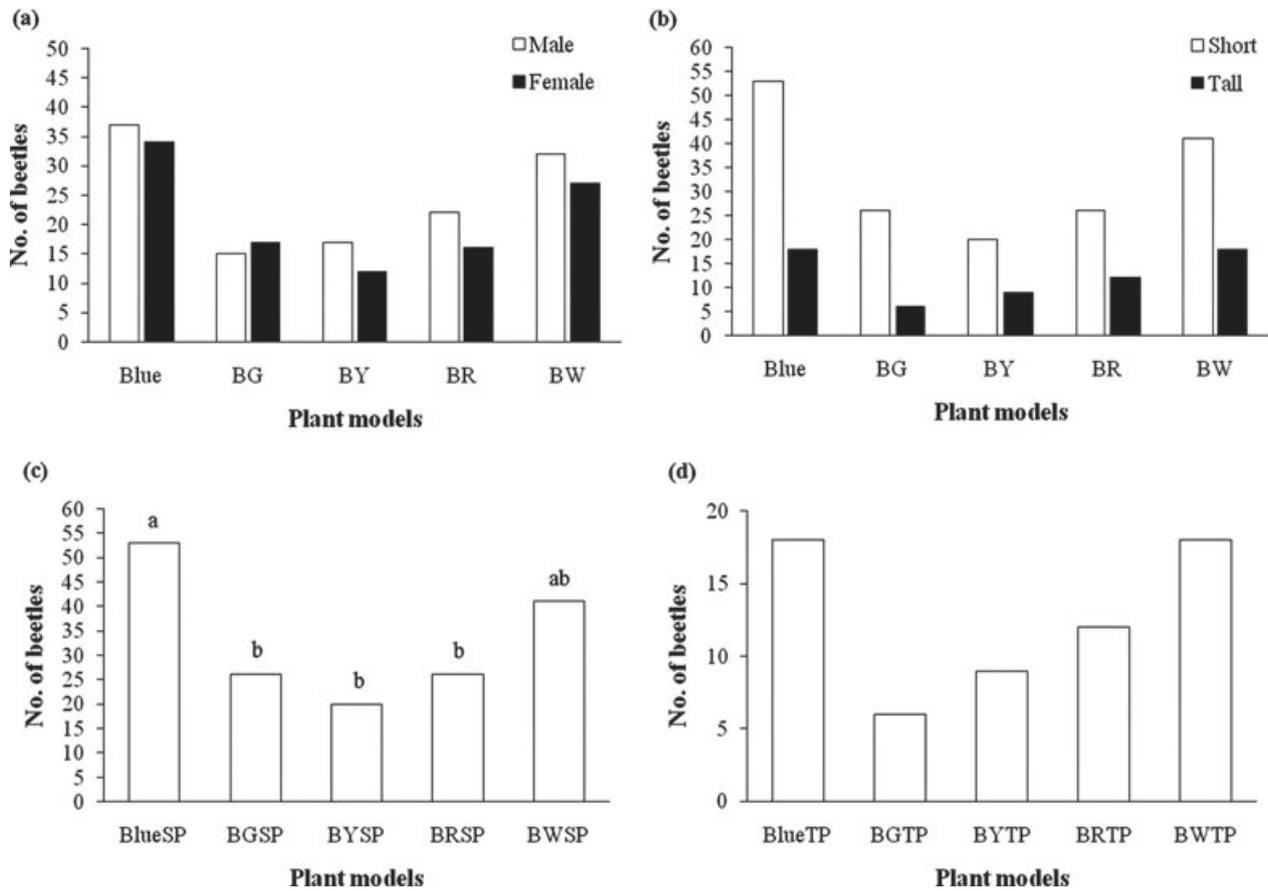
Fig. 3. Responses of blister beetles to the sky blue plant models single colour (blue) and in combination with bright green (BG), red (BR), yellow (BY) and white (BW): (a) males and females, (b) short and tall plant models (0.5 and 1.0 m high, respectively), (c) short plant models (SP) and (d) tall plant models (TP). Different letters above bars indicate significant differences ($P < 0.05$) (paired chi-square tests corrected with Bonferroni adjustment).

wavelengths at 430 and 446 nm. Yellow and red had the next highest percentage reflectance (both above 80%) peaking at 530 and 630 nm, respectively. Black had the lowest percentage reflectance at approximately 5% (Fig. 5a). Sky blue, on the other hand, had the highest percentage reflectance (72%) among the hues of blue, and this occurred at the peak wavelength of 450 nm, whereas the reflectance of dark blue (39%) at the dominant wavelength of 450 nm was the lowest (Fig 5b). The data on total number of beetles responding to the different colours (including different hues of blue) suggest that beetles could have a high preference for colours with reflectance in the range 440–500 nm Fig. 5(a,b).

Discussion

In the present study, *H. apicicornis* exhibits a strong preference for blue. This finding is similar to a previous study by Hall (1984), involving another species of blister beetle (*Mylabris designata* var. *hacolyssa*), in which larger numbers are reported to be caught by blue traps. The present study shows that white, yellow, red and green targets attract relatively few

beetles. However, white is the next most attractive colour after blue. The preference for sky blue followed by white is demonstrated further when evaluating plant models of two colours combined (sky blue, together with any other colour except black). Although white paper in a blue-white combination does not elicit a significantly different response from *H. apicicornis* to plant models with blue paper alone, the presence of yellow, red or green results in fewer beetles landing on those models.

Black reflects the minimum level of light (approximately 5% throughout the whole reflectance spectra) and failure to attract any beetles to this colour shows that *H. apicicornis* needs reflective materials to be attracted to a target. This is also the finding of a preliminary experiment in which differently coloured nonshiny cotton fabrics fail to attract *H. apicicornis* in significant numbers (L. N. Lebesa, unpublished observations). Previously, Blight & Smart (1999) report that a black coloured trap fails to attract pollen beetles, *Meligethes aeneus* (Coleoptera: Nitidulidae), presumably because of poor reflectance. Green is preferred by many insects because it falls within one of their common sensitive areas (Prokopy & Owens, 1983) and is presumably associated with
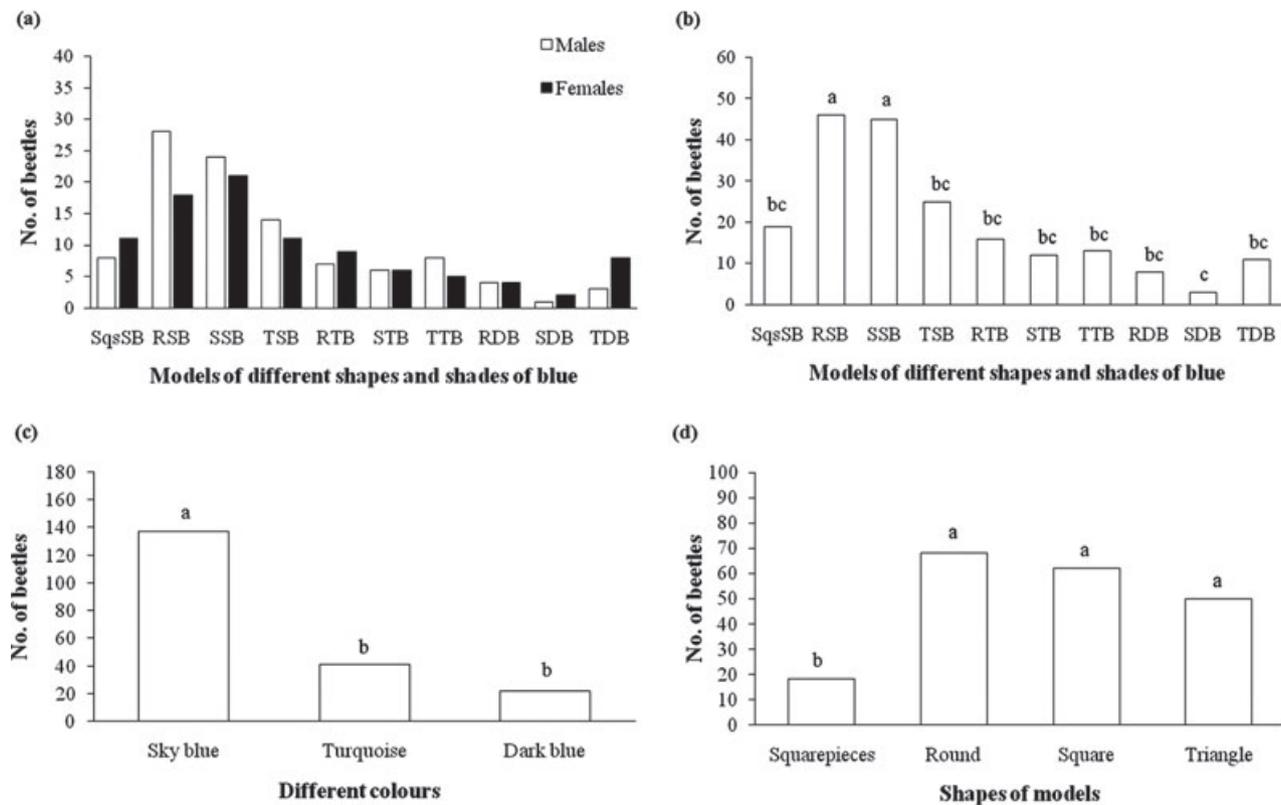
Fig. 4. Responses of blister beetles to plant models of different shapes and hues of blue: (a) males and females, (b) shapes and hues combined, (c) hues of blue (SB, sky blue; TB, turquoise; DB, dark blue) and (d) shapes (SqsSB, sky blue square pieces models; LLSB, leaflike sky blue models; RSB, round sky blue models; SSB, square sky blue models; TSB, triangular sky blue models; RTB, round turquoise blue models; STB, square turquoise blue models; TTB, triangular turquoise blue models; RDB, round dark blue models; SDB, square dark blue models; TDB, triangular dark blue models). Different letters above bars indicate significant differences ($P < 0.05$) (paired chi-square tests corrected with Bonferroni adjustment).

foliage. However, in the present study, only a few beetles land on green plant models, suggesting that, even if *H. apicicornis* adults can detect green, they are not attracted to it. This makes sense because adults feed on flowers not foliage. Although green and yellow have very close maximum wavelengths (510 and 530 nm, respectively), they differ with respect to their percentage reflectance (43% and 85%, respectively). Yellow attracts more beetles, indicating that reflectance intensity plays a role in attracting *H. apicicornis*.

White, which has a high reflectance (approximately 99%), is the next preferred colour after sky blue among short plant models and is more attractive to *H. apicicornis* than red and green. In addition to having a high percentage reflectance, white has a broader wavelength reflectance range (peaking from 430–650 nm) that overlaps with blue (whose maximum reflectance falls in the range 440–480 nm), which has the highest attraction for *H. apicicornis*. Therefore, the broad wavelength reflectance covering the sky blue maximum reflectance region coupled with the high intensity could explain why white models are the next attractive models to *H. apicicornis*. This may argue against intensity being the main factor behind the choice because the red and yellow colours used in the present study have intensities that are higher than that of sky blue. The high spectral reflectance (80% and above)

and broad wavelength range for white is well known from previous studies (Hardie *et al.*, 1996; Strom *et al.*, 1999; Yaku *et al.*, 2007) despite the differing reflectances of the various white papers and materials used. Because the white models reflect a wider range of wavelengths, which is generally the case with white flowers as perceived by the human eye (Chittka *et al.*, 1994; White *et al.*, 1994; Kevan *et al.*, 1996), these models may also be seen by *H. apicicornis* as violet flowers (as perceived by humans).

According to Chittka *et al.* (1994), violet, pink, purple and blue flowers (in accordance with human perception) are known as blue (bee ultraviolet-blue) flowers. Although the colour space for *H. apicicornis* is not known, it is possible that majority of flowers of its host plants fall into this category. To the human eye, flowers of most host plants of *H. apicicornis* (e.g. silverleaf desmodium, sweetpotato, cowpea, morning glory and beans) have a light purplish (violet) appearance. Sky blue models attract more beetles than turquoise or dark blue targets, irrespective of shape. Of the reflectance spectra, sky blue has the highest percentage reflectance. Although percentage reflectance of turquoise is close to that of sky blue, its maximum reflectance range is slightly outside the blue region (440 nm). This indicates that *H. apicicornis* has the ability to distinguish flowers of host plants from those
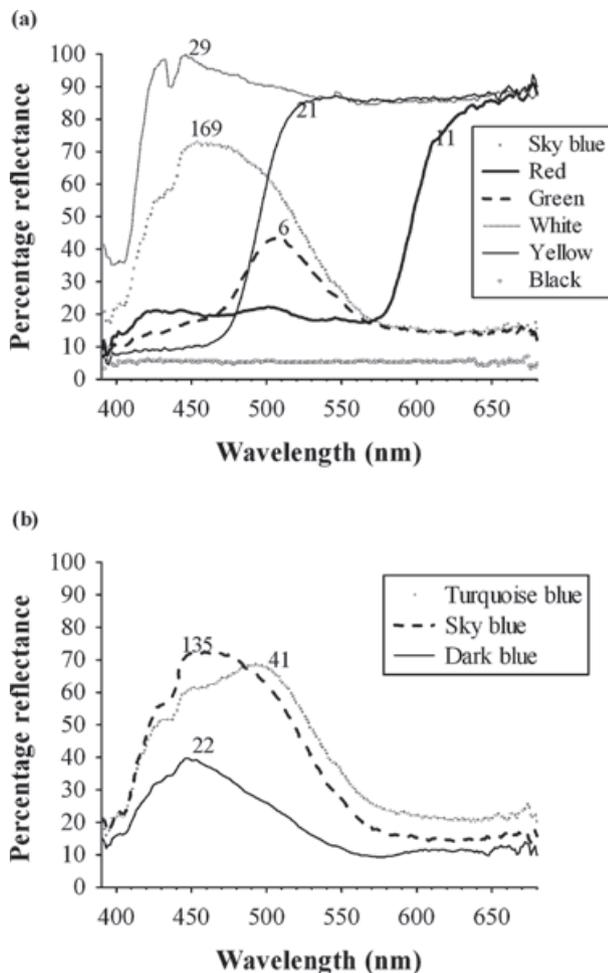
Fig. 5. Spectral reflectance of printed coloured papers used as visual targets for *Hycleus apicicornis*: (a) reflectance of papers used in plant models to test attractiveness of different colours and (b) three hues of blue. The numbers close to lines show the number of beetles that landed on a colour represented by that line.

more attractive, simultaneously blooming small flowers may be equally attractive because insects view them as a combined image. However, visual cues such as shape, pattern or size may not necessarily complement colour during a host search and may have to be coupled with olfactory cues for an insect to make correct choices (Rojas & Wyatt, 1999; Kulahci *et al.*, 2008).

In the present study, there is no significant difference between the landing preferences of males and females, suggesting that the distribution of the photoreceptors in the eyes of the two sexes is similar. It is possible that *H. apicicornis* does not use visual cues for mate location or for locating oviposition sites. In insects that do, there are differences in the arrangement of receptors to allow for mate recognition (Bernard & Remington, 1991; Brunton & Majerus, 1995) and recognition of host plants for oviposition (Prokopy *et al.*, 1983b; Kelber, 1999; Briscoe & Chittka, 2001).

Although the spectral sensitivity of *H. apicicornis* is not known and is not determined in the present study, the results show that adult *H. apicicornis* is attracted mainly to targets that reflect in the blue wavelength region (440–500 nm), suggesting the possibility of sensitivity of the main receptor in this region. However, physiological and behavioural studies, including experiments on any relationship between colour targets and rewards (Giurfa *et al.*, 1995; Kelber & Hénique, 1999; Kelber *et al.*, 2003a; Balkenius & Kelber, 2006; Goyret & Raguso, 2006), would need to be undertaken to determine whether the observed feeding behaviour in *H. apicicornis* is a result of true colour vision or is just wavelength dependent.

The present findings should be of use in the design of traps for use in the management of *H. apicicornis* on *Desmodium* spp. on which they have recently become a pest in East Africa. No studies are available reporting on the possible mediation of attractive plant semiochemicals for *H. apicicornis* or indeed any other blister beetle. If such semiochemicals also play a role in attracting *H. apicicornis*, they could be used together with sky blue coloured materials to design an effective trap for the management of this beetle.

References

Allan, S.A., Day, J.F. & Edman, J.D. (1987) Visual ecology of biting flies. *Annual Review of Entomology*, **32**, 297–316.

Balkenius, A. & Kelber, A. (2004) Colour constancy in diurnal and nocturnal hawkmoths. *Journal of Experimental Biology*, **207**, 3307–3316.

of similarly coloured nonhost plants. This ability may be important if *H. apicicornis* uses primarily colour for locating its host plants, as is the case with the butterfly *Vanessa indica* (Lepidoptera: Nymphalidae) (Ômura & Honda, 2005). This means that an insect can avoid wasting energy on visiting flowers that would not be rewarding (Chittka & Menzel, 1992).

Other important traits in the visual system of insects for correct host recognition are shape and/or patterns and size of flowers (Wäckers & Lewis, 1999; Ne'eman & Kevan, 2001; Dyer & Chittka, 2004a). Shape may be a complementary factor ensuring that insects do not make mistakes when confronted with a choice of similarly coloured host and nonhost plants (Dyer & Chittka, 2004a). However, in the present study, shape does not appear to be of importance for *H. apicicornis*, possibly because this species feeds on several host plant species that are often unrelated and that differ in their morphological appearance. Ne'eman & Kevan (2001) suggest that floral area is important and that, although larger flowers may appear

Balkenius, A. & Kelber, A. (2006) Colour preferences influences odour learning in the hawkmoth, *Macroglossum stellatarum*. *Naturwissenschaften*, **93**, 255–258.

Balkenius, A., Rosén, W. & Kelber, A. (2006) The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *Journal of Comparative Physiology A*, **192**, 431–437.

Bernard, G.D. & Remington, C.L. (1991) Color vision in *Lycaena* butterflies: spectral tuning of receptor arrays in relation to behavioral ecology. *Proceedings of the National Academy of Science of the United States of America*, **88**, 2783–2787.

Bernays, E.A. & Chapman, R.F. (1994) *Host-Plant Selection by Phytophagous Insects. Contemporary Topics in Entomology 2.* Chapman & Hall, U.K.

Blight, M.M. & Smart, L.E. (1999) Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aeneus* in field traps. *Journal of Chemical Ecology*, **25**, 1501–1516.

Briscoe, A.D. & Chittka, L. (2001) The evolution of color vision in insects. *Annual Review of Entomology*, **46**, 471–510.

Brunton, C.F.A. & Majerus, M.E.N. (1995) Ultraviolet colours in butterflies: intra- or inter-specific communication? *Biological Sciences*, **260**, 199–204.

Campbell, S.A. & Borden, J. (2006a) Integration of visual and olfactory cues of hosts and non-hosts by three bark beetles (Coleoptera: Scolytidae). *Ecological Entomology*, **31**, 437–449.

Campbell, S.A. & Borden, J. (2006b) Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. *Entomologia Experimentalis et Applicata*, **120**, 91–98.

Chittka, L. & Menzel, R. (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A*, **171**, 171–181.

Chittka, L., Shmida, A., Troje, N. & Menzel, R. (1994) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. *Vision Research*, **34**, 1489–1508.

Döring, T.F. & Skorupski, P. (2007) Host and non-host leaves in the colour space of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Entomologia Generalis*, **29**, 81–95.

Dyer, A.G. & Chittka, L. (2004a) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *Journal of Comparative Physiology A*, **190**, 105–114.

Dyer, A.G. & Chittka, L. (2004b) Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A*, **190**, 759–763.

Epsky, D.E. & Heath, R.R. (1998) Exploiting the interactions of chemical and visual cues in behavioral control measures for pest tephritid fruit flies Behavioral Ecology Symposium '97. *Florida Entomologist*, **81**, 273–282.

Evans, E.W., Smith, C.C. & Gendron, R.P. (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. *Oecologia*, **78**, 220–230.

Fukaya, M., Wakamura, S., Arakaki, N. *et al.* (2006) Visual 'pinpoint' location associated with pheromonal cue in males of the black chafer *Holotrichia loochooana loochooana* (Coleoptera: Scarabaeidae). *Applied Entomology and Zoology*, **41**, 99–104.

Gahukar, R.T. (1991) Pest status and control of blister beetle in West Africa. *Tropical Pest Management*, **37**, 415–420.

Giurfa, M. (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften*, **91**, 228–231.

Giurfa, M., Backhaus, W. & Menzel, R. (1995) Color and angular orientation in the discrimination of bilateral symmetric patterns in the honeybee. *Naturwissenschaften*, **82**, 198–201.

Giurfa, M., Vorobyev, M., Brandt, R. *et al.* (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A*, **180**, 235–244.

Goyret, J. & Raguso, R.A. (2006) The role of mechanosensory input in flower handling efficiency and learning by *Manduca sexta*. *Journal of Experimental Biology*, **209**, 1585–1593.

Goyret, J., Markwell, P.M. & Raguso, R.A. (2007) The effect of decoupling olfactory and visual stimuli on the foraging behavior of *Manduca sexta*. *Journal of Experimental Biology*, **210**, 1398–1405.

Hall, M.J.R. (1984) Trap-oriented behaviour of red-banded blister beetles, *Mylabris designata* var. *hacolyssa* Rochebrune (Coleoptera: Meloidae), in the Sudan. *Bulletin of Entomological Research*, **74**, 103–112.

Hardie, J., Storer, R.J., Cook, F.J. *et al.* (1996) Sex pheromone and visual trap interactions in mate location strategies and aggregation by host-alternating aphids in the field. *Physiological Entomology*, **21**, 97–106.

Harris, M.O. & Miller, J.R. (1983) Color stimuli and oviposition behavior of the onion fly, *Delia antique* (Meigen) (Diptera: Anthomyiidae). *Annals of the Entomological Society of America*, **76**, 766–771.

Harris, M.O., Rose, S. & Malsch, P. (1993) The role of vision in the host plant-finding behaviour of the Hessian fly. *Physiological Entomology*, **18**, 31–42.

Hausmann, C., Samietz, J. & Dorn, S. (2004) Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae). *Environmental Entomology*, **33**, 1410–1415.

Hesler, L.S. & Sutter, G.R. (1993) Effect of trap color, volatile attractants, and type of toxic bait dispenser on captures of adult corn rootworm beetles (Coleoptera: Chrysomelidae). *Environmental Entomology*, **22**, 743–750.

Hirota, T. & Kato, Y. (2004) Color discrimination on orientation of female *Eurema hecabe* (Lepidoptera: Pieridae). *Applied Entomology and Zoology*, **39**, 229–233.

Jiggins, C.D., Naisbit, R.E., Coe, R.L. & Mallet, J. (2001) Reproductive isolation caused by colour pattern mimicry. *Nature*, **411**, 302–305.

Jönsson, M., Rosdahl, K. & Anderson, P. (2007) Responses to olfactory and visual cues by over-wintered and summer generations of the pollen beetle, *Meligethes aeneus*. *Physiological Entomology*, **32**, 188–193.

Kelber, A. (1999) Ovipositing butterflies use a red receptor to see green. *Journal of Experimental Biology*, **202**, 2619–2630.

Kelber, A. & Hénique, U. (1999) Trichromatic colour vision in the hummingbird hawkmoth, *Macroglossum stellatarum* L. *Journal of Comparative Physiology A*, **184**, 535–541.

Kelber, A., Balkenius, A. & Warrant, E.J. (2003a) Colour vision in diurnal and nocturnal hawkmoths. *Integrative and Comparative Biology*, **43**, 571–579.

Kelber, A., Vorobyev, M. & Osorio, D. (2003b) Animal colour vision – behavioural tests and physiological concepts. *Biological Reviews*, **78**, 81–118.

Kevan, P., Giurfa, M. & Chittka, L. (1996) Why are there so many and so few white flowers? *Trends in Plant Science*, **1**, 280–284.

Khan, Z.R., Pickett, J.A., Van den Berg, J. *et al.* (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. *Pest Management Science*, **56**, 957–962.

Khan, Z.R., Hassanali, A., Overholt, W. *et al.* (2002) Control of witchweed *Striga hermonthica* by intercropping with *Desmodium* spp. and the mechanism defined as allelopathic. *Journal of Chemical Ecology*, **28**, 1871–1885.

Khan, Z.R., Midega, C.A.O., Amudavi, D.M. *et al.* (2008) On-farm evaluation of the push-pull technology for the control of stemborers

and striga weed on maize in western Kenya. *Field Crops Research*, **106**, 224–233.

Kulahci, I.G., Dornhaus, A. & Papaj, D.R. (2008) Multimodal signals enhance decision making in foraging bumble-bees. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **275**, 797–802.

Lale, N.E.S. & Sastawa, B.M. (2000) Evaluation of host plant resistance, sowing date modification and intercropping as methods for the control of *Mylabris* and *Coryna* species (Coleoptera: Meloidae) infesting pearl millet in the Nigerian Sudan savanna. *Journal of Arid Environments*, **46**, 263–280.

Lunau, K. (1993) Interspecific diversity and uniformity of flower colour patterns as cues for learned discrimination and innate detection of flowers. *Experientia*, **49**, 1002–1010.

Menzel, R. (1979) Spectral sensitivity and color vision in invertebrates. Spectral sensitivity and color vision in invertebrates. *Handbook of Sensory Physiology, Vol. VII/6A, Vision in Invertebrates* (ed. by H. Autrum), pp. 503–580. Springer-Verlag, Germany.

Ne'eman, G. & Kevan, P.G. (2001) The effect of shape parameters on maximal detection distance of model targets by honeybee workers. *Journal of Comparative Physiology A*, **187**, 653–660.

Nikbakhtzadeh, M.R. (2004) *Transfer and distribution of cantharidin within selected members of blister beetles (Coleoptera: Meloidae) and its probable importance in sexual behaviour*. PhD Dissertation, Universität Bayreuth.

Norman, G.R. & Streiner, D.L. (1994) *Biostatistics, the Bare Essentials*. Mosby-Year Book, Inc., St Louis, Missouri.

Ômura, H. & Honda, K. (2005) Priority of color over scent during flower visitation by adult *Vanessa indica* butterflies. *Oecologia*, **142**, 588–596.

Payne, R.W., Murray, D.A., Harding, S.A. *et al*. (2007) *GenStat for Windows® Introduction*, 10th edn. VSN International, Hempstead, New York.

Prokopy, R.J. & Owens, E.D. (1983) Visual detection of plants by herbivorous insects. *Annual Review of Entomology*, **28**, 337–364.

Prokopy, R.J., Collier, R.H. & Finch, S. (1983a) Leaf color used by cabbage root flies to distinguish among host plants. *Science*, **221**, 190–192.

Prokopy, R.J., Collier, R.H. & Finch, S. (1983b) Visual detection of plants by cabbage root flies. *Entomologia Experimentalis et Applicata*, **34**, 85–89.

Raguso, R.A. (2001) Floral scent, olfaction, and scent-driven foraging behavior. *Cognitive Ecology of Pollination* (ed. by L. Chittka and J. Thomson), pp. 83–105. Cambridge University Press, U.K.

Raguso, R.A. & Willis, M.A. (2002) Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths. *Manduca Sexta Animal Behavior*, **64**, 685–695.

Rojas, J.C. & Wyatt, T.D. (1999) Role of visual cues and interactions with host odour during the host-finding behaviour of the cabbage moth. *Entomologia Experimentalis et Applicata*, **91**, 59–65.

Schmera, D., Tóth, M., Subchev, M. *et al*. (2004) Importance of visual and chemical cues in the development of an attractant trap for *Epicometis* (*Tropinota*) *hirta* Poda (Coleoptera: Scarabaeidae). *Crop Protection*, **23**, 939–944.

Selander, R.B. (1986) Rearing blister beetles. *Insecta Mundi*, **1**, 209–220.

Seyoum, A., Pålsson, K., Kung'a, S. *et al*. (2002) Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96**, 225–223.

Sokal, R.R. & Rohlf, F.J. (1969) *Biometry – The Principle and Practice of Statistics in Biological Research*, 2nd edn. W.H. Freeman and Company, New York, New York.

Strom, B.L., Roton, L.M., Goyer, R.A. & Meeker, J.R. (1999) Visual and semiochemical disruption of host finding in the southern pine beetle. *Ecological Applications*, **9**, 1028–1038.

Yaku, A., Walter, G.H. & Najar-Rodriguez, A.J. (2007) Thrips see red – flower colour and the host relationships of a polyphagous anthophilic thrips. *Ecological Entomology*, **32**, 527–535.

Wäckers, F.L. & Lewis, W.L. (1999) A comparison of color-, shape- and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. *Journal of Comparative Physiology A*, **184**, 387–393.

Weiss, M.R. (1997) Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour*, **53**, 1043–1052.

White, R.H., Stevenson, R.D., Bennett, R.R. *et al*. (1994) Wavelength discrimination and the role of ultraviolet vision in the feeding behavior of hawkmoths. *Biotropica*, **26**, 427–435.

Zaccardi, G., Kelber, A., Sison-Mangus, M.P. & Briscoe, A.D. (2006) Color discrimination in the red range with only one long-wavelength sensitive opsin. *Journal of Experimental Biology*, **209**, 1944–1955.

Zar, H.J. (1984) *Biostatistical Analysis*, 2nd edn. Prentice Hall International, U.K.