



## Response of *Trichogramma* egg parasitoids to colored sticky traps

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**Abstract.** The response of *Trichogramma* spp. egg parasitoids to colored sticky traps was evaluated in the field during two seasons (1995/1996, 1996/1997). Traps consisted of a glass tube coated with Bird-Tanglefoot® into which colored paper was inserted or clear traps without paper. Colors tested were white, green, blue, yellow and red in the first season and white, green, yellow and black in the second season. The proportion of both female and male parasitoids caught on the sticky traps was significantly different among colors, indicating that the parasitoids actively move between plants and are not solely carried along passively by wind. White was the color most preferred by female parasitoids, followed by clear and green traps. Yellow was preferred over black but was less attractive than green. Visual cues may be used by *Trichogramma* spp. during the habitat location process. The color preference of male *Trichogramma* spp. differed significantly from females with yellow and green being more attractive than white. For all colors, more female *Trichogramma* spp. were caught on the sticky traps (>85% of all wasps caught), indicating a lower activity level and/or shorter lifespan for males. The use of white cylindrical sticky traps for monitoring *Trichogramma* spp. populations in the field is recommended.

**Résumé.** La réaction des parasitoïdes des oeufs *Trichogramma* spp. aux pièges gluants colorés a été évaluée au champ au cours de deux saisons (1995/1996 et 1996/1997). Les pièges étaient constitués d'un tube en verre enrobé de Bird-Tanglefoot®. Du papier coloré était inséré dans les tubes. Les tubes sans papier représentaient des pièges clairs. Les couleurs utilisées pour l'expérience étaient le blanc, le vert, le bleu, le jaune et le rouge pour la première saison, et le blanc, le vert, le jaune et le noir pour la deuxième saison. La proportion de parasitoïdes femelles attrapés aux pièges gluants était sensiblement différente entre les couleurs, ce qui indique que les parasitoïdes se déplacent activement d'une plante à l'autre et qu'ils ne se laissent pas seulement emportés passivement par le vent. La couleur la plus préférée par les parasitoïdes femelles était le blanc, suivie par les pièges clairs et les pièges verts. Le jaune était plus préféré que le noir, mais il était moins attrayant que le vert. Il est probable que les *Trichogramma* spp. se servent de signaux visuels au cours de la recherche d'habitat. La préférence de couleurs des *Trichogramma* mâles était sensiblement différente de celle des femelles, le jaune et le vert étant plus préférés que le blanc. Pour toutes les couleurs, un nombre plus important de *Trichogramma* femelles étaient piégés (>85% des parasitoïdes

piégés), ce qui indique un niveau d'activité moins élevé et/ou une durée de vie plus courte pour les mâles. L'article recommande l'emploi des pièges gluants cylindriques et blancs pour la suivie des populations de *Trichogramma* spp. au champ.

**Key words:** color preference, habitat location, parasitoids, sticky traps, *Trichogramma* spp.

## Introduction

Species of *Trichogramma* (Hym.: Trichogrammatidae) egg parasitoids are important natural enemies of a large number of insect pests and are successfully used in biological control programs worldwide (Li, 1994; Smith, 1996). *Trichogramma* spp. are perhaps the most thoroughly studied natural enemy genus, yet little has been published on how they locate their host habitat. Response to odors of some host plants has been reported (Nordlund, 1994; Romeis et al., 1997), but volatiles were found to arrest rather than attract the searching parasitoids. Orientation using visual cues such as plant color could also be involved in the host-habitat location process of *Trichogramma* spp. as discussed for several hymenopteran parasitoids belonging to various families: Aphelinidae (Ekbohm, 1980; Moreno et al., 1984; Romeis and Zebitz, 1997), Aphidiidae (Vater, 1971; Goff and Nault, 1984), Braconidae (Weseloh, 1972; Ridgway and Mahr, 1986; Wäckers and Lewis, 1994; Wäckers, 1994), Cynipidae (Vater, 1971), Encyrtidae (Weseloh, 1972) and Ichneumonidae (Hollingsworth et al., 1970; Schmidt et al., 1978; McAuslane et al., 1991; Ma et al., 1992).

The visual system of *Trichogramma* spp. has been almost exclusively studied with regard to the visual detection of host eggs (Glas et al., 1981; Pak et al., 1991; Bruins et al., 1994). At present, only one study has reported information on the color preference of *Trichogramma embryophagum* (Hartig) (Kadlubowski, 1970); yellow was found to be more attractive than blue or white. Unfortunately, the experimental methodology including spectral analysis of the colors used is not available for that study. The following investigation was made to test the color preference of naturally occurring *Trichogramma* spp. populations in the field.

## Material and methods

Experiments were conducted between 19 December 1995 and 15 March 1996 (Season 1), and between 29 November 1996 and 10 March 1997 (Season 2) in flowering sorghum fields at ICRISAT Asia Center near Hyderabad, India.

Glass tubes (3.4 cm diameter; 14.5 cm in length) coated with a thin layer of transparent glue (Bird-Tanglefoot<sup>®</sup>, Tanglefoot Company, Grand Rapids, MI, USA) were used as sticky traps. The surface area of each trap was approximately 132 cm<sup>2</sup> (12 × 11 cm). Colored paper was rolled inside the glass tube. Colors tested in Season 1 were: white, green, blue, yellow and red, and in Season 2: white, green, yellow and black. In Season 1, one set of traps were used without a paper inside ('clear' traps).

The reflectance spectrum of the colored papers and a sorghum leaf and flowering panicle was recorded between 400 and 650 nm, representing the range within the electromagnetic energy spectrum perceptible to insects via ocular receptors (Menzel, 1979), but excluding ultraviolet. Measurements were taken at noon in the field from the plant material, the colored papers, and after the papers were inserted into the glue-coated glass tubes using a portable photometer (model 2703, Abe Sekkei Inc., Japan). The spectral reflectance was measured as a percentage of the reflectance from a white standard (Lab-sphere, SRT-99-100). Measuring spectral reflectance in the environment is not absolute, but does provide a relative estimate of visual contrasts (Prokopy and Owens, 1983).

The glue-coated glass tubes were placed on sticks at sorghum panicle height (approximately 1.5 m above ground level). For each sample, five sets of traps of different colors were placed in a systematic design at different locations within the sorghum field. The distance between different colored traps was a minimum of 2 m. Traps were left in the field for 1–3 days depending on the parasitoid population. The location of different colors was rotated between samples. For easier handling, traps were covered with clear plastic wrap before removal. The number of female and male *Trichogramma* spp. caught on each trap was counted under a dissecting microscope in the laboratory. For each sample the numbers of female parasitoids on the five traps belonging to the same color were pooled and the proportion caught on the different colors was calculated. Only samples with more than an average of 7 female parasitoids per trap were used in the analysis. The mean proportion of female *Trichogramma* spp. caught on the different colored traps was calculated based on a total of 13 samples (65 traps per color) in Season 1 and 22 samples (110 traps per color) in Season 2.

The proportions of females caught on the different colors were arcsin transformed and analyzed separately for both seasons using analysis of variance. Means were separated using Fisher's PLSD (Abacus Concepts, 1996). Because of the generally low number of males caught, numbers were pooled across samples within a season. The distribution was compared with an equal distribution using a  $\chi^2$ -test. The number of males expected on the differ-

ent colors, using the actual distribution of females as null hypothesis, was compared with the actual number caught using a  $\chi^2$ -test.

The *Trichogramma* spp. caught on the sticky traps were not identified, but *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) egg collections from sorghum fields during the same seasons at ICRISAT Asia Center have shown that 99% of the *Trichogramma* spp. which emerged belonged to *T. chilonis* Ishii (Monje et al., in press), the most important egg parasitoid of *H. armigera* in India (Romeis and Shanower, 1996).

## Results and discussion

The number of *Trichogramma* spp. females and males caught on the sticky traps was significantly different among colors (females: Season 1,  $F = 26.68$ ,  $df = 5, 72$ ,  $P < 0.001$ ; Season 2,  $F = 69.63$ ,  $df = 3, 84$ ,  $P < 0.001$ ; males: Season 1,  $\chi^2 = 90.0$ ,  $df = 5$ ,  $P < 0.001$ ; Season 2,  $\chi^2 = 22.5$ ,  $df = 3$ ,  $P < 0.001$ ) (Table 1). This result indicates that the parasitoids differentiated among colors and moved actively between plants. A more equal distribution among colors would have resulted if the wasps were carried passively by the wind.

Colors are described by three characteristics: intensity (reflected energy), hue (dominant wavelength), and saturation (spectral purity of the reflected light). Phytophagous insects use all three characters, either alone or in combination, for orientation (Prokopy and Owens, 1983; Bernays and Chapman, 1994). Similarly, color vision in Hymenoptera has been shown to be influenced by intensity (Hollingsworth et al., 1970; Labhart, 1974), hue (Menzel, 1967; Hollingsworth et al., 1970), and/or saturation (Lunau, 1990).

White was the most attractive color for female *Trichogramma* spp., followed by clear and green traps (Table 1). Clear traps were probably as attractive as green traps because the plants were visible through the glass tube (Weseloh, 1972). White and green traps had a high reflection in the same spectral region where the sorghum plant surface had its reflection maximum (Figure 1). In Season 1, yellow was as unattractive as blue and red (Table 1). In Season 2, yellow was again less attractive than green, but preferred over black. However, this spectral-specific behavioral response does not establish the existence of true color vision as it is not clear whether the parasitoids can discriminate hue independent of intensity, requiring a minimum of two receptor types (Menzel, 1979). It is likely that intensity is not responsible for the observed color preference. Yellow, which was less attractive to the female parasitoids than green, also has its maximum reflection at 550 nm but at a higher intensity than green (Figure 1). Therefore it is probably the

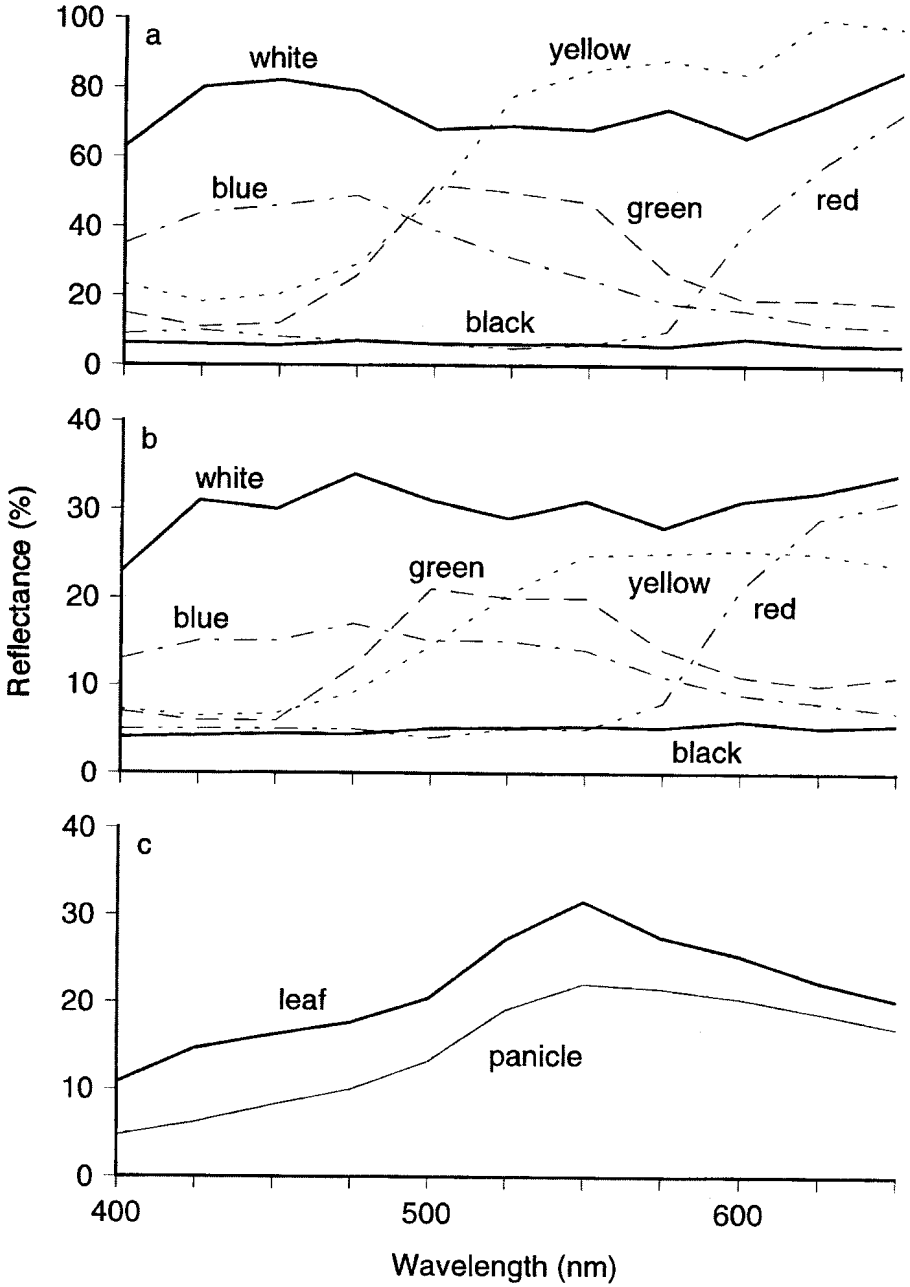


Figure 1. Spectral reflectance curves of: a, the colored papers alone; b, the colored papers inside the glue-coated glass tube; and c, sorghum leaf and panicle.

Table 1. Proportion of female and male *Trichogramma* spp. caught on colored sticky traps during two seasons (Season 1, n = 13; Season 2, n = 22). The total number of parasitoids caught on the different colors is given in parentheses

Trap color	Females <sup>a</sup>		Males <sup>b</sup>	
	Season 1	Season 2	Season 1	Season 2
White	0.28 ± 0.016 a (1401)	0.40 ± 0.012 a (2922)	0.15 (124)	0.19 (117)
'Clear'	0.19 ± 0.014 b (983)	— <sup>c</sup> —	0.22 (188)	— —
Green	0.18 ± 0.012 b (835)	0.25 ± 0.013 b (1893)	0.19 (161)	0.32 (200)
Blue	0.13 ± 0.009 c (672)	— —	0.11 (89)	— —
Red	0.11 ± 0.009 c (570)	— —	0.09 (79)	— —
Yellow	0.11 ± 0.014 c (472)	0.21 ± 0.015 c (1411)	0.24 (197)	0.25 (153)
Black	— —	0.15 ± 0.010 d (1106)	— —	0.24 (151)

<sup>a</sup>Proportion ± S.E.; means within a column followed by the same letter are not significantly different ( $P > 0.05$ ; Fisher's PLSD).

<sup>b</sup>Because of the generally low number of males caught, numbers were pooled across samples within a season and the S.E. was not calculated.

<sup>c</sup>(—) not tested.

hue or saturation of the colors which attract female *Trichogramma* spp. This is advantageous as intensity, unlike hue or saturation, can vary considerably with the angle and degree of illumination (Gates, 1980). Our results indicate that *Trichogramma* spp. could use visual cues during their host habitat location process. To evaluate if the parasitoids respond to hue or saturation, colored papers with an attractive hue, i.e. green, but with a different saturation should be tested (Finch, 1986).

Herbivorous insects using visual cues for host plant recognition usually show a preference not only for green but also for yellow (Prokopy and

Owens, 1983). Yellow is often more attractive than green and is considered a 'super normal foliage stimulus' as it reflects in the same bandwidth as green foliage, but with a greater intensity (Prokopy and Owens, 1983). A positive response to yellow has been reported for many hymenopteran parasitoids (Vater, 1971; Schmidt et al., 1978; Dowell and Cherry, 1981; Goff and Nault, 1984; Moreno et al., 1984; Trimble and Brach, 1985; Ridgway and Mahr, 1986; Weseloh, 1986; Ma et al., 1992; Wäckers, 1994; Romeis and Zebitz, 1997). However, several other studies, in addition to ours, have found yellow less attractive than green (references in Prokopy and Owens, 1983 for herbivores; for hymenopteran parasitoids see Vater, 1971; Goff and Nault, 1984). Wäckers (1994) showed that yellow is not a 'super normal foliage stimulus' for *Cotesia rubecula* (Marshall) (Hym.: Braconidae). Female parasitoids landed preferentially on small pieces of yellow paper attached to cabbage plants only when they were starved. Satiated parasitoids were not attracted by the yellow paper and landed almost exclusively on the plant foliage. This suggests that the yellow color is used as a cue to locate flowers during food-foraging.

Hymenoptera, with some exceptions, do not have receptors sensitive to wavelengths in the red part of the spectrum (Peitsch et al., 1992), which may explain the low response to red traps exposed in Season 1. Many insects are also sensitive to ultraviolet (UV)-light (Menzel, 1979; Peitsch et al., 1992). The colored papers used in this study will reflect UV-light with different intensities. A response of *Trichogramma* spp. to UV-light has been reported before (Brower and Cline, 1984) but we believe that our results were not affected by UV-light since at 400 nm much of the light (>45%) was already absorbed by the glass tube and glue (Figure 1). Tangle-Trap<sup>®</sup> was reported earlier to significantly decrease the percentage reflectance between 300 and 390 nm (Brach and Trimble, 1985). In addition, UV-light is unlikely to have a strong impact on foliage finding as it is generally reflected at low intensities from green plant surfaces. To clearly separate the effect of UV-light, *Trichogramma* females should be exposed to monochromatic light in the laboratory (Finch, 1986).

Our results contradict the findings by Kadlubowski (1970) who found yellow to be preferred over blue and white by *T. embryophagum*. Unfortunately, no description was given of the methodology or the characteristics of the colors used in that study, making a discussion of the differences in the color preference difficult.

The response of male *Trichogramma* spp. was significantly different from that of the females (Season 1,  $\chi^2 = 183.7$ ,  $df = 5$ ,  $P < 0.001$ ; Season 2,  $\chi^2 = 122.6$ ,  $df = 3$ ,  $P < 0.001$ ) (Table 1). In contrast to females, white was not the most preferred color by males and more parasitoids were caught on yellow

or green than on white. The reasons for these differences are not known but may be due to the different life strategies of the two sexes. For example, male *Trichogramma* spp. searching for a mate may be more attracted to black parasitized eggs while females may respond more strongly to white unparasitized eggs. For comparison, Hollingsworth et al. (1970) reported that both sexes of *Campoletis perdinctus* (Viereck) (Hym.: Ichneumonidae) showed a similar wavelength preference but female response was consistently higher for all wavelength and intensities. Trimble and Brach (1985) found no differences in the response of female and male *Pholetesor ornigis* Weed (Hym.: Braconidae) to colored sticky traps.

*Trichogramma* spp. caught on the sticky traps were predominately females. From a total of 5771 and 7953 wasps caught in Seasons 1 and 2, only 838 (14.5%) and 621 (7.8%) were male. The low percentage of male *Trichogramma* spp. caught on the sticky traps in both seasons is in contrast to an average of 48% males emerging from parasitized *H. armigera* eggs collected in sorghum fields at ICRISAT Asia Center during the same period (Romeis, 1997). This difference is likely due to the fact that male *Trichogramma* spp. are less active than females (Stern et al., 1965; Hendricks, 1967; Forsse et al., 1992) and that they have a shorter lifespan (Hoffmann et al., 1995).

The results of this study demonstrate that white sticky traps are the most effective in trapping female *Trichogramma* spp. Preliminary studies (Romeis et al., 1996; Romeis, 1997) have shown that they are a useful tool for monitoring the relative population dynamics of these natural enemies in the field.

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