Integrated protection and production in viticulture
IOBC/ wprs Bulletin Vol. 67, 2011
pp. 281-284

Attractiveness of different colours to *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) adults

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Abstract: Laboratory and field tests were performed to ascertain a possible role of visual cues for *Scaphoideus titanus* during the location of a host plant. In laboratory, two choice colour tests were made in a Y-tube to compare the attractiveness of blue (peak wavelength: 475nm), green (521nm), yellow (573nm) and red (650nm). The observed preference order was yellow > red > green > blue for males and red > yellow > green > blue for females. In field, nine groups of three coloured (yellow, red, blue) sticky traps were positioned in an organic vineyard and replaced once a week, for two summers (2008 and 2009). In both seasons the yellow traps captured significantly more individuals (either males or females) and had the highest sex rate (percentage of males on the total captures) followed by red and blue. Our investigation suggests that, despite a partial different response between laboratory and field tests, *S. titanus* adults are influenced by visual cues present in their habitat. The possible reasons of the different responses observed in lab and field are discussed.

Key words: hemiptera, leafhopper, choice tests, y-tube, sticky traps

Introduction

The leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) is the insect vector of the Flavescence dorée, a phytoplasma grapevine disease quarantined in Europe.

When mating, *S. titanus* adults communicate by means of substrate-borne vibrational signals (Mazzoni et al., 2009a), whereas the host plant recognition and identification by nymphs are partially mediated by volatile cues (Mazzoni et al., 2009b). The role of vision in environmental orientation has been poorly investigated. Studies on "Auchenorrhyncha" showed that several foraging leafhoppers and planthoppers discriminate using primarily visual stimuli, even combined with olfaction (Bullas-Appleton et al., 2004; Patt & Sétamou, 2007; Todd et al., 1990a, b). In the case of *S. titanus*, Lessio & Alma (2004) employed sticky traps of 4 colours (yellow, red, blue and white). In their specific experimental conditions, they found that all were extremely more effective in capturing males than females and, in particular, red traps captured significantly more males than other colours while no differences were detected for females.

The aim of the present study was to investigate the role of vision in orientating *S. titanus* adults. Laboratory tests were made to isolate the colour factor from other possibly interactive factors (e.g. odours and background colours). In addition, field studies were made to verify the laboratory results, in which the insects had to choose among the colours with completely different environmental conditions.

Material and methods

Insects for laboratory tests

In the winter 2008, two year-old grapevine canes were collected from organic farms at Villazzano (Trento, Italy) and stored in a cold room at 4°C. Eggs were hatched in climatic chamber (25±1°C, L16:D8, RH:75±5%). Nymphs were kept in plastic cylinders (10x5cm) at 25±1˚C, 65±5% relative humidity, and 16:8 (L:D) photoperiod. Adult males and females were removed from the nymphal culture on the day of emergence and kept separated by gender. Grapevine leaves were provided as food source: a grapevine leaf disk was laid over a 1-cm layer of technical agar solution [0.8% (wt⁄vol)] at the bottom of a plastic cylinder and replaced twice a week. All tests were done with virgin males and females.

Colours

The chosen colours were blue, green, red and yellow for laboratory tests and blue, red and yellow for field tests. The spectral reflectance (range: 360-1160nm) of each employed colour was measured with a EPP-2000 spectroradiometer (Stellarnet). Peaks of reflectance were: blue = 475nm (46%), green = 521nm (54%), yellow = 573nm (56%) and red = 650nm (56%).

Laboratory tests

To observe the behaviour of *S. titanus* when exposed to colour stimuli, two-choice tests were performed in a Y-tube (stem and arms, 40cm; diameter, 7cm; side arms at 60°). The tube was kept vertical since in preliminary tests the insect showed a noteworthy negative geotropism. Coloured disks (6.9cm i.d.) were inserted in each arm 10cm before the fork and kept still with an iron wire fixed at the upper mouth of the same arm. Groups of 2-3 insects of the same gender were inserted from the base of the stem. Tests were conducted in a dark room (25±1°C, 60±5%) and each coloured disk was enlightened with a fluorescent light (~300 lux). Two-choice tests were made by crossing any colour with each others. In tests involving blue, 20 males and 10 females were released; in all other tests 20 males and 20 females were released.

Results were analyzed by log-likelihood ratio (G-test), after Williams' correction. The statistics included also individuals that did not make a choice.

Field tests

Nine groups of three differently coloured (blue, red and yellow) sticky traps (Rebell, Andermatt, Switzerland) of 15x8cm were homogeneously positioned in a Chardonnay vineyard trained on 'Trentino Pergola' system at Villazzano (Trento, Italy), from 10th of July to 30th of September 2008 and 2009. Traps were hung at the grapevine supporting iron wire (height 120 cm) and weekly replaced. Within each group the traps were separated by a distance of 50cm. Each group was distant from the closest one of a range of 50-70m.

A Friedman test (non-parametric two-way Anova) followed by Bonferroni-Dunn multiple comparison tests was used to discriminate among colours and genders in terms of number of captured individuals (Siegel & Castellan, 1988). The sex rate (percentage of captured males) was measured along each season, by calculating the average of each month (four samplings) for each colour.

Results and discussion

Laboratory tests

The two-choice tests in Y-tube showed that *S. titanus* responds differently to red, yellow, green and blue colours (Tab. 1). Blue disks were never significantly preferred by the tested group of specimens, while green prevailed only over blue. The yellow disks were significantly more attractive to males whereas females were more attracted by the red colour.

Table 1. Results from the two-choice tests conducted on males (grey cells) and females (white cells) of *S. titanus*. Inside each cell are indicated the first letter of the preferred colour and the statistical level of significance after G-test (* < 0.05; ** < 0.01; *** < 0.001; ns = not significant). For each cell n = 20 except those cells involving blue where n = 10.

	Blue	**Green**	**Red**	**Yellow**
Blue		G **	ns	Y **
Green	G *		R ***	ns
Red	R ***	R *		R ***
Yellow	Y ***	Y *	Y *	

Field tests

The numbers of captured individuals and seasonal trends were similar between the two years of study. Yellow sticky traps collected always the highest number of both males and females, followed by red and blue traps (Tab. 2). Male and female captures were in similar quantity in 2008, while in 2009 the females slightly prevailed. During each season the sex rate (percentage of males on the total of captures) greatly varied: the highest value was found at the first seasonal sampling, then it constantly decreased until the end of the surveying period (values around 50% were recorded in August), exactly mirroring the insect life biological cycle. In general, yellow traps always kept the highest and blue traps the lowest values of sex rate.

Table 2. The rank mean (after Friedman's Test) of *S. titanus* males and females captured by red, yellow and blue sticky traps in 2008 and 2009 and the mean value of sex rate (Sr) of individuals captured for each month of each year. Different letters indicate significant difference among the rank means of the same year (Bonferroni-Dunn multiple comparison test).

Year	Gender	Blue	Red	Yellow
2008	Males	21.3 a	28.5 bc	36.7 d
	Females	23.0 a	26.3 b	29.1 c
	Sr July	0.62	0.78	0.87
	Sr August	0.56	0.56	0.62
	Sr September	0.28	0.28	0.37
2009	Males	23.5 a	27.2 b	36.6 d
	Females	28.1 bc	30.2 c	37.4 d
	Sr July	0.69	0.78	0.80
	Sr August	0.47	0.52	0.54
	Sr September	0.21	0.18	0.25

The results are partially different between field and laboratory, in particular for the female preference, to red in Y-tube tests and to yellow in field tests. This difference could be explained by the following three factors: 1) the different physiological state of the females in the field (mated and virgin) and in the lab (virgin), 2) the presence of a background of colours in the field, 3) the variable light conditions during the day in the field.

Moreover, the peculiarity of experimental conditions, such as the grapevine training system, the pedoclimate, and the monitoring method, may strongly affect the field results and determine remarkable discrepancies also between field tests if conducted in different localities.

Further research will focus on the above mentioned factors. Nonetheless, these results clearly suggest that *S. titanus* adults are influenced and oriented by the visual cues present in their habitat. An improved understanding of colour attraction is an important knowledge to take into account when creating a monitoring system of *S. titanus*.

Acknowledgements

This study was funded by Interneuron Project (Fondazione E. Mach), Host Project (Autonomous Province of Trento) and Fondi Ateneo of Pisa University.

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