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Standardization and criticism of sampling procedures using sticky card traps: monitoring sap-sucking insect pests and *Anagrus atomus* inhabiting European vineyards

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Abstract

Coloured sticky card traps are widely used for sampling and control of sap-sucking insect pests. In European vineyards they are used for monitoring leafhoppers [i.e., *Empoasca vitis* (Gothe), *Zygina rhamni* Ferrari, *Scaphoideus titanus* Ball], the vine thrips *Drepanothrips reuteri* Uzel and the leafhopper egg parasitoid *Anagrus atomus* (L.). A study was conducted to establish the trap factors that influence captures of these insects (i.e., size, inclination, exposure days, colour, position within canopy and side orientation). The total captures of grapevine leafhoppers increased as trap size increased, without a significant decline in captures relative to unit area. All leafhopper species were more attracted by vertical traps than horizontal traps, and in the latter case, *E. vitis* and *Z. rhamni* were mainly captured on the underside of the trap, while *S. titanus* on the upper side. For all leafhoppers and *D. reuteri*, efficiency decreased with the number of days the traps remained in the field. Yellow was a colour preferred by all insects, with *Z. rhamni* showing a strong preference for lighter yellows. *S. titanus* was also captured by red traps and *A. atomus* by colourless ones. *Z. rhamni* and *S. titanus* showed a preference for traps placed in shady positions, whereas *E. vitis* and *D. reuteri* preferred traps in sunny positions. *E. vitis* and *D. reuteri* preferred trap sides exposed to sunlight in the late afternoon and early morning, respectively. Our results were compared with the literature and discussed in relation to the feeding preference and behaviour of the different species.

Key words: leafhoppers, thrips, Hymenoptera Mymaridae, grapevines, behaviour, sticky cards.

Introduction

In field and greenhouse crops coloured sticky card traps are widely used as sampling and control method for sap-sucking insect pests (e.g., thrips, whiteflies, leafhoppers) and fruit flies (Prokopy and Owens, 1983; Chu *et al.*, 2000; Pinto-Zevallos and Vänninen, 2013). Their attractiveness is based on the role of visual stimuli in host plant selection (Döring and Chittka, 2007; Brévault and Quilici, 2010). For sap-sucking insects, host plant selection involved both visual and chemical stimuli, with the former often effective at long distance and the latter only at very close range (Saxena and Saxena, 1975; Zhang *et al.*, 2018). The same traps can also capture natural enemies belonging to different arthropods' orders (Wallis and Shaw, 2008; Reddy and Rajan, 2016).

In the context of Integrated Pest Management for sustainable agriculture, sampling of adult insect pests with sticky traps is commonly used to establish the timing of control measures (Bažok *et al.*, 2012) and the exceeding of economic thresholds (Shipp *et al.*, 1998; Pinto-Zevallos and Vänninen, 2013). Within experimental studies, captures with sticky traps have been used to verify the efficacy of control measures against pests (Markò *et al.*, 2008; Pavan *et al.*, 2012) and to know their host plant preference (Pavan and Picotti, 1993; Bentz and Townsend, 2004; Gharekhani *et al.*, 2014), adult phenology, spatial distribution and migration from natural habitats to cultivated fields and *vice versa* (e.g., for leafhoppers and planthoppers: McClure, 1982; Purcell and Suslow, 1982; Whitney and Meyer, 1988; Blua and Morgan, 2003; Bentz and Townsend, 2004; Lessio *et al.*, 2011; 2016; Riolo *et al.*, 2014; Mori *et al.*, 2016; e.g., for thrips: Allan and Gillett-Kaufman, 2018; Rodriguez-Saona *et al.*, 2010). Sticky traps are also used in faunistic surveys of the potential insect vectors of pathogens (Klein *et al.*, 2001; Dellapé *et al.*, 2016).

Yellow sticky traps have been widely used in European vineyards to monitor the Palaearctic leafhoppers *Empoasca vitis* (Gothe) and *Zygina rhamni* Ferrari (Hemiptera Cicadellidae) (Pavan *et al.*, 1988; Bosco *et al.*, 1996; Pavan, 2000; Decante and van Helden, 2006; 2008; Mazzoni *et al.*, 2008), the Nearctic leafhoppers *Scaphoideus titanus* Ball (Pavan *et al.*, 1987; 2012; Jermini *et al.*, 1992; Lessio and Alma, 2004a; 2004b; Grižon *et al.*, 2012) and *Erasmoneura vulnerata* (Fitch) (Hemiptera Cicadellidae) (Duso *et al.*, 2019; 2020), the vine thrips *Drepanothrips reuteri* Uzel (Thysanoptera Thripidae) (Strapazzon, 1989; Jenser *et al.*, 2010) and the grape phylloxera *Viteus vitifoliae* (Fitch) (Hemiptera Phylloxeridae) (Stevenson and Jubb, 1976; Strapazzon, 1987).

In vineyards, yellow sticky traps have also been used to study the biology of *Anagrus* spp. (Hymenoptera Mymaridae) egg parasitoids of leafhoppers (Antolin and Strong, 1987; Picotti and Pavan, 1993; Corbett and Rosenheim, 1996; Ponti *et al.*, 2003; Viggiani *et al.*, 2006; Prischmann *et al.*, 2007; Zanolli and Pavan, 2011).

To compare data on captures with sticky traps collected in different grape-growing areas and years, it is necessary to standardize the capture methods. In this regard, different aspects such as trap colour, size and position have been considered in a number of studies carried out on different crops.

The role of sticky trap colour has been demonstrated for many insects and the preference for different colours is associated with the plant part on which the insect pests feed (e.g., Chu *et al.*, 2000; Chen *et al.*, 2004). *D. reuteri*, which preferentially infests newly sprouted leaves of broad-leaf plants like grapevines, is mainly captured by yellow traps (Jenser *et al.*, 2010), whereas thrips that primarily infest herbaceous plants, such as *Frankliniella* spp. and *Thrips tabaci* Lindeman (Thysanoptera Thripidae), are most often captured by blue and red traps that resemble the colours of host plant flowers (Vernon and Gillespie, 1990; Blumthal *et al.*, 2005; Yaku *et al.*, 2007; Demirel and Yıldırım, 2008; Broughton and Harrison, 2012; Gharekhani *et al.*, 2014). Sap-sucking leafhoppers belonging to the Empoascini, included *E. vitis* on tea plants, are mostly captured by yellow traps, which is in agreement with leaf reflectance (Mensah, 1996; Demirel and Yıldırım, 2008; Bian *et al.*, 2014). Yellow traps are also often preferred by *S. titanus*, but in some cases red traps are even more attractive (Lessio and Alma, 2004b; Mazzoni *et al.*, 2011). However, colours other than yellow are also preferred by other leafhoppers (Rodriguez-Saona *et al.*, 2012). Among natural enemies, captures of Aeolothripidae, Anthocoridae, Coccinellidae and Hymenoptera parasitoids are higher on yellow traps, whereas those of Syrphidae on blue traps (Romeis *et al.,* 1998; Chen *et al.*, 2004; Rodriguez-Saona *et al.*, 2012; Gharekhani *et al.*, 2014; Tang *et al.,* 2016). *Anagrus* spp. are mostly captured by yellow and colourless traps with a preference for the former noted in Larsen *et al.* (2014) and the latter in Wallis and Shaw (2008). However, yellow traps have the advantage of being repellent to honey bees (Rodriguez-Saona *et al.*, 2012).

In terms of trap size, yellow sticky traps of small size (2.2 × 21 cm) have been reported to capture more *D. reuteri* adults per cm^2 than medium (11 × 21 cm) and large (22 × 21 cm) sizes (Strapazzon *et al.*, 1990).

The position of sticky traps with respect to the plant canopy influences the number of captured insects. The captures of thrips are usually larger when the traps are positioned in the higher parts of canopy (Rodriguez-Saona *et al.*, 2010; Gharekhani *et al.,* 2014), or even some centimetres above it (Gencsoylu, 2007). The captures of leafhoppers in some cases are higher at canopy levels, such as for *S. titanus* on grapevine (Lessio and Alma, 2004b) and *Empoasca* spp. on cotton (Gencsoylu, 2007), but in the case of *E. vitis* on tea, higher captures were recorded above the canopy (Bian *et al*., 2014). The captures of *V. vitifoliae* were greater in the upper part of the canopy, but decreased when the traps were positioned progressively higher above the grapevine (Stevenson and Jubb, 1976).

In the northern hemisphere, thrips preferred the east-west oriented traps on both cotton and tea (Gencsoylu, 2007; Bian *et al.*, 2016), whereas north-south oriented traps captured more *Empoasca* spp. on cotton (Gencsoylu, 2007). On cotton, vertical traps with respect to the ground captured more *Empoasca* spp. and *Frankliniella* spp. than horizontal traps (Gencsoylu, 2007), whereas the contrary was reported on grapevine for *S. titanus* (Jermini *et al.*, 1992).

The aim of this study was to acquire new insights on sticky trap factors that can influence adult captures (i.e., size, inclination, exposure days, colour, position within canopy and side orientation) of some grapevine sap-sucking insects and *Anagrus atomus* (L.) (*sensu* Triapitsyn *et al.*, 2020) in vineyards in order to standardize the sampling procedures.

Materials and methods

To set up the monitoring of grapevine sap-sucking insects and *A. atomus* with sticky traps, four different experiments were carried out under field conditions.

Experimental vineyard and sampling methods

The study was conducted in one hectare vineyard located in northeastern Italy (Pasiano di Pordenone, Pordenone district, 45°50'41"N 13°39'24"E, 13 m a.s.l., cultivar Verduzzo Trevigiano). The climate of the area is of humid moderate continental type with an average annual temperature of 12 °C and an average annual rainfall of 1300 mm. The soil has a silty clay structure. The vineyard had eight rows oriented N50°W-S40°E, with the grapevines trained to the Sylvoz system and planted at distances between and within rows of 4.0 and 2.0 m, respectively. The soil was periodically tilled along the rows, while spontaneous herbaceous vegetation was present in the inter-rows. In the vineyard, standard fungicide programs were followed, and no insecticide was sprayed before and during this study.

Sticky traps used in the field experiments, unless otherwise specified, were obtained from yellow plastic sheets with a thickness of 0.1 cm (Plastibor S.r.l., Ponte San Nicolò, Padova, Italy) cut to have traps of three different sizes: 2.2 × 21 cm, named "small"; 11 × 21 cm, named "medium"; 22 × 21 cm, named "large". The small and large traps were five times narrower and twice larger than the medium, respectively. The traps were smeared on both sides for 4/5 of the surface with colourless glue (Temo-O-Cid, Kollant S.r.l., Vigonovo VE, Italy). In the vineyard, unless otherwise specified, the traps were hung on support wires at about 1.5 m from ground level in a vertical position facing the outer sides of the canopy, and then parallel to the direction of the grapevine row; traps were immersed in the foliage of the canopy with their faces unobscured by leaves, so as to be visible looking from the inter-rows (shady position).

In the laboratory, captured adults of the grapevine leafhoppers *E. vitis*, *Z. rhamni* and *S. titanus*, the vine thrips *D. reuteri* and the parasitoid *A. atomus* were identified on the traps (after Vidano, 1963; 1964; Mound *et al.*, 1976; Zanolli *et al*., 2016; Nugnes *et al*., 2017; Triapitsyn *et al.*, 2020) and counted under a dissecting microscope.

Experiment I: trap size

The influence of trap size on the captures of *E. vitis*, *Z. rhamni* and *S. titanus* adults was studied from 11th July to 31st August, replacing the traps weekly for a total of eight sampling intervals. For this purpose, the captures on yellow traps of small, medium and large sizes were compared. These three trap sizes had been previously compared for *D. reuteri* captures (Strapazzon *et al.*, 1990). For each sampling interval, the three trap sizes were randomly replicated in three different rows 4 m apart from each other. The distance along the rows among the traps of different size was 4 m.

Experiment II: trap inclination with respect to the ground

The influence of trap inclination with respect to the ground on captures of *E. vitis*, *Z. rhamni* and *S. titanus* adults was studied by exposing yellow traps of medium size from the 17th to the 24th of September, without ever replacing them. For this purpose, the captures on traps inclined at 90 and 0 degrees to the ground (hereafter named "vertical" and "horizontal" traps, respectively) were compared. The sides of vertical traps faced north-west and south-east and those of horizontal traps faced up and down. Both vertical and horizontal traps were located in shady position as described in the "Experimental vineyard and sampling methods" paragraph. To keep horizontal traps in position, they were fixed onto paired wires located about 30 cm above the horizontal cordon, with the longer side of the trap parallel to the row. The two trap inclinations were randomly replicated in six different rows 4 m apart from each other. The distance along the rows among the traps of different inclination was 4 m.

Experiment III: trap exposure days

The influence of trap exposure days on the captures of *E. vitis*, *S. titanus* and *D. reuteri* adults was studied from 7th to 18th August. Captures recorded on yellow traps of medium size exposed for 12 consecutive days were compared with those replaced after six days, every three days and daily. The four sets of exposure days were randomly replicated on three different rows 4 m apart from each other. The distance along the rows among the traps of different exposure days was 4 m.

Experiment IV: trap colour, position within the canopy and side orientation

The study on the influence of the trap colour on the captures of *E. vitis*, *Z. rhamni*, *S. titanus*, *D. reuteri* and *A. atomus* was replicated over three weekly sampling intervals (5-12 July, 7-14 August and 25 September-2 October, i.e. early July, mid-August and late September). This experiment was carried out with traps of small size (i.e., 2.2 × 21 cm). The yellow trap of small size described in Experiment I (here named control yellow) was compared with a colourless trap and seven coloured traps (3 yellow, 1 blue, 1 green, 1 white and 1 red). To make the colourless traps, a transparent plastic sheet (thickness 4 mm) was used. To make the coloured traps, the colourless plastic sheet was covered with the following commercial pigments: three yellow and blue (Garzanti Specialities S.p.a., Milano, Italy), white (Colorificio San Marco S.p.a., Marcon, Venezia, Italy) and red (Saratoga S.p.a., Trezzano sul Naviglio, Milano, Italy). The green colour was obtained by mixing light yellow and blue pigments in a 1:1 ratio. The spectral reflectance curves of the different colours were measured using a Zeiss microscope with a photometer head consisting of an HBO100W lamp, a monochromator for selecting the incident wavelength, and a Plan-Neofluar 25× objective. The intensity of the reflectance of the area under examination was measured, at intervals of 10 nm, as a percentage relative to standard white (calcium carbonate). The reflectance curves for the colours used are shown in figure 1. The colours of traps obtained with the three yellow pigments were named light yellow, mid yellow and dark yellow, respectively. The control yellow showed a tonality similar to light yellow but at a lower intensity.

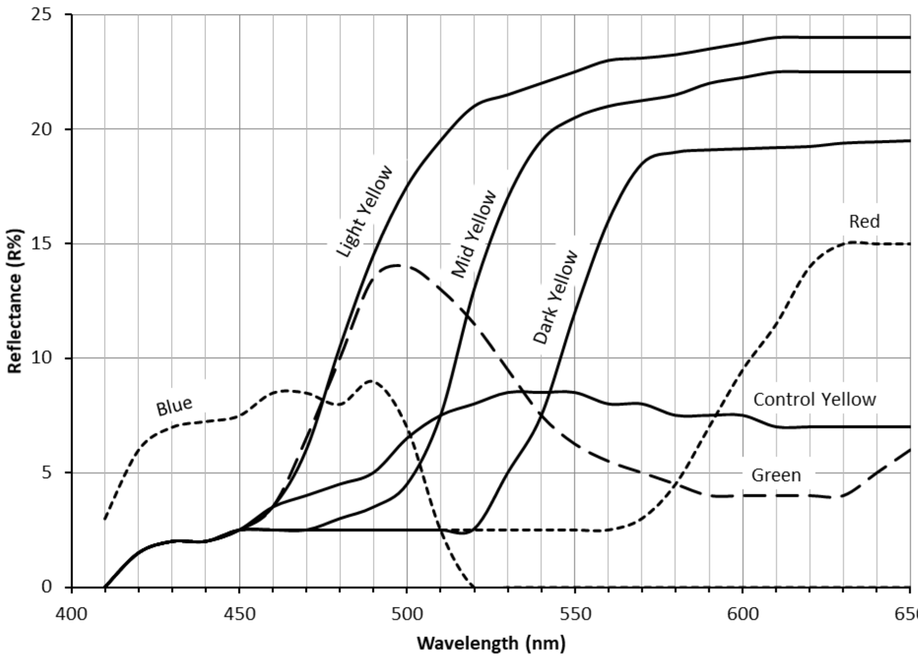

Figure 1. Spectral reflectance curves of different coloured traps used in the experiment on the effect of trap colour on the captures of the studied species.

For both the colourless and coloured traps, a shady position, as described in the "Experimental vineyard and sampling methods" paragraph, was compared to a sunny position. For this latter position, the traps were exposed to direct sunlight during the day by removing the foliage above them. In contrast, the traps in shady positions could be hit by direct sunlight only in the early morning and late afternoon, i.e. for some hours after sunrise and before sunset. Moreover, the different sunlight exposure of the two faces of each trap, both those in sunny and shady positions, was taken into account as orientation side effects. At each sampling interval, the nine colour traps and the two canopy positions were randomly replicated on four different rows 4 m apart from each other. The distance along the rows among the traps of different colours was 4 m. In the laboratory, the adult captures of the studied taxa on the two trap sides were counted separately in order to distinguish those on the northwest-oriented side (i.e., that in direct sun in the late afternoon) from those on the southeast-oriented side (i.e., that in direct sun in the early morning).

Statistical analysis

In Experiments I and IV, a mixed ANOVA was performed to compare the differences in adult captures, considering treatments as between-subjects factor and time as within-subjects factor. For data of Experiment I, trap size, time, and their interactions were considered as sources of variation and their effects were tested with an F test. The captures on traps of different sizes were also compared by considering the total captures relative to the size of the small trap, i.e. by dividing captures on the medium and large traps by 5 and 10, respectively. For data of Experiment IV, trap colour, trap position within canopy, trap-side orientation, time, and their interactions were considered as sources of variation and their effects were tested with an F test. For *S. titanus*, time was not considered because captures occurred only in one sampling interval. Treatments were compared using a t test to the least-square means with Bonferroni adjustment of the P values ($\alpha = 0.05$). The Kenward-Roger method was used for degrees of freedom estimation. This method can produce not integer value for degree of freedom. Data were checked for analysis assumptions and were log ($x + 1$) transformed prior to the analyses. Post hoc comparisons were performed with a Tukey's test.

To compare adult captures in Experiments II and III, after logarithmic transformation of data, a t test and a one-way ANOVA followed by a Tukey's post hoc test were performed, respectively. For the horizontal traps of Experiment II, adult captures between underside and upper side were compared with a paired t test.

Analyses were performed with IBM SPSS Statistics 20.

Results

Experiment I: trap size

The sampling interval significantly influenced the number of captures of the three leafhoppers, i.e. *E. vitis* (F = 76.55; d.f. = 2.85, 17.11; P < 0.0001), *Z. rhamni* (F = 17.47; d.f. = 2.25, 13.54; P < 0.0001) and *S. titanus* (F = 9.50; d.f. = 2.46, 14.76; P < 0.001). In particular, captures were highest for *E. vitis* in early August, *Z. rhamni* in mid-July and *S. titanus* in mid-August (data not shown).

The trap size significantly influenced the number of captures of the three leafhoppers, i.e. *E. vitis* (F = 97.69; d.f. = 2, 6; P < 0.0001), *Z. rhamni* (F = 40.98; d.f. = 2, 6; P < 0.0001) and *S. titanus* (F = 32.33; d.f. = 2, 6; P < 0.001). For all three species, captures observed on the small traps were significantly lower than on the medium and large traps, which were respectively five and 10 times larger, but significant differences between medium and large traps were only observed for *E. vitis* (figure 2A).

When the number of captures on the medium and large traps was adjusted relative to the surface of the small trap, there were no significant differences between trap size for any of the three leafhoppers, i.e. *E. vitis* (F = 1.70; d.f. = 2, 6; P = 0.26), *Z. rhamni* (F = 1.80; d.f. = 2, 6; P = 0.25) and *S. titanus* (F = 0.13; d.f. = 2, 6; P = 0.88) (figure 2B). This occurred because the preferred trap size, on average, was not the same in all samplings. Indeed, the interaction between the sampling interval and the trap size were significant for *S. titanus* (F = 3.16; d.f. = 4.98, 14.94; P = 0.039) and close to significance for *E. vitis* (F = 2.21; d.f. = 5.68, 17.04; P = 0.10) and *Z. rhamni* (F = 1.93; d.f. = 4.35, 13.07; P = 0.16).

Experiment II: trap inclination with respect to the ground

The number of captures was significantly greater on the vertical traps than on the horizontal traps for *E. vitis* (t = 3.77; d.f. = 10; P = 0.0036) and *S. titanus* (t = 2.69; d.f. = 10; P = 0.023), but not for *Z. rhamni* (t = 1.24; d.f. = 10; P = 0.24) (figure 3A).

Captures were significantly higher on the underside of the horizontal traps for *E. vitis* (t = 7.38; d.f. = 5; P = 0.0007) and *Z. rhamni* (t = 5.81; d.f. = 5; P = 0.0021) (figure 3B). On the contrary, the captures of *S. titanus* were higher on the upper side but the difference did not reach statistical significance (t = 2.46; d.f. = 5; P = 0.057).

Experiment III: trap exposure days

The number of captures was significantly influenced by the number of continuous days that the traps were left out in the field for the leafhoppers *E. vitis* (F = 4.21; d.f. = 3, 8; P = 0.046) and *S. titanus* (F = 9.08; d.f. = 3, 8; P = 0.006) and the thrips *D. reuteri* (F = 6.52; d.f. = 3, 8; P = 0.015). For all these species the captures over 12 days were significantly higher on the traps replaced every day than on those exposed for 12 consecutive days (i.e., "never") (figure 4). For *E. vitis* the captures gradually decreased with the increasing consecutive trap exposure days, whereas for *S. titanus* and *D. reuteri* the decrease occurred mostly between six and 12 and between three and six consecutive trap exposure days, respectively.

Experiment IV: trap colour, position within the canopy and side orientation

The sampling interval significantly influenced the number of captures of *E. vitis* (F = 304.76; d.f. = 2, 216;

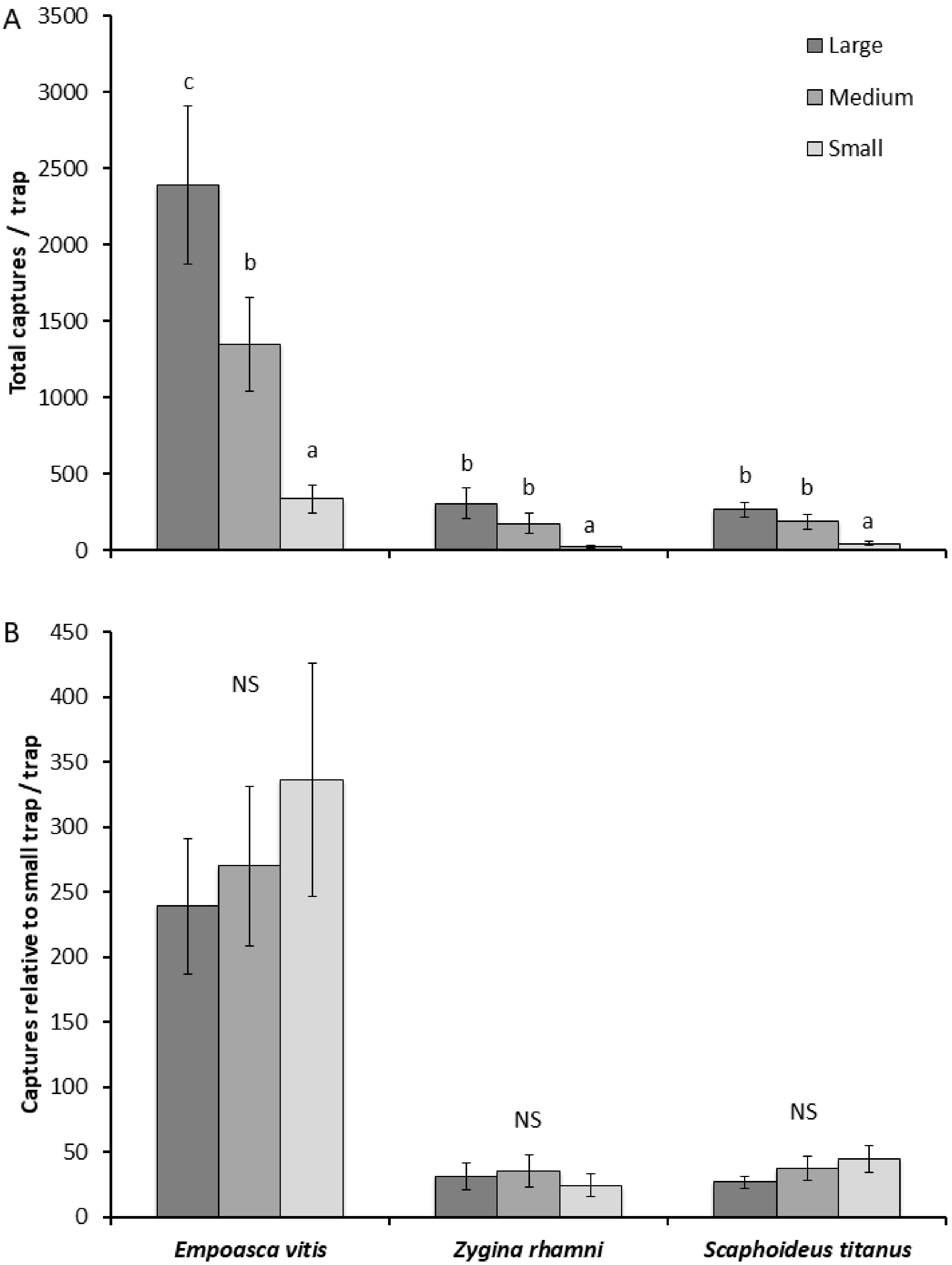

Figure 2. Experiment I. Captures (mean ± SE) of three grapevine leafhoppers, *E. vitis*, *Z. rhamni* and *S. titanus*, on yellow traps of different size (large, 22 × 21; medium, 11 × 21; small 2.2 × 21 cm) replaced weekly from early July to late August. Both total captures (**A**) and captures relative to the size of the small trap (**B**) are reported. Different small letters above columns indicate significant differences among size according to Tukey's test (α = 0.05). NS = non-significant differences.

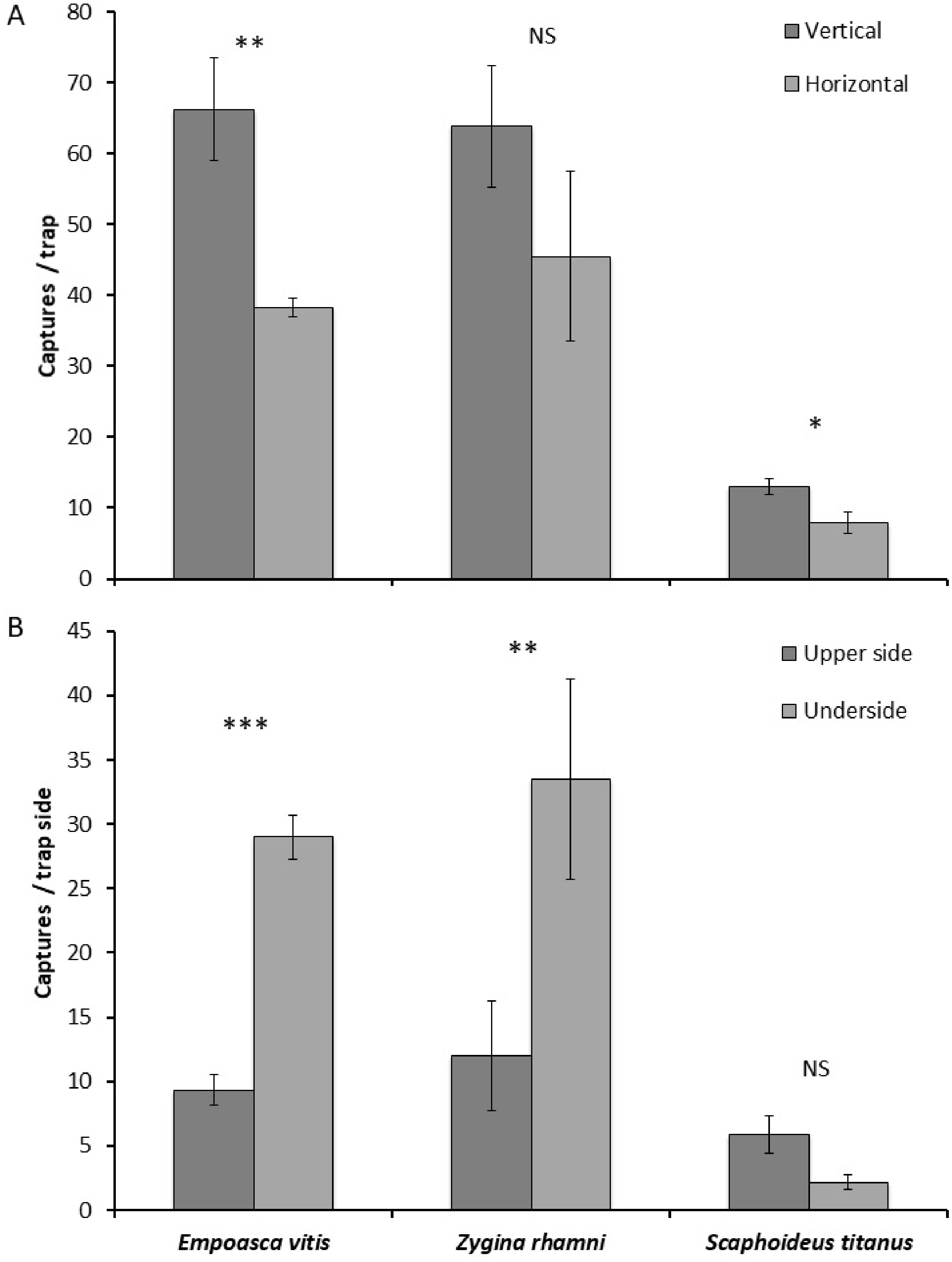

Figure 3. Experiment II. Captures per trap (mean ± SE) of three grapevine leafhoppers, *E. vitis*, *Z. rhamni* and *S. titanus*, on vertical or horizontal yellow traps of medium size with respect to the ground. For horizontal traps the captures on the upper side and underside are also reported. NS = non-significant differences; *, **, *** = significant differences at 0.05, 0.01, 0.001 levels using an unpaired t test for vertical vs horizontal inclination and a paired t test for upper side vs underside.

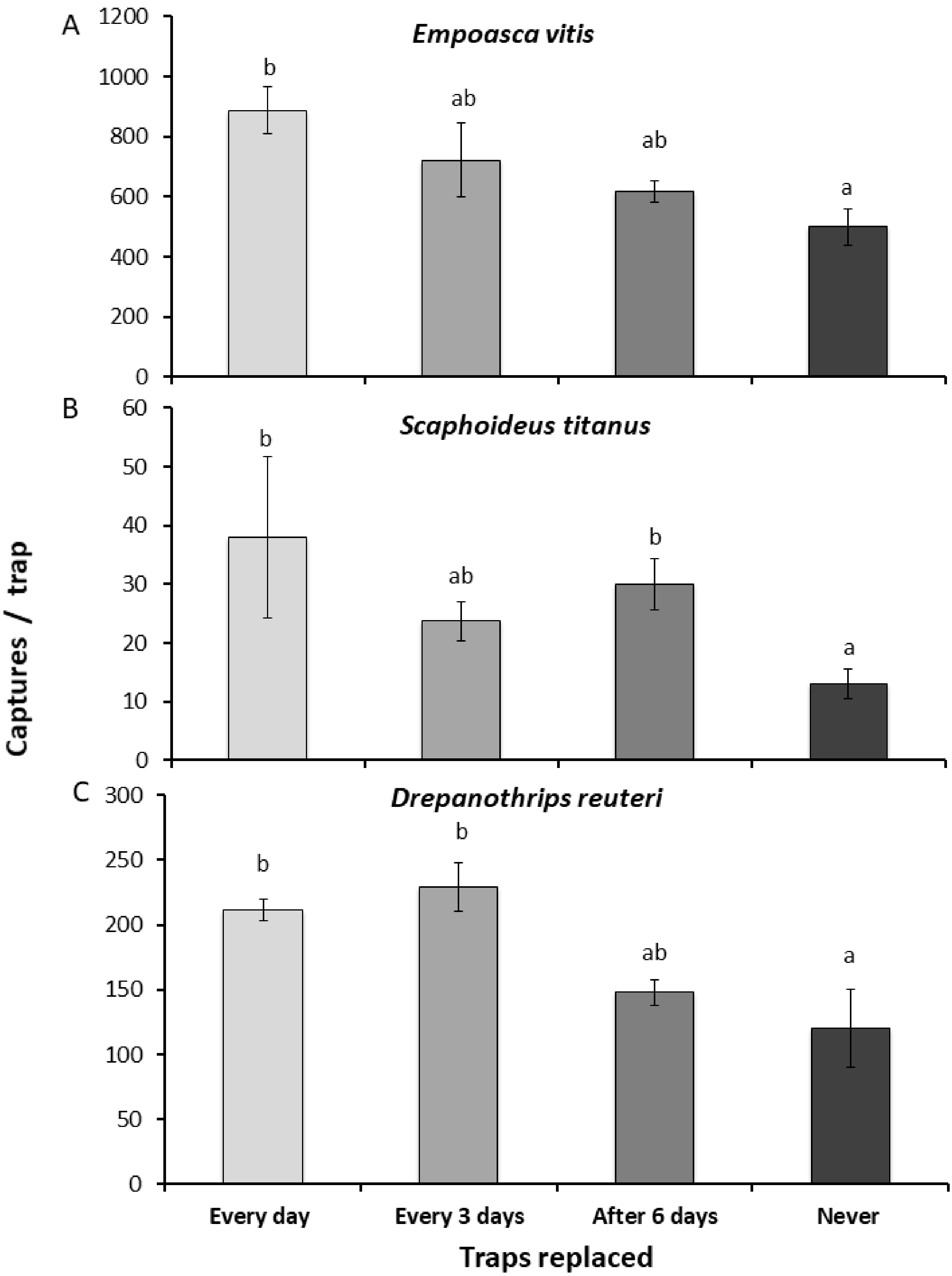

Figure 4. Experiment III. Captures per trap (mean ± SE) over 12 days of two grapevine leafhoppers *E. vitis* and *S. titanus*, and the vine thrips *D. reuteri*, depending on the number of continuous days that the traps were left out in the field. Yellow traps of medium size were used.

P < 0.0001), *Z. rhamni* (F = 121.90; d.f. = 2, 216; P < 0.0001), *D. reuteri* (F = 81.85; d.f. = 2, 216; P < 0.0001) and *A. atomus* (F = 51.72; d.f. = 2, 216; P < 0.0001). The highest captures of *E. vitis* occurred in mid-August, those of *Z. rhamni* in late September, those of *D. reuteri* in early July and those of *A. atomus* in mid-August (data not shown). For *S. titanus* the captures were abundant only in mid-August (N. 596), whereas they were negligible in early July (N. 7) and absent in late September, so these last sampling intervals were not considered in the statistical analysis.

The trap colour significantly influenced the number of captures of all sampled species, i.e. *E. vitis* (F = 172.42; d.f. = 8, 108; P < 0.0001), *Z. rhamni* (F = 7.02; d.f. = 8, 108; P < 0.0001), *S. titanus* (F = 3.87; d.f. = 8, 105; P < 0.001), *D. reuteri* (F = 134.16; d.f. = 8, 108; P < 0.0001) and *A. atomus* (F = 13.03; d.f. = 8, 108; P < 0.0001).

The trap colour preference varied concerning to the species (figure 5). For *E. vitis*, all of the yellow traps captured significantly more adults than the other colours, with control yellow being significantly less attractive than mid yellow; the blue, red and white traps did not differ from colourless traps, and the green traps even seemed repellent because they captured significantly fewer adults than the colourless traps. For *Z. rhamni*, the four yellow traps and the green traps captured the largest numbers of adults without significantly differing from each other, whereas captures with the other colours were significantly lower than at least one of the trap types among the yellow and green group. For *S. titanus*, the mid yellow and dark yellow traps captured the largest number of adults, both being significantly different from the blue and green traps, but only the dark yellow traps were significantly different from the white traps. For *D. reuteri*, the two lighter yellow traps (i.e., light yellow and control yellow) captured significantly more adults than the two darker yellow traps (i.e., mid yellow and dark yellow) and all the other traps. For *A. atomus*, all of the yellow traps were more attractive than the other colours, but not than colourless ones. For both *D. reuteri* and *A. atomus* the blue and red traps even seemed to be repellent because they captured significantly fewer adults than the colourless traps.

The trap position within the canopy (i.e., shady or sunny) significantly influenced the number of captures of all sampled species, i.e. *E. vitis* (F = 40.31; d.f. = 1, 108; P < 0.0001), *Z. rhamni* (F = 236.49; d.f. = 1, 108; P < 0.0001), *S. titanus* (F = 19.57; d.f. = 1, 105; P < 0.0001), *D. reuteri* (F = 607.91; d.f. = 1, 108; P < 0.0001) and *A. atomus* (F = 11.86; d.f. = 1, 108; P < 0.001). *Z. rhamni*, *S. titanus* and *A. atomus* had a significantly greater preference for shady positions than sunny positions, and the differences were substantial for *Z. rhamni* (5×, but only 1.3× and 2.1× for *S. titanus* and *A. atomus*, respectively) (figure 6). In contrast, for *E. vitis* and *D. reuteri*, traps in sunny positions were significantly more attractive than those in shady positions, but the multiplication coefficient was very high for the vine thrips (29×) and rather low for the leafhoppers (1.7×).

The trap side orientation (i.e., northwest or southeast) significantly influenced the number of captures of *E. vitis* (F = 4.29; d.f. = 1, 108; P = 0.041) and *D. reuteri* (F = 10.33; d.f. = 1, 108; P = 0.002). For *E. vitis*, the northwest-oriented side of traps had significantly more captures than the southeast-oriented side, but the differences were negligible (1.1×) (figure 6). Contrastingly, for *D. reuteri* the southeast-oriented side captured significantly more adults than the northwest-oriented side, the difference being fairly substantial (2.9×). For *Z. rhamni*, the captures were higher on the northwest-oriented side of traps with differences very close to level of significance (F = 3.85; d.f. = 1, 108; P = 0.052). No preference was observed for *S. titanus* (F = 0.36; d.f. = 1, 105; P = 0.55) and *A. atomus* (F = 0.037; d.f. = 1, 105; P = 0.85).

The sampling interval × trap colour interaction was significant for *E. vitis* (F = 2.42; d.f. = 16, 216; P = 0.002), *Z. rhamni* (F = 2.33; d.f. = 16, 216; P = 0.003), *D. reuteri* (F = 8.42; d.f. = 16, 216; P < 0.0001) and *A. atomus* (F = 2.86; d.f. = 16, 216; P < 0.0001). For *E. vitis*, the captures on mid yellow compared to light yellow trap were on average higher in early July (46.8 ± 6.2 vs 26.8 ± 4.2) and lower in late September (7.4 ± 1.0 vs 9.4 ± 1.9). For *Z. rhamni*, the captures on light yellow compared to dark yellow trap was on average higher in early July (3.1 ± 0.9 vs 1.4 ± 0.4) and mid-August (4.0 ± 1.6 vs 2.1 ± 0.7) and lower in late September (16.6 ± 4.8 vs 18.8 ± 5.4). For *D. reuteri*, the differences between the two lighter yellow traps (i.e., light yellow and control yellow) and the two darker yellow traps (i.e., mid yellow and dark yellow) were the lowest in late September (3.4 ± 1.0 vs 2.2 ± 0.7, i.e. 1.6×), intermediate in early July (67.9 ± 20.0 vs 19.1 ± 6.5, i.e. 3.6×) and the highest in mid-August (63.5 ± 23.3 vs 9.5 ± 3.5, i.e. 6.7×). In the case of *A. atomus* there was a greater preference for light yellow than dark yellow traps in early July (1.1 ± 0.3 vs 0.8 ± 0.2) and mid-August (3.8 ± 1.5 vs 2.4 ± 0.6), whereas the opposite occurred in late September (0.25 ± 0.11 vs 0.44 ± 0.11).

The sampling interval × trap position interaction was significant for *E. vitis* (F = 6.40; d.f. = 2, 216; P = 0.002), *Z. rhamni* (F = 5.70; d.f. = 2, 216; P = 0.004) and *D. reuteri* (F = 47.24; d.f. = 2, 216; P < 0.0001), but not for *A. atomus* (F = 1.70; d.f. = 2, 216; P = 0.19). For *E. vitis*, there was a greater preference for sunny positions in late September (4.9 ± 1.0 vs 2.5 ± 0.5 in shady positions, i.e. 2×) than in August (32.2 ± 6.2 vs 22.9 ± 3.9 in shady positions, i.e. 1.4×) when there were no significant differences between the two positions. For *Z. rhamni*, there was a greater preference for shady positions than sunny positions in mid-August (4.3 ± 0.7 vs 0.3 ± 0.1, i.e. 13×) over the other two sampling intervals (in early July: 3.2 ± 0.5 vs 0.7 ± 0.2, i.e. 4.3×; in late September: 17.1 ± 2.9 vs 3.7 ± 0.7, i.e. 4.6×). For *D. reuteri*, there was a greater preference for sunny positions in mid-August (33.1 ± 12.2 vs 0.6 ± 0.3, i.e. 53×) than in late September (2.1 ± 0.6 vs 0.6 ± 0.3, i.e. 4×).

The sampling interval × trap side orientation interaction was not significant for any of the species, i.e. *E. vitis* (F = 0.15; d.f. = 2, 216; P = 0.86), *Z. rhamni* (F = 0.15; d.f. = 2, 216; P = 0.86), *D. reuteri* (F = 2.40; d.f. = 2, 216; P = 0.093) and *A. atomus* (F = 1.16; d.f. = 2, 216; P = 0.31).

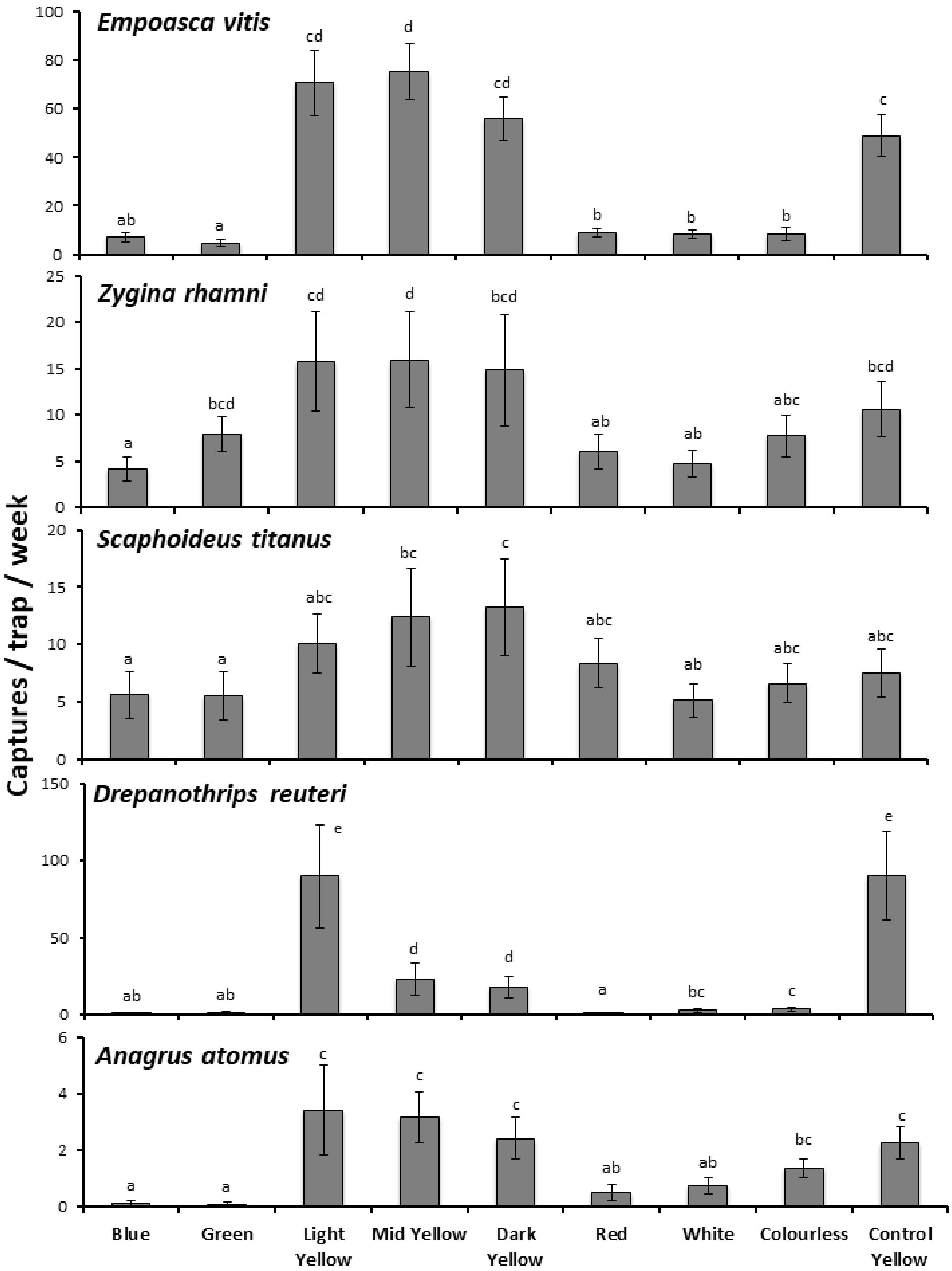

Figure 5. Captures of *E. vitis*, *Z. rhamni*, *S. titanus*, *D. reuteri* and *A. atomus* on different colour traps. Different small letters above columns indicate significant differences among colours according to Tukey's test (α = 0.05).

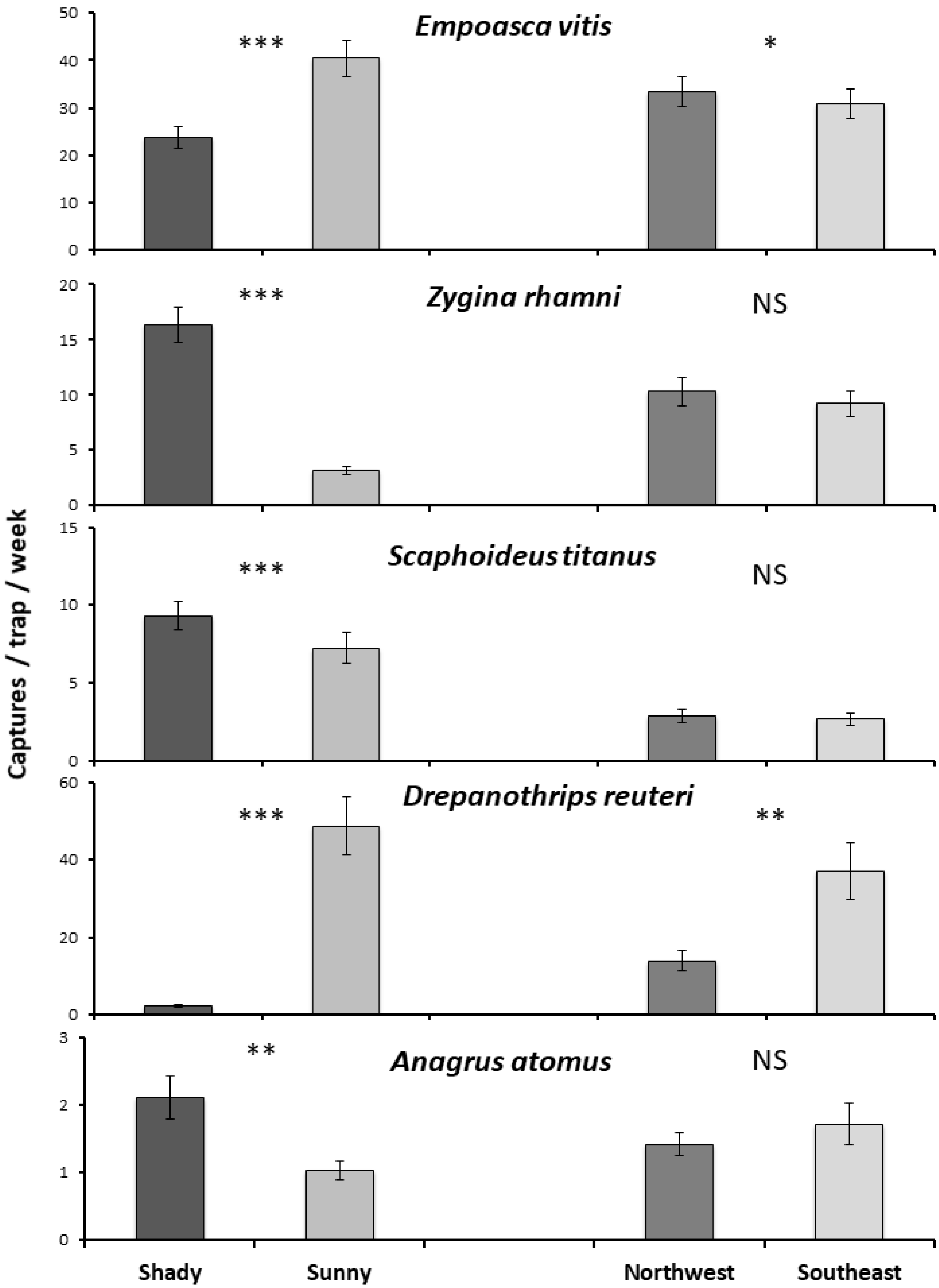

Figure 6. Captures of *E. vitis*, *Z. rhamni*, *S. titanus*, *D. reuteri* and *A. atomus* (mean ± SE) on traps placed in two different positions with respect to the canopy (i.e., shady and sunny). Captures on the two trap sides (i.e., northewest and southeast oriented, respectively) were also reported. NS = non-significant differences; *, **, *** = significant differences at 0.05, 0.01, 0.001 levels using ANOVA.

The trap colour × trap position interaction was significant for *E. vitis* (F = 8.47; d.f. = 8, 108; P < 0.0001), *Z. rhamni* (F = 2.38; d.f. = 8, 108; P = 0.021), *D. reuteri* (F = 43.95; d.f. = 8, 108; P < 0.0001) and *A. atomus* (F = 3.15; d.f. = 8, 108; P = 0.003), but not for *S. titanus* (F = 0.49; d.f. = 8, 113; P = 0.99). In sunny positions, the preference of *E. vitis* for yellow traps was even higher compared to other colours. Considering shady positions, *Z. rhamni* preferred more yellow traps than most of the other trap colours. In the case of *S. titanus*, the preference for all the yellow traps and the red traps compared to the other colours was even higher in sunny positions. Compared to the other colours, the two most attractive traps for *D. reuteri* (i.e., light yellow and control yellow) were much more attractive in sunny positions than in shady positions. For *A. atomus*, the preference for all yellow traps was higher in shady positions than in sunny positions.

The trap colour × trap side orientation interaction was significant for *D. reuteri* (F = 2.20; d.f. = 8, 108; P = 0.032), but not for *E. vitis* (F = 0.94; d.f. = 8, 108; P = 0.49), *Z. rhamni* (F = 0.52; d.f. = 8, 108; P = 0.84), *S. titanus* (F = 0.13; d.f. = 8, 113; P = 1.00) and *A. atomus* (F = 0.48; d.f. = 8, 108; P = 0.87). The significance of the interaction for *D. reuteri* is due to the fact that the preference for southwest-oriented side occurred only for the four yellow traps (data not shown).

Discussion

Influence of trap size

The number of captures of grapevine leafhoppers increased as the trap size increased, suggesting that it might be necessary for trap size to be consistent across different vineyards, plots and years to enable comparisons. However, because the captures referred to the same unit area were not significantly different, comparisons could be made with reference to captures per unit area.

In an earlier study on *D. reuteri*, the average captures per unit area of the smallest trap (the same used in the present study) were significantly higher than for the medium and large traps (Strapazzon *et al*., 1990). This effect was not observed for the three leafhopper species in the present study. Although the progressive decrease in the average captures of *E. vitis* and *S. titanus* per unit area from the large to the small traps suggests that progressive reduction of the number of individuals, that the traps may still attract, cannot be excluded.

Influence of trap inclination with respect to the ground

Although the data collected refer to a single weekly period, the vertical traps with respect to the ground captured significantly more adults of *E. vitis* and *S. titanus* than horizontal traps. For *E. vitis*, this result agreed with findings for *Empoasca* spp. on cotton (Gencsoylu, 2007). For *S. titanus*, this result was apparently in contrast with Jermini *et al.* (1992), even though the methods used in this last study may have underestimated the captures on vertical traps and overestimated those on horizontal traps. Indeed, in the study of Jermini *et al.* (1992), the vertical traps were placed under the canopy (i.e., in a sunny position), where captures could have been reduced because *S. titanus* prefers shady positions within the foliage (data reported in the present study) and only the upper side of the horizontal trap was smeared with glue, i.e. the surface preferred by the leafhopper (data reported in the present study). In any case, it can be assumed that for monitoring purposes, vertical orientation of traps is preferable to horizontal orientation due to easier installation.

On horizontal traps, the preference of *E. vitis* and *Z. rhamni* for the underside might suggest that adults prefer to land on lower surface of leaf laminae. The higher captures of *S. titanus* on upper side of traps would suggest a preference of this species for landing on upper surface of leaf laminae.

Influence of trap exposure days

For the leafhoppers *E. vitis* and *S. titanus* and the thrips *D. reuteri*, the number of captures per days decreased as the exposure days of the traps increased. The sharp drop in *S. titanus* captures observed during the traps' second week in the field could be due to this species being larger than *E. vitis* and *D. reuteri*, and it may thus have been more affected by reductions in free space as captures of other insects covered the trap surface. Additionally, the bigger size of *S. titanus* compared to the other two species could be associated with a greater number of specimens landing on traps but flying away due to loss of stickiness.

During monitoring, if the traps are replaced at different intervals, the comparison among samplings is usually carried out with reference to the average daily captures. However, based on the present study, more inaccurate conclusions can be drawn by daily average as the sampling intervals increase. In particular, this fact appears important for *S. titanus* because the comparison among vineyards and years is reliable only if the traps were left in the field for the same number of days (Pavan *et al.*, 2012).

Influence of trap colour

The three grapevine leafhoppers preferred yellow traps over colourless and other coloured traps, in accordance with the fact that yellow is usually the colour preferred by phytophagous insects (Prokopy and Owens, 1983).

The preference of *E. vitis* for yellow traps agrees with the literature on both *E. vitis* specifically (Bian *et al.*, 2014) and other members of the Empoascini (Mensah *et al.*, 1996; Demirel and Yıldırım, 2008). In late summer, when solar radiation decreases, light yellows are relatively more preferred than dark yellow.

Data on trap colour attractiveness for *Z. rhamni* were not found in the literature. In late summer, when solar radiation decreases, light yellow traps (i.e., light yellow and control yellow) were relatively less attractive than dark yellow ones (i.e., mid yellow and dark yellow).

For *S. titanus*, the preference data collected in this study (yellow > red > blue > white) agree with those of Mazzoni *et al.* (2011) (yellow > red > blue), but do not agree with Lessio and Alma (2004b) (red > white > yellow > blue). The difference could be that in the Lessio and Alma (2004b) study, the yellow and blue traps were commercial traps that were already coated in glue, whereas the red and white traps were manually smeared with glue and a larger amount of glue on the latter two traps might be the reason for the larger number of captures on them. In fact, adults of *S. titanus* have been observed to escape after contact with the surface of yellow commercial traps due to the low amount of glue (Pavan F., personal observation). Both the laboratory and field data of Mazzoni *et al.* (2011) showed that red is relatively preferred by females and yellow by males of this species. This phenomenon probably occurred due to female behaviour that, laying eggs under the bark, are attracted by wavelengths that are more reflected by bark (i.e., red) (Prokopy and Owens, 1983). The same wavelengths are attractive to xylophagous insects such as the Scolytidae (Coutal and Courtois, 2008) as well as Pseudococcidae species that overwinter and develop under the bark of their host plants (Negishi *et al.*, 1980; Hwang and Chu, 1987).

The vine thrips *D. reuteri* showed a clear preference for yellow traps in comparison to the colourless and other coloured traps. Among the yellow traps, the lighter shades (i.e., light yellow and control yellow) were much more preferred than the darker shades (i.e., mid yellow and dark yellow). This clear preference for yellow traps agrees with the data of Jenser *et al.* (2010). Indeed, blue traps were even repellent to *D. reuteri*, whereas thrips associated with herbaceous plants (e.g., *Frankliniella occidentalis* Pergande and *T. tabaci*) have often shown a marked preference for blue traps (Vernon and Gillespie, 1990; Blumthal *et al.*, 2005; Demirel and Yıldırım, 2008; Broughton and Harrison, 2012; Gharekhani *et al.*, 2014). This difference can be explained by parts of the host plants that these species feed on; *D. reuteri* only feeds on green organs (i.e., leaves and grapevine flowers), whereas *F. occidentalis* and *T. tabaci* also feed on coloured flowers. The strong preference of *D. reuteri* for light yellow traps (i.e., light yellow and control yellow) could be associated with the pale green of the newly sprouted leaves on which it preferentially feeds. In fact, sap-sucking insects have demonstrated the ability to distinguish between young leaves and mature leaves based on colour intensity (Prokopy and Owens, 1983; Bian *et al.*, 2020).

For both the leafhoppers *E. vitis* and *Z. rhamni* and the thrips *D. reuteri*, the relative attractiveness of preferred colours was enhanced when the traps were placed in the preferred position on the canopy, i.e. sunny positions for *E. vitis* and the vine thrips and shady positions for *Z. rhamni*. It can be assumed that in the least preferred positions with respect to the light source the incidence of random captures would be relatively more important.

For all of the leafhopper species considered in the present study, control yellow, which was the least intense of yellows used in the comparison, was also the least attractive, and this suggests that colour intensity is important.

A. atomus was captured more on yellow traps, just like its leafhopper hosts (i.e., *E. vitis* and *Z. rhamni*), but many captures also occurred on the colourless traps, in agreement with other studies on *Anagrus* spp. (Wallis and Shaw, 2008; Larsen *et al.*, 2014). In general, hymenopteran parasitoids are better captured by traps of yellow tones than traps of other colours (Rodriguez-Saona *et al.*, 2010).

Influence of trap position within the canopy

S. titanus and especially *Z. rhamni* were more abundantly captured when the traps were in shady positions, whereas *E. vitis* preferred traps in sunny positions. These results agree with the negligible captures of *S. titanus* above the canopy (Lessio and Alma, 2004b) and with the higher number of captures of *E. vitis* 40-60 cm above the canopy in tea (Bian *et al.*, 2014). It is interesting to observe that for *E. vitis* in early autumn, when solar radiation decreases (12600 kJ/m^2 per day vs 26000 and 27000 kJ/m^2 per day in early- and mid-summer, respectively; https://www.osmer.fvg.it) traps in sunny positions are even more preferred than those in shady positions, and that for *Z. rhamni* in early-mid summer, when solar radiation is highest, this species has a greater preference for traps in shady positions.

D. reuteri showed a marked preference for traps in sunny positions. This agrees with data reported for *T. tabaci* and other thrips that are mostly captured above the canopy of their host plants (Gharekhani *et al.*, 2014; Bian *et al.*, 2016). For *D. reuteri*, the preference for traps exposed all day to direct sunlight could be associated with their feeding preference for the apical part of the shoot. In late summer, when the vine thrips ceases laying eggs on apical leaves and moves to overwintering under the bark, captures on the traps in shady positions are relatively more abundant.

A. atomus was preferentially captured by traps in shady positions. This seems to agree with the fact that leaves located in more protected positions inside the canopy are those most used by *E. vitis* and *Z. rhamni* adults for oviposition (Vidano *et al.*, 1988; Pavan and Pavanetto, 1989; Fos *et al.*, 1997; Pavan and Picotti, 2009).

The differences between the more attractive yellow traps and other colours were greater for *E. vitis* and *D. reuteri*, which preferred sunny positions, than for *S. titanus* and *Z. rhamni*, which preferred shady positions, and again this suggests that random captures are relatively more important in the latter case.

These results have some important practical implications for sampling purposes: (i) it is important to place all traps in the same position with respect to the canopy (i.e., in shady or sunny positions) in order to reduce statistical variability and increase the accuracy of the estimate; (ii) when population densities are low, traps should be placed in shady positions to monitor *Z. rhamni* and placed in sunny positions to monitor *D. reuteri*.

Influence of trap side orientation

The leafhopper *E. vitis* was mostly captured on the northwest-oriented side of traps, whereas *D. reuteri* was mostly present on the southeast-oriented side of traps. Since the northwest-oriented side of traps is exposed to direct sun in the late afternoon and the southeast in the early morning, this difference could be associated with the period of maximum flight activity of different species. In fact, thrips are usually more active in the morning (Yaku *et al.*, 2007; Aliakbarpour and Rawi, 2010), whereas leafhoppers are more active from late afternoon (Kersting and Bascedillapinar, 1995; Chancellor *et al.*, 1997; Lessio and Alma, 2004a). This hypothesis is indirectly confirmed by the preference of thrips on northsouth-oriented traps (Gencsoylu, 2007; Bian *et al.*, 2016), which are sun-exposed in the middle of the day, and of *Empoasca* spp. for eastwest-oriented traps (Gencsoylu, 2007), which are sun-exposed in the early morning and the late afternoon. To confirm this hypothesis vineyards with different orientation of rows should be compared.

Concluding remarks

In vineyards, coloured sticky card traps are a partially selective sampling method and have the advantage of allowing the simultaneous monitoring of several species. However, not all species have the same preferences for the colour and position of traps with respect to the canopy. The choice of colour and position should be made according to the most important species in each context.

Traps of yellow colours are preferred by all species considered in this study, but for monitoring of *D. reuteri*, lighter yellow traps must be used. Yellow colours also allow easy counting of the captured insects under a dissection microscope.

In European vineyards, the trap position must be chosen with consideration for monitoring of *E. vitis*, *S. titanus* and *D. reuteri* because *Z. rhamni* is not particularly harmful. Because *E. vitis* and *D. reuteri* are better captured in sunny positions and since *S. titanus* captures were only 20% lower in sunny positions than in shady positions, sunny positions are to be preferred. Such positioning also minimizes the negative effect of capturing *A. atomus*. However, if the monitoring of *D. reuteri* is not important and *S. titanus* is the sampling target, shady positions must be preferred.

Therefore, in order to compare captures of the same species in different grape-growing areas and years, it is not only necessary to use traps with the same features (colour, size, glue type and amount) but the traps must be placed in the same position with respect to the canopy.

Finally, in this study we have highlighted the advantages of the most appropriate use of sticky traps to monitor some grapevine sap-sucking pests and *A. atomus*. This information can be useful to refine the sampling protocols to help winemakers take control measures in the context of Integrated Pest Management strategies.

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