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The effect of trap colour and aggregation pheromone on trap catch of *Frankliniella occidentalis* and associated predators in protected pepper in Spain

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Abstract: In a greenhouse experiment, there was a strong effect of colour on sticky trap catch of *Frankliniella occidentalis* in a sweet pepper crop. Blue traps attracted more thrips than yellow traps (×2.4), clear traps (×9.3), or black traps (×34.7). The *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, increased trap catch in inverse proportion to the attractiveness of the trap colour (blue ×1.3, yellow ×1.7, clear x1.9, black x3.4). It is proposed that in greenhouse crops, the most visually attractive trap colours are already catching a large proportion of the thrips present in the area surrounding each trap, so the addition of scent cannot increase trap catch by as much. The mirid predator *Orius laevigatus* was caught in low numbers on traps and showed no attraction to specific trap colours, the predatory thrips *Aeolothrips tenuicornis* was most frequent on yellow and blue traps and the staphylinid beetle *Oxypoda exoleta* was only found on black and clear traps. None of these predatory species were attracted to the *F. occidentalis* aggregation pheromone which can therefore be used to enhance *F. occidentalis* trap catch without affecting natural enemy establishment.

Key words: Thrips, sticky traps, trap colour, aggregation pheromone, predators, *Frankliniella occidentalis, Orius laevigatus, Aeolothrips tenuicornis, Oxypoda exoleta*

Introduction

Scents and colours are used by flower-inhabiting thrips to locate flowers (Kirk, 1984; Terry, 1997) and both are utilised to increase trap catch for the monitoring or control of thrips. The western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), shows preference for traps of specific colours and blue (440-460nm) is the most attractive in greenhouse-grown crops (Brødsgaard, 1989; Vernon & Gillespie, 1990; Matteson & Terry, 1992). The addition of various scents such as the *F. occidentalis* aggregation pheromone (neryl (*S*)-2-methylbutanoate), kairomones or their analogues (e.g. anisaldehyde, methyl isonicotinate) increase trap catch by variable amounts between < x2 and x15 e.g. (Brødsgaard, 1990Teulon *et al.*, 1999; Smits *et al.*, 2000; Hamilton *et al.*, 2005; Gómez *et al.*, 2006).

Predators also use visual and odour cues for host finding and it is important to know the effect of trap colour and scents on predators for monitoring and also to ensure that essential biocontrol agents are not removed by trapping. *Orius* spp. (Heteroptera: Anthocoridae) have been released commercially to control *F. occidentalis* in European pepper crops since the early 1990s (Chambers *et al.*, 1993). They also feed on pollen and show attraction to flowers (Hansen *et al.*, 2003), their own pheromones (Aldrich *et al.*, 2007) and plant volatiles emitted from infested plants (Venzon *et al.*, 1999). *Orius* spp. are recorded on blue (Boone, 1999), yellow (Muvea *et al.*, 2011) and white traps (Atakana & Bayramb, 2011), although sticky traps have been used extensively for monitoring of thrips without having any apparent negative impact on *Orius* populations. *Aeolothrips tenuicornis* (Thysanoptera: Aeolothripidae) are thrips predators found in Spanish greenhouses, surrounding weeds (Lacasa *et al.*, 1996) and on blue and yellow sticky traps (Lacasa *et al.*, 1993). Very little is known about the behaviour or feeding habits of *Oxypoda* species (Coleoptera: Staphylinidae), although members of the same sub-family (Aleocharinae), such as *Atheta coriaria* Kraatz, are thrips predators (Bennison *et al.*, 2008) and other staphylinid species are found on yellow (Chen *et al.*, 2004) and blue sticky traps (Sampson pers. obs., 2011).

This paper examines the effects of and interaction between trap colour and the *F. occidentalis* aggregation pheromone on trap catches of *F. occidentalis* in protected pepper, as well as the effect on the released predator *Orius laevigatus* and naturally occurring predators *Ae. tenuicornis* and *Oxypoda exoleta* (det. R. Booth).

Material and methods

The experiment was carried out in a commercial sweet pepper crop (*Capsicum annuum* cv. Guepard) near Los Infiernos (N 37° 57.073´ W 0° 54.776´) in the Murcia region of Spain. The crop was grown in soil under a 6,000m^2 multi-tunnel plastic greenhouse. Crop height was about 90cm, planted in rows 1m apart with 3 plants/1.2m length of row. The maximum and minimum temperatures and humidities were 14°C:37°C and 27%:88% RH respectively. Thrips occupancy was 3.12 adults/flower (82%) and *O. laevigatus* occupancy was 0.65 adults/flower (51%). *Aeolothrips tenuicornis* and *O. exoleta* were not observed in the flower samples. There was an average of 1.88 open flowers per plant (n = 27 plants, 51 flowers).

On 14th April 2011, the experiment was laid out in a randomised complete block design with 20 blocks and one replicate per block with 3.6m between traps within a block and 6 m between blocks. Treatments included four different colours of sticky trap, each with or without the *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate. Traps were yellow, blue, clear or black dry sticky cards with a black grid on the back (10cm x 25cm, Russell IPM, UK). Traps were suspended vertically with the base of the trap about 10cm above crop height by attaching them with wooden clothes pegs to vertical strings supporting the crop. A single natural rubber septum (6.3mm x 10.8mm; International Pheromone Systems Ltd.) that had been solvent cleaned and dried in an oven at 50°C was placed centrally on each trap. The pheromone septum was impregnated with 30µg neryl (*S*)-2-methylbutanoate dissolved in 30µl hexane and the control septum with 30µl hexane only. All traps were oriented so that the septum side faced north to avoid direct sunlight on the release device. After 24 hours the traps were removed from the crop, wrapped individually in plastic and stored in a freezer.

Trap catches were counted under a binocular microscope and selected thrips mounted on slides in Hoyer's solution for identification. The majority of thrips (> 97%) were *F. occidentalis.* The identification was confirmed by randomly selecting (using a grid acetate and random numbers) and identifying one thrips from each of 100 traps. All aeolothripid thrips (with broad wings), *Orius* spp. and staphylinids were counted and identified separately. *Thrips* species and phlaeothripid thrips (with elongated end of abdomen) were excluded from the counts.

Tables and figures show untransformed data. The counts of *F. occidentalis* were transformed to $\log_{10}$ (n+1) for statistical analyses (Minitab 16). Despite differences in variance between thrips numbers on different trap colours, the ratio of the largest cell variance to the smallest (*F* max) was less than 10, so analysis of variance was used (Tabachnick & Fidell, 2001) and residuals were normally distributed. Pair-wise comparisons between pheromone and control traps of each trap colour used Tukey's test for significance. The ratio of trap catch between each treatment and the control was calculated by comparing the untransformed trap catch on the two trap types within each block, and these ratios were compared using analysis of variance. The predator data were not normally distributed and were analysed using Kruskal-Wallis tests.

Results

Trap catches of *F. occidentalis* on different coloured traps with and without the aggregation pheromone are shown in Figure 1 and for predators in Table 1.

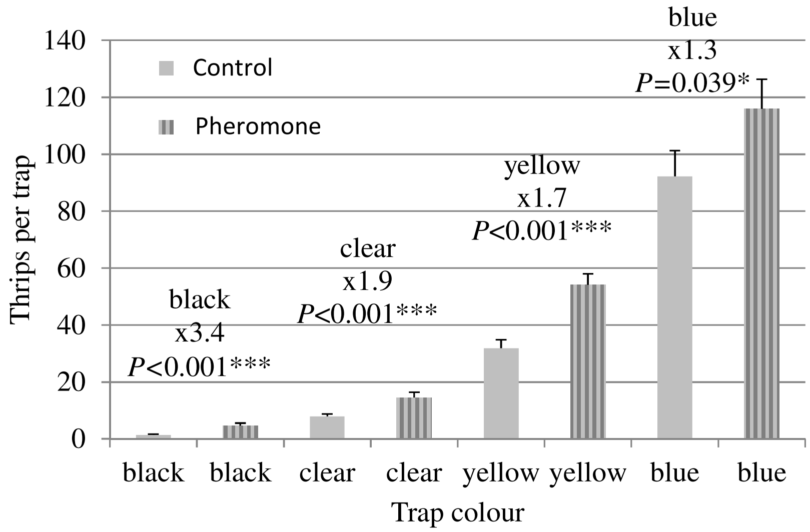

Figure 1. Mean catch + S.E. of *F. occidentalis* on different coloured traps with and without the aggregation pheromone. Pair-wise comparisons between trap catch on control and pheromone treated traps for each trap colour are shown by *P* values and factor of increase.

Table 1. The total number of predators a) on different coloured sticky traps and b) on sticky traps with and without the *F. occidentalis* aggregation pheromone.

Predator	(a) Total number of predators caught on sticky traps of different colours (n=40)					(b) Total no. of predators on traps ± pheromone (n=80)		
	Black	Clear	Yellow	Blue	*P*	Control	Phero-mone	*P*
Orius	3	15	9	10	0.12 ns	16	21	0.73 ns
Aeolothrips	0	1	9	7	0.004**	9	8	0.79 ns
Oxypoda	8	9	0	0	0.001***	7	10	0.59 ns

There was a strong effect of trap colour on trap catch of *F. occidentalis* ($F_{(3,133)} = 175$, $P < 0.001$***). Blue traps caught significantly more thrips than yellow traps (×2.4), clear traps (×9.3) or black traps (×34.7) (Figure 1). The aggregation pheromone increased trap catch ($F_{(1, 133)} = 16$, $P < 0.001$***) in inverse proportion to the attractiveness of the trap colour (blue ×1.3, yellow ×1.7, clear x1.9, black x3.4). A general linear model with a regression showed that there was a negative association between the relative effectiveness of pheromone (ratio of treatment:control catch) and the attractiveness of the trap colour (total catch on control traps for each colour) ($F_{(1, 55)} = 7.18$, $P = 0.01$**).

Orius laevigatus was found on all the trap colours tested, but in very low numbers with no clear preference for any specific colour ($H_{(3)} = 5.9$, $P = 0.12$ ns; Table 1). A total of 37 *O. laevigatus* were caught on 160 traps when there was an estimated 22,000 adult *O. laevigatus* in the crop.

Aeolothrips tenuicornis was most frequent on blue and yellow traps ($H_{(3)} = 13.1$, $P = 0.004$; Table 1).

Oxypoda exoleta was found on black and clear traps but not on blue and yellow traps ($H_{(3)} = 17.65$, $P = 0.001$; Table 1).

Discussion

Our results confirmed the attractiveness of blue to *F. occidentalis* in greenhouse crops (Brødsgaard, 1989; Gillespie & Vernon, 1990) and the importance of visual cues in trap attraction. Addition of the aggregation pheromone increased trap catches. The absolute increase with additional pheromone was greater for the more attractively coloured traps, although the proportional increase was lower. A similar interaction between colour and scent has been observed with plant kairomones (Kirk, 1987; Teulon *et al.*, 1999; Davidson *et al.*, 2012). The reduced response to pheromone on the most visually attractive traps may be explained, at least in part, by the fact that they are already extremely attractive, drawing in a large number of thrips, thereby reducing the numbers of thrips available for the pheromone to attract unless an invasion of thrips is occurring. For example, the blue traps in this experiment were catching thrips equivalent to all the adult thrips in flowers of about $5m^2$ of crop.

Low numbers of *O. laevigatus* were caught on traps. As *O. laevigatus* is highly mobile and was widespread, the few caught on traps could be explained by accidental catch rather than by attraction, indicating that sticky traps are unlikely to pose a risk to *O. laevigatus* released for the control of *F. occidentalis*.

Aeolothrips spp. feed on pollen as well as invertebrate prey and are similar to plant feeding thrips (Bournier *et al.*, 1979). The higher catch of *Ae. tenuicornis* on blue and yellow traps than on clear and black traps supports this and might be predicted (Kirk, 1984). No population estimate was made of *Ae. tenuicornis* in this experiment but it is likely that numbers were low and further work is needed to determine whether yellow and blue sticky traps could pose a risk to *Ae. tenuicornis* populations.

Oxypoda exoleta has not previously been reported in Spanish pepper greenhouses. This species may have been under-recorded as it is small (2-3mm long), difficult to identify, possibly nocturnal and not found on the yellow and blue traps used for monitoring pests. Little is known about its diurnal activity (Assing, pers. comm., 2012), but some staphylinids are nocturnal and it could be that the coloured traps are more visible at night than black or clear traps and so avoided. Previous records of *O. exoleta* have been in cereal crops and field margins (Pietraszko & de Clercq, 1983) and some basic information on its occurrence, biology and prey preference in greenhouse crops would be of interest.

None of the predatory species examined was attracted to the *F. occidentalis* aggregation pheromone. The pheromone can therefore be used to enhance trap catch of *F. occidentalis* in European pepper crops without affecting natural enemy establishment.

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