Research Article

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Nontarget Coleoptera Species Captured in Coloured Sticky Traps in Maize Crops in Bulgaria

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Abstract: Coloured sticky traps are useful for detection, monitoring and capturing of different insect groups. In order to study the presence of Cicadomorpha and Fulgoromorpha species and the trapping efficiency of different coloured sticky traps (CSALOMON®, Hungary) in Bulgaria, parallel tests were performed in maize crops in Knezha and Sofia in 2014. Apart from the numerous target species captured in traps, several species belonging to coleopteran families Coccinellidae, Chrysomelidae, and Elateridae were found. Coccinellidae was the most numerous family with eight species in Knezha and five species in Sofia. In both maize crops, the most abundant were *Propylea quatuordecimpunctata* and *Harmonia axyridis,* and captures of these species were registered during the whole period of the study in all traps tested. In Knezha, fluorescent yellow colour was highly attractive to *P. quatuordecimpunctata,* while *H. axyridis* showed no preference between fluorescent yellow, yellow, orange, blue, white and transparent traps. At the same site captures of *Diabrotica virgifera virgifera* adults were registered in all types of traps, with the exception of red traps in the period end of July – middle of September. A single specimen of *D. v. virgifera* was registered in a fluorescent yellow trap in Sofia at the end of August – the beginning of September. Adults of *Agriotes ustulatus* were recorded only in Knezha during the period from the second half of June to the second half of August. The largest captures of *A. ustulatus* were registered in the fluorescent yellow traps followed by those in the white traps.

Key words: Traps with visual stimuli, *Propylea quatuordecimpunctata, Harmonia axyridis*, *Diabrotica virgifera virgifera*, *Agriotes ustulatus*

Introduction

Coloured sticky traps are useful for detection, monitoring and capturing of different groups of insects, including thrips, leafhoppers, sawflies, anthomyiid flies, fruit flies, beetles, and others (Al-Ayedh &Al-Doghairi 2004, Wallis & Shaw 2008, Thein et al. 2011, Bažok et al. 2012, Rodriguez-Saona et al. 2012, Atakan & Pehlivan 2015, Pustai et al. 2015, Bian et al. 2016, Spears et al. 2016, Vétek et al. 2016).

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In order to study the presence of Cicadomorpha and Fulgoromorpha species and the trapping efficiency of various coloured sticky traps in Bulgaria, parallel tests were performed in maize crops in Sofia and Knezha (Pleven District). Apart from the numerous Cicadomorpha and Fulgoromorpha species that were captured in traps, several species, belonging to three coleopteran families were also found, including two non-native species – the Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, 1868, and the multicoloured Asian lady beetle (or harlequin ladybird), *Harmonia axyridis* Pallas, 1773. The results regarding recorded coleopteran species are presented in this paper.

Materials and Methods

Field studies

In 2014, field tests were carried out in maize crops (0.1-0.2 ha), belonging to the Maize Research Institute in Knezha (N 43°28’48.85”; E 24°3’22.03”), and the Training and Experimental Field Station (University of Forestry) in Vrazhdebna, Sofia (N 42°42’20.75”; E 23°26’19.11”), using CSALOMON® (Plant Protection Institute, CAR HAS, Budapest, Hungary) coloured sticky traps (10 x 16 cm) made of a PVC foil. In each maize crop, white, blue, red, orange, yellow, and fluorescent yellow traps were tested in six replications, while transparent traps were used as control. The reflectance spectra of the coloured traps were measured and reported by Koczor et al. (2013). Periods of the studies were 5 June – 23 September 2014 and 2 July – 17 September 2014 for Knezha and Sofia, respectively. The traps were attached to wooden poles and the trap height was initially 1.00 m and later was raised to 1.80 m above ground level. The traps were inspected in one or two-week intervals, collected and replaced with new traps. During each inspection the positions of traps were randomly changed. In the laboratory, the insects were removed from the glue, wrapped in a paper towel, placed in a paper bag and labeled.

The nomenclature of detected species follows Fauna Europaea database (de Jong 2013).

Daily meteorological data records on minimum and maximum air temperatures (°C), relative humidity and precipitation (mm) were obtained from the meteorological station located near the experimental field in Knezha. No such data were available for the maize field in Sofia.

Statistical analyses

The trap capture data were tested for normality, using a Kolmogorov-Smirnov test. Because these data were not normally distributed, non-parametric Kruskal-Wallis test and Mann-Whitney U post hoc test were conducted to compare the number of beetles captured in different treatments. The influence of mean air temperatures, relative humidity and precipitation on the abundance of the most numerous beetle species in the traps was analysed by Spearman’s rho correlation nonparametric test.

All statistical analyses were performed, using the STATISTICA 7.0 Software (StatSoft, Inc. 2004). The level of significant difference was set at $P < 0.05$.

Results

Eleven species, belonging to three families of Coleoptera – Coccinellidae, Chrysomelidae and Elateridae, were recorded in the traps. Coccinellidae was the most numerous family presented with nine species and 300 captured specimens. The relative abundance of ladybird species of the family Coccinellidae (in percentages) captured in all inspected traps in the maize crops in Knezha and Sofia is presented in Fig. 1.

In Knezha, eight coccinellid species were recorded: *Adalia (Adalia) decempunctata* (Linnaeus, 1758), *Ceratomegilla (Ceratomegilla) undecimnotata* D. H. Schneider, 1792, *Chilocorus bipustulatus* (Linnaeus, 1758), *Coccinella (Coccinella) septempunctata* Linnaeus, 1758, *H. axyridis*, *Hippodamia (Hippodamia) variegata* Goeze, 1777, *Propylea quatuordecimpunctata* (Linnaeus, 1758), and *Psyllobora vigintiduopunctata* (Linnaeus, 1758) (Table 1). *Propylea quatuordecimpunctata* was the most abundant, followed by *H. axyridis* and *C. septempunctata*. Both species *P. quatuordecimpunctata* and *H. axyridis* were registered during the whole period of investigation. The most of *C. septempunctata* adults were captured in June, and only single captures were registered until the beginning of September 2014 (Fig. 2). The species *C. undecimnotata* and *C. bipustulatus* were scarce and only single specimens were detected in the period from the end of June to the beginning of July and during the end of September, respectively. It was noticed that the most numerous species (*P. quatuordecimpunctata*, *H. axyridis,* and *C. septempunctata*) were captured in each type of trap. We registered strong preference of *P. quatuordecimpunctata* for fluorescent yellow colour. The highest captures were found in the fluorescent yellow traps, and the mean obtained was significantly higher than the mean captures in the other tested variants (transparent and coloured traps). For *H. axyridis*, no significant statistical

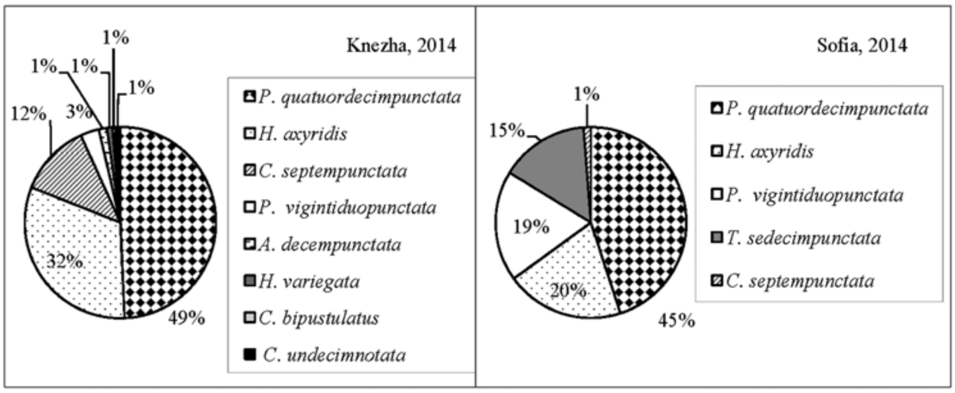

Fig. 1. Relative abundance of ladybird species from the family Coccinellidae (in percentages) in traps in maize crops in Knezha and Sofia

difference was detected between the mean captures in transparent, fluorescent yellow, yellow, orange, blue, and white traps. Although the highest number of *C. septempunctata* adults was found in the yellow traps, no significant difference was detected between the mean captures in the tested traps (Table 1).

In the maize field in Sofia, five coccinellid species were captured: *C. septempunctata*, *H. axyridis*, *P. quatuordecimpunctata*, *P. vigintiduopunctata,* and *Tytthaspis sedecimpunctata* (Linnaeus, 1758) (Fig. 1). The most numerous were *P. quatuordecimpunctata* with 36 captured adults, followed by *H. axyridis* with 16 adults. *C. septempunctata* was recorded only with one specimen at the beginning of the study, in the period of 2-16 July 2014 (Table 1).

Twenty one *D. v. virgifera* adults were registered in Knezha in the period of 22 July – 17 September, 2014 (Fig. 3A) and a single one in Sofia in the period of 23 August – 6 September, 2014. In Knezha, the pest was found in all variants of traps, with the exception of the red traps. The highest number of adults was captured in the fluorescent yellow traps, although no significant differences were detected between the mean captures in the tested traps (Fig. 3B). In Sofia, a single specimen was recorded in a fluorescent yellow trap at the end of August – the beginning of September.

Adults of *Agriotes ustulatus* Schäller (Elateridae) were caught only in Knezha during the period from 13 June to 19 August 2014 (Fig. 4A). The highest number of beetles was registered in the middle of July. The fluorescent yellow traps captured 52% of beetles, followed by white and yellow traps, which captured 20% and 14% of beetles, respectively. No significant differences were found between the mean captures in the fluorescent yellow traps and white traps, and those in white and yellow traps. No attractiveness was detected for blue, orange and red traps (Fig. 4B).

During the monitoring period in Knezha, a significant correlation was found only between air temperature parameters and the captures of *P. quatuordecimpunctata* and *D. v. virgifera* beetles. The captures of these two species were positively influenced by the minimum (*P. quatuordecimpunctata*: Spearman's rank correlation coefficient, $r_s = 0.54$, $P = 0.04$; *D. v. virgifera:* $r_s = 0.61$, $P = 0.02$) and mean (*P. quatuordecimpunctata*: $r_s = 0.65$, $P = 0.01$; *D. v. virgifera:* $r_s = 0.75$, $P = 0.002$) air temperature patterns.

Discussion

Eight coccinellid species were captured in the coloured and transparent sticky traps in Knezha and five species – in Sofia. At both investigated sites, the most numerous coccinellid species was *P. quatuordecimpunctata* (50% of all captured coccinellids in Knezha and 45% of all captured coccinellids in Sofia), followed by *H. axyridis* (32% in Knezha and 20% in Sofia). Among the species recorded, four are common – *P. quatuordecimpunctata*, *H. axyridis*, *C. septempunctata*, and *P. vigintiduopunctata*. Nicolae & Roşca (2010) also report *P. quatuordecimpunctata* as a dominant species in maize crops in Romania, followed by *C. septempunctata. P. quatuordecimpunctata* is abundant in some fabaceous crops in Bulgaria and Romania (Dimitrov 2008, Murariu & Andriev 2008). However, *H. axyridis* is the most abundant species in various crops – alfalfa, broad bean, maize, potato, and wheat (Vandereycken et al. 2013, 2015, Jovičić et al. 2016).

Harmonia axyridis, native to East Asia (Orlova-Bienkowskaja et al. 2015), has been distributed in many countries in North America, South America, Europe and Asia, and parts of Africa. After the establishment its rapid spreading in many countries has bee-n documented (Roy et al. 2016).

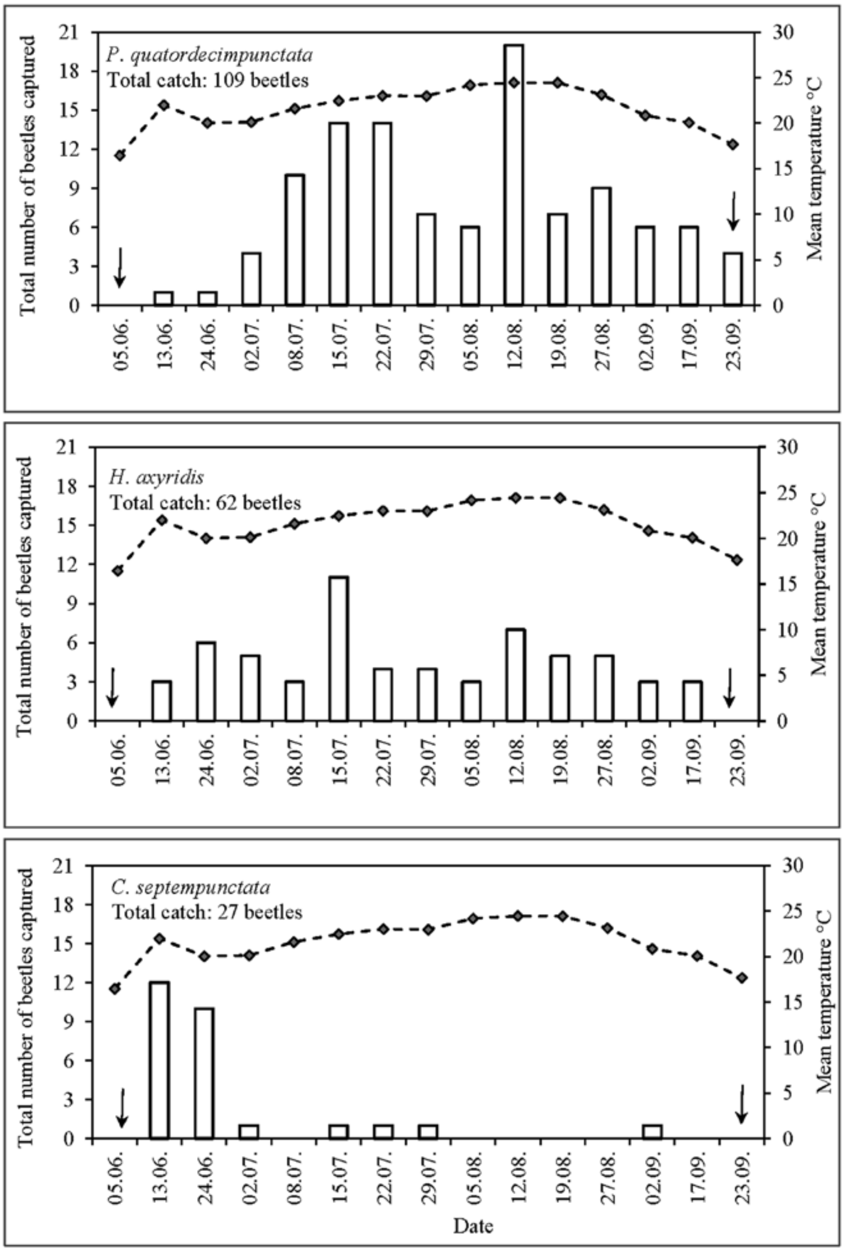

Fig. 2. Total captures of the most abundant Coccinellidae species in maize crops in Knezha. The bars show the captures, and the curve - the mean air temperature in the given period. The arrows (↓) indicate the dates of the beginning and the end of investigation

Recently, this non-native species has been reported in Albania (Ibrahimi et al. 2016), FYR Macedonia (Kulijer 2016), Montenegro (Gligorović et al. 2016), Tanzania (Zanzibar) (Nedvěd & Háva 2016), and the larvae were observed for the first time in New Zealand (http://naturewatch.org.nz/observations/3175895). It was detected for the first time in Bulgaria in Sofia in 2008 (Tomov et al. 2009). After the first findings, the species spread within the next year to 16 new localities in Bulgaria – Ardino, Belogradchik, Bladoevgrad, Botevgrad, Dupnitsa, Elin Pelin, Gabrovo, Kresna defile, Montana, Pravets, Smolyan, Varna, Veliko Turnovo, Velingrad, Vidin, and Vratsa, exclusively on broadleaved trees heavily infested by aphids (Tomov et al. 2009, 2010). Harizanova et al. (2012) and Vasilev (2016) reported the presence of this species in the region of Plovdiv. Several years after the establishment of *H. axyridis* in Bulgaria, it can be found in most anthropogenic-altered landscapes, and overwintering aggregations exist in houses in large numbers in the autumn and winter in different regions of the country. The species is recorded in various habitats and on more than 100 plant species, including maize (Vandereycken et al. 2012, 2013, Panigaj et al. 2014, Prescott & Andow 2016).

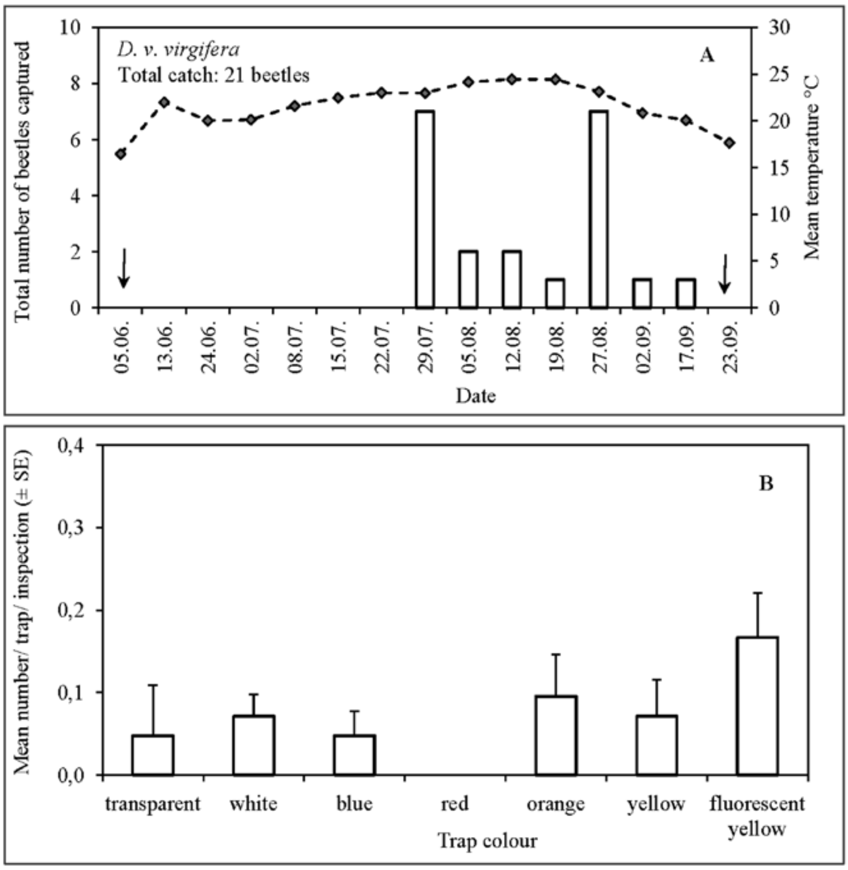

Fig. 3. Seasonal activity of the adults of *Diabrotica virgifera virgifera* in Knezha in 2014 (A); distribution of the mean captures in transparent and coloured sticky traps (B). For the legend, see Fig. 2

Preference for yellow colour traps by ladybird species has been registered in many studies (Mensah 1997, Rodriguez-Saona et al. 2012, Atakan et al. 2016). Kemp & Cottrell (2015) report that yellow pyramidal traps capture significantly more *H. axyridis* adults than orange, red, green, blue, purple, black, and white traps. The results of the current study in Bulgaria showed no colour preference in *H. axyridis*. One of a possible reason may be the type of the trap used and the ability of adults to escape the sticky traps (Stephens & Losey 2004). The population of *C. septempunctata* was more numerous in Knezha than in Sofia and similarly to Maredia et al. (1992), the highest capture of conspecifics was recorded in the yellow sticky traps. It was noticed that the fluorescent yellow coloured traps were highly attractive to *P. quatuordecimpunctata* beetles, followed by the yellow and orange colours, but only the fluorescent yellow colour attracted significantly higher numbers of adults than the traps without visual stimulus (transparent).

The results of this study also showed lower abundance of *C. septempunctata* in comparison to *H. axyridis* in investigated maize fields in Knezha and Sofia. This could be due to the competitive influence of *H. axyridis* on other coccinellids and the effect of temporal distribution factors (aphid density, crop maturity, planting date, nearby vegetation, and maize type) (Bozsik 2009, Musser & Shelton 2014, Kemp & Cottrell 2015). In a study of the phenology of *C. septempunctata* in Greece, Katsoyannos et al. (1997) reported that the larvae are abundant between April and June, and scarce in July until the end of the warm period of the year. According to Grigorov (1980), the native coccinellid species in Bulgaria are more abundant in the spring when the food availability is higher.

With regard to the food preference, most coccinellid species captured during this study were predaceous. Both larvae and adults feed on aphids and other crop pests, and they are an important component of agricultural ecosystems and ecological compensation areas nearby crops (Lami et al. 2016). *P. vigintiduopunctata* and *T. sedecimpunctata* are mycophages. Facultative mycophagy is reported for *P. quatuordecimpunctata* and *C. septempunctata* (Sutherland & Parrella 2009). *H. axyridis* is considered a polyphagous species with a wide host

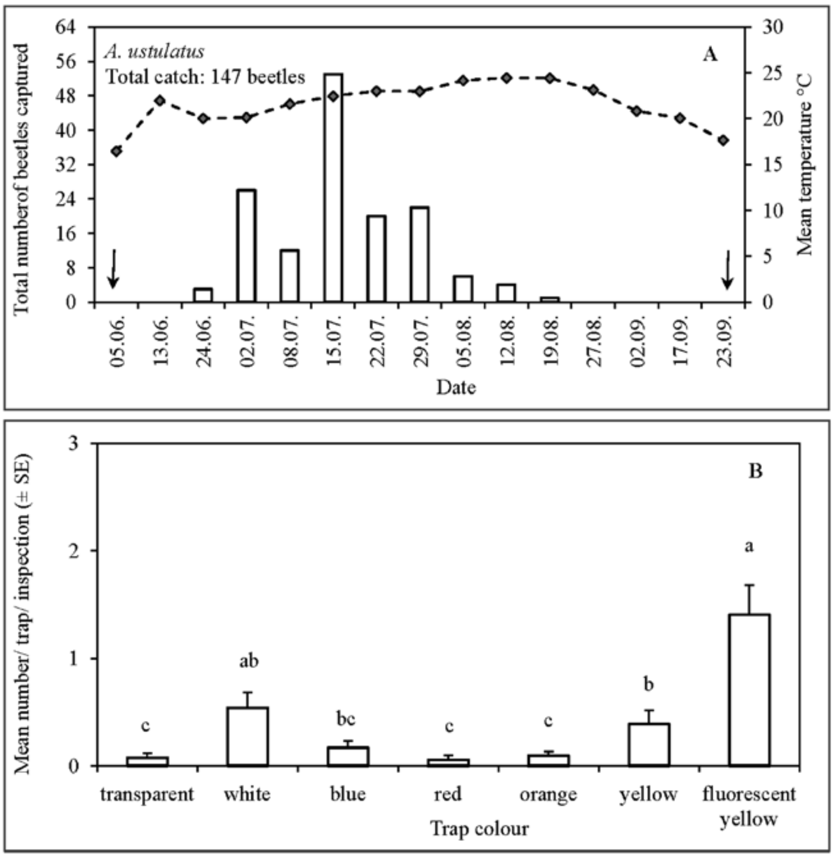

Fig. 4. Seasonal activity of the adults of *Agriotes ustulatus* in Knezha in 2014 (A); distribution of the mean captures in transparent and coloured sticky traps (B). The bars marked with the same letter in a column are not significantly different at $P < 0.05$, Kruskal-Wallis analysis of variance followed by a Mann-Whitney U test for pair-wise comparisons. For the legend, see Fig. 2

range, including aphids, psyllids, coccids, adelgids, other invertebrates (including conspecific eggs and other coccinellid species), as well as nectar, pollen, honeydew, plant sap, a juice of ripe fruits, and young plant tissues (Roy et al. 2016). Moser et al. (2008) reported feeding of third and fourth instars of *H. axyridis* on leaf tissue of non-*Bt* and *Bt*-corn seedlings. Recently, Camacho-Cervantes et al. (2017) have reviewed published records about *H. axyridis,* and reported that at least one quarter of the studies consider it a potential threat to native Coccinellidae species, and pay special attention to *Adalia bipunctata* (Linnaeus, 1758), which is under serious risk of population decline in Europe. *A. bipunctata* was not recorded during the present study.

D. v. virgifera was reported for the first time in Bulgaria in 1998 (Ivanova 2002a, 2002b), in the region of Lom (the northwest border of Bulgaria). In the frame of the long-term phytosanitary monitoring programme for the Western corn rootworm in Bulgaria conducted by the Central Laboratory for Plant Quarantine, Bulgarian Food Safety Agency, the spreading of the pest continued southward and eastward in the country, and now it is widespread and established in all districts in Bulgaria with the exception of Varna, Kardzhali and Smolyan (MAF 2015). *D. v. virgifera* adults were caught in 2001 in Knezha (Ivanova 2002b), but no more records were published from this area.

In Knezha, the largest captures of *D. v. virgifera* were registered in the fluorescent yellow traps, while a single specimen was caught in Sofia in the same type of trap. The population density in both investigated maize fields was too low to estimate the role of colour of the trap on *D. v. virgifera* attractiveness. It is reported that *D. v. virgifera* adults are attracted to yellow colour (Tollefson et al. 1975, Ball 1982). Testing unbaited transparent, yellow and fluorescent yellow PAL (CSALOMON®) sticky traps, Tóth et al. (2003) found that fluorescent yellow traps capture a higher number of *D. v. virgifera* adults, but without significant differences between captures of the tested traps. According to BFSA (2016), the adults of this pest appear at the end of June. Using pheromone traps and coloured sticky traps, Ivanova (2002a, 2002b) and Toshova et al (2017) reported about a later appearance

Table 1. Mean captures (± SE) of Coccinellidae species in coloured and transparent traps in maize crops in Knezha (12.06 – 23.09.2014) and Sofia (02.07 – 17.09.2014). For the results at Knezha, mean captures marked with the same letter in a column are not significantly different at $P < 0.05$, Kruskal-Wallis analysis of variance followed by a Mann-Whitney U test for pair-wise comparisons. Legend: '–'– species without captures in the test; *A. d. – A. decempunctata*, *Ch. b. – Ch. bipustulatus, C. s. – C. septempunctata*, *C. u. – C. undecimnotata*, *H. a. – H. axyridis*, *H. v. – H. variegata*, *P. q. – P. quatuordecimpunctata, P. v. – P. vigintiduopunctata,* and *T. s. – T. sedecimpunctata*

Site	Trap colour	A. d.	Ch. b.	C. s.	C. u.	H. a.	H. v.	P. q.	P. v.	T. s.
Knezha	transparent	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.03	0.00 ± 0.00	0.24 ± 0.06 a	0.01 ± 0.01	0.18 ± 0.05 bc	0.01 ± 0.01	–
	white	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.02	0.01 ± 0.01	0.05 ± 0.02 ab	0.00 ± 0.00	0.04 ± 0.02 c	0.00 ± 0.00	–
	blue	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.02	0.00 ± 0.00	0.06 ± 0.03 ab	0.00 ± 0.00	0.05 ± 0.02 bc	0.01 ± 0.01	–
	red	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.03	0.00 ± 0.00	0.01 ± 0.01 b	0.00 ± 0.00	0.05 ± 0.02 bc	0.00 ± 0.00	–
	orange	0.04 ± 0.03	0.00 ± 0.00	0.03 ± 0.02	0.00 ± 0.00	0.15 ± 0.05 ab	0.00 ± 0.00	0.25 ± 0.07 ab	0.05 ± 0.04	–
	yellow	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.02	0.00 ± 0.00	0.14 ± 0.04 ab	0.01 ± 0.01	0.30 ± 0.07 ab	0.00 ± 0.00	–
	fluorescent yellow	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.23 ± 0.06 ab	0.00 ± 0.00	0.50 ± 0.10 a	0.01 ± 0.01	–
Sofia	transparent	–	–	0	–	0.06 ± 0.04	–	0.11 ± 0.07	0.06 ± 0.04	0
	white	–	–	0	–	0.06 ± 0.04	–	0.03 ± 0.03	0	0.06 ± 0.04
	blue	–	–	0.03 ± 0.03	–	0.06 ± 0.04	–	0.06 ± 0.04	0.03 ± 0.03	0.03 ± 0.03
	red	–	–	0	–	0.06 ± 0.04	–	0.03 ± 0.03	0	0
	orange	–	–	0	–	0.06 ± 0.04	–	0.28 ± 0.10	0.14 ± 0.07	0.08 ± 0.05
	yellow	–	–	0	–	0.14 ± 0.06	–	0.22 ± 0.08	0.08 ± 0.06	0.03 ± 0.03
	fluorescent yellow	–	–	0	–	0.03 ± 0.03	–	0.28 ± 0.10	0.11 ± 0.07	0.14 ± 0.11

of *D. v. virgifera* adults in northern Bulgaria in 1998 and 2014, at the beginning of August and the middle of July, respectively. The results of the present study showed the first captures of *D. v. virgifera* adults at the end of July 2014 in Knezha.

According to Penev & Tarnawski (1987), *A. ustulatus* is widely distributed in Bulgaria. Using sex pheromone traps it was caught in different habitats in the country, including maize crops (Subchev et al. 2004, 2005, 2006, 2010, Toshova et al. unpublished data). Based on the results of previous studies, it can be concluded that the swarming period of *A. ustulatus* in Bulgaria varies within the period from the beginning of June until the end of August. According to the results of the present study from 2014, the period of captures overlapped with the mentioned period. The colour preference of *A. ustulatus* adults to fluorescent yellow and white colour of the trap was also observed. These results are similar to those of Furlan (1996), who compared the efficiency of coloured sticky traps, and showed that white and light yellow traps are significantly more attractive than red, green, and black traps. Horton & Landolt (2001) showed similar colour preference in other click beetle species, *Limonius canus* LeConte, 1853, when yellow traps and white traps captured more beetles than red, green, dark blue, and black traps.

Testing of traps with different yellow hues in the present study showed that this feature plays a significant role in capturing *A. ustulatus* since more beetles were captured in fluorescent yellow traps than in the yellow traps. Within Coleoptera, similar preference for fluorescent yellow colour has been shown for two *Oxythyrea* species (Coleoptera, Cetoniidae) – *O. funesta* (Poda, 1761) (Vuts et al. 2008) and *O. cinctella* (Schaum, 1841) (Vuts et al. 2012), two longhorn beetle species – *Plagionotus floralis* (Pallas, 1776) (Imrei et al. 2014) and *Pseudovadonia livida* (Fabricius, 1776) (Toshova et al. 2016), and the pollen beetle *Brassicogethes aeneus* (Fabricius, 1775) (= *Meligethes aeneus* F., 1775) (Coleoptera, Nitidulidae) (Döring et al. 2012).

Conclusions

The coloured sticky traps are useful tool to study the occurrence, relative abundance, seasonal appearance and activity, as well as the colour preference of different groups of insects, including Coleoptera species.

A total of 11 species belonging to three Coleoptera families, Coccinellidae, Chrysomelidae, and Elateridae, were recorded in maize crops in Bulgaria (Knezha and Sofia) by means of coloured sticky traps CSALOMON®. It was found that the unbaited fluorescent yellow traps are more effective in capturing of the adults of *P. quatuordecimpunctata* and *A. ustulatus* than the other coloured traps tested.

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