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Detection and Monitoring of *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae) by KLP+ Traps with Dual (Pheromone and Floral) Lures in Bulgaria

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Abstract: The potential of KLP+ traps baited with dual (pheromone and floral) lures (Csalomon®, Plant Protection Institute, CAR HAS, Budapest, Hungary) as a new tool for detection and monitoring of the Western corn rootworm, *Diabrotica virgifera virgifera*, was tested in 2015 and 2016 in Bulgaria. Four locations were chosen: Knezha (northwestern Bulgaria), Lozitsa village (north-central Bulgaria), Sofia (western Bulgaria), and Plovdiv (southern Bulgaria). *D. v. virgifera* was recorded in Knezha, Lozitsa and Sofia. The traps showed very high efficiency at different population densities of the pest. During our investigation, captures of *D. v. virgifera* adults were detected from the middle of July until the middle of September. The peak catches of the pest were registered at the end of July – the beginning of August in Knezha and Lozitsa, and in August in Sofia. In Knezha, the captures of beetles were influenced strongly by the local climatic factors – negatively by the air humidity in August 2015, and positively by the temperature variables in July 2016 (soil temperature) and August 2016 (air and soil temperatures).

Key words: Western corn rootworm, non-sticky trap, combination (pheromone and kairomone) lures, climatic factors, Bulgaria

Introduction

The Western corn rootworm, *Diabrotica virgifera virgifera* LeConte, 1868 (Coleoptera: Chrysomelidae), is one of the most damaging pests of maize *(Zea mays* L.) in North America and Europe. In Europe, this species was firstly reported in 1992 in a maize field near the Belgrade Airport in former Yugoslavia (Bača 1994). However, based on annual growth rate of this invasive alien species, Szalai et al. (2011) estimated that the introduction event has occurred between 1979 and 1984. Since its introduction, *D. v. virgifera* has been spreading in Europe, and by 2012, the pest has spread to almost all important maize growing areas in Europe, which resulted in well-established populations in many regions (Kiss 2014).

The first detection of *D. v. virgifera* adults in Bulgaria occurred in 1998, during a monitoring by pheromone traps and yellow sticky traps within the FAO project TCP/RER/6712(A) 'Development and implementation of containment of the Western Corn Rootworm in Europe', in Orsoya and Slivata villages (Lom Municipality, Montana District) located near

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the northern and north-western borders of Bulgaria. In the same year, adults of this species were also caught at other localities in Vidin and Montana districts (Ivanova 2002a, 2006). The spread of the pest in the country continued to the south and east, and the total area infested by the Western corn rootworm in Bulgaria increased rapidly – from 200 km^2 in 1998 to 12,000 km^2 at the end of the 2003 maize-growing season (Ivanova 2002b, Kiss et al. 2005). According to Ivanova (2006), seven years after its first record in Bulgaria, the pest has been already spread in Vidin and Montana districts, the bigger part of Vratsa District, and in parts of the districts of Sofia and Pleven.

According to Chiang (1973), *D. v. virgifera* develops one generation per year, and overwinters as diapausing egg in the soil. The soil-inhabiting larvae of this pest seriously damage roots of maize and lead to yield losses. Adults cause damage by feeding on pollen, silk and young kernels. Despite its importance and spread in Bulgaria, this species has been poorly studied in this country – to the best of our knowledge, there are records concerning only the distribution and the seasonal activity of the adults established by traps with pheromone or visual stimuli (Ivanova 2002a, 2002b, 2006, Toshova et al. 2017). In contrast to this, there are detailed studies on the phenology, ecology, damages, control measures and management of *D. v. virgifera*, as well as on the influence of weather factors and altitude on pest abundance, in the countries neighbouring to Bulgaria – Serbia and Romania, where the pest was also established at the end of the last century (Sivčev 2001, Baca et al. 2003, Tančić et al. 2006, Ciobanu et al. 2007, 2009, Grozea et al. 2008, 2009, 2010a, 2010b, 2011, 2014, 2015, Crisan et al. 2009, Dinnesen et al. 2009a, 2009 b, Sivcev et al. 2009, Sarajlić et al. 2011, Rancov & Cârciu 2012, Sivčev et al. 2012, Florian et al. 2013, Fora & Lauer 2013, Inđić et al. 2014, Popović et al. 2016, Manole et al. 2017).

In the countries of the European Union, the Western corn rootworm was considered at first as a regulated harmful organism with a quarantine status. However, after the failure to prevent the spread of the pest, as it had spread over a half of the Union maize-growing area, in February 2014, the European Commission repealed the Commission Decision *2003/766*/EC and lifted the quarantine status of this species. According to the Commission Recommendation 2014/63/EU, the integrated pest management of *D. v. virgifera* should be based on application of crop rotation, appropriate monitoring of the pest populations, and other relevant measures preventing the spreading of harmful organisms. The measures for controlling the Western corn rootworm in the current situation were reviewed by Furlan & Kreutzweiser (2015). In Bulgaria, the control measures against the pest include the applying of crop rotation, use of early maize hybrids, cleaning of agricultural machinery used in maize fields, soil tillage (plowing after harvesting maize in autumn), monitoring the adults flight by means of pheromone traps and yellow unbaited Pherocon AM® traps, taking soil samples in maize crops, monitoring the larvae by examining the maize roots for root pruning, tunneling or scarring, and application of insecticides for the larvae and the adults (when chemical control is necessary) (BFSA 2016).

The aim of the current study was to investigate the potential of KLP+ traps baited with dual (pheromone and floral) lures (Csalomon®) as a new tool for detection and monitoring of the Western corn rootworm, *D. v. virgifera* in Bulgaria.

Materials and Methods

Traps and lures

Commercially available non-sticky KLP+ ('hat') traps with dual semiochemical (pheromone and floral) lures for *D. v. virgifera* adults from Csalomon® (Plant Protection Institute, CAR HAS, Budapest, Hungary, www.csalomontraps.com) were used in our field investigations. Vaportape® (Hercon Environmental Inc., Emigsville, PA, USA) insecticide strip was placed in each trap to kill the caught beetles.

Monitoring sites

The seasonal monitoring was organised at four sites in three districts of Bulgaria in 2015 and 2016:

Knezha (Pleven District, north-western Bulgaria): Five baited and two unbaited (control) KPL+ traps were set up in a maize field (N 43°28'48.85"; E 24°3'22.03") (0.1 ha), belonging to the Maize Research Institute, Knezha, on 17 June 2015 and 12 July 2016. In both years, the traps were visited one to three times per week until the end of September. The traps were attached to wooden poles (2 m) at a height of 1.5-1.8 m, depending on the growing stage of the maize plants. The replications were designed in order to cover the whole maize field and the distance between them was about 20 m. The control traps were placed at the second and fifth replication, and the distance between the baited and control traps was 10-15 m.

Lozitsa Village (Pleven District, north-central Bulgaria): Two baited traps were set up in a private

vineyard with Mavrud variety (N 43°35'55.41"; E 24°58'26.57"), located close to the border with a large private maize crop on 24 July 2015 and inspected weekly until 30 September 2015. The traps were fixed to the iron wire between two neighbouring vine plants. The maize crop was harvested for silage on 15 August 2015. No investigations were provided in 2016.

Sofia (Sofia City District, western Bulgaria): Four baited traps were set up in a maize field (N 42°42'20.75"; E 23°26'19.11"), belonging to the Training and Experimental Field Station (University of Forestry) in Vrazhdebna suburb of Sofia on 24 June 2015 and inspected weekly until 28 September 2015. The traps were fixed with a wire at a height of 1.50-1.80 m on stone poles located regularly in the maize field, in a square shape. The distance between the traps was about 20 m. No investigations were made in 2016.

Plovdiv (Plovdiv District, southern Bulgaria): Two baited traps were placed on stone poles at the edge of a maize field (N 42°08'08.16"; E 24°48'18.01"), belonging to Agricultural University (Plovdiv) and inspected weekly in the period of 8 July – 30 September 2016.

The beetles caught were collected on each inspection date, and the lures and toxicant stripes were replaced with fresh ones at 5-6-week intervals.

Meteorological data were available only for the maize field in Knezha. The daily meteorological data records were provided by the meteorological station of Knezha, located at a distance of 0.7 km from the maize field. For the analysis, these data were averaged to get means for each particular time period of trap inspection. The influence of the minimum (T min), maximum (T max) and mean air temperature (T mean), along with the soil temperature at 10 cm depth, relative humidity and precipitation at the site of *D. v. virgifera* captures in the traps, was analysed by Spearman's rho correlation nonparametric test for 2015 and 2016. The statistical analyses were performed by a STATISTICA 7.0 Software (StatSoft, Inc. 2004). The significant difference was set at $P < 0.05$.

Results

Diabrotica virgifera virgifera captures (females and males) were recorded at three of the four investigated sites – Knezha, Lozitsa village and Sofia.

The highest population density of the pest was established in Knezha in 2016. At this site all adults were caught in the baited traps with the exception of a single specimen caught in one of the control traps in August 2016. In 2015, although the traps were set up in the field in the middle of June, the first captures of the beetles were registered only in the period of 22-24 July (Fig. 1). The largest captures were recorded at the end of July, while the last were in the period of 5-12 August. In 2016, the appearance of *D. v. virgifera* adults was in the period of 23-25 July. The pest was most abundant at the end of July – the beginning of August. The adults were recorded until the beginning of September. In 2016, the total captures of the pest exceeded about 60 times those obtained in 2015.

In Sofia, the first captures of *D. v. virgifera* were registered about three weeks after installing the traps in the maize crop, in the period of 15-22 July 2015 (Fig. 2). The seasonal flight of the pest was in the period from the middle of July to the middle of September, with a peak in August.

In Lozitsa Village, the beginning of the flight of the Western corn rootworm was missed because of the late installing of the traps (Fig. 3). Captures were observed in the period from the end of July to the middle of August 2015, with a peak at the end of July – the beginning of August.

The analysis of the data showed that the air humidity and temperatures were the climatic variables that had affected the total captures of *D. v. virgifera* in Knezha in 2015 and 2016, respectively. The significant correlations between captures and the climatic variables are showed in Table 1. In 2015, the abundance of the pest was negatively influences by the air humidity in August. In 2016, the soil temperature at 10 cm depth had a significant positive effect on appearance of the beetles in July. Significant correlations between the temperature variables investigated and beetle captures were found in August 2016 (all temperature variables). We did not find a significant correlation between precipitation and the total captures of the *D. v. virgifera* adults for each sampling period (Spearman's rank correlation: $P > 0.05$).

Discussion

The Bulgarian Food Safety Agency is responsible for the supervision of detection, recording and preventing the spread of the Western corn rootworm in Bulgaria, and the pest has been a subject of monitoring programmes since its detection in Bulgaria in 1998. The pest is distributed in all districts in Bulgaria, excepting Varna, Kardzhali, and Smolyan (MAF 2015). In 2015, the species was registered in Yambol District as well (MAF 2015). In Knezha, *D. v. virgifera* was caught for

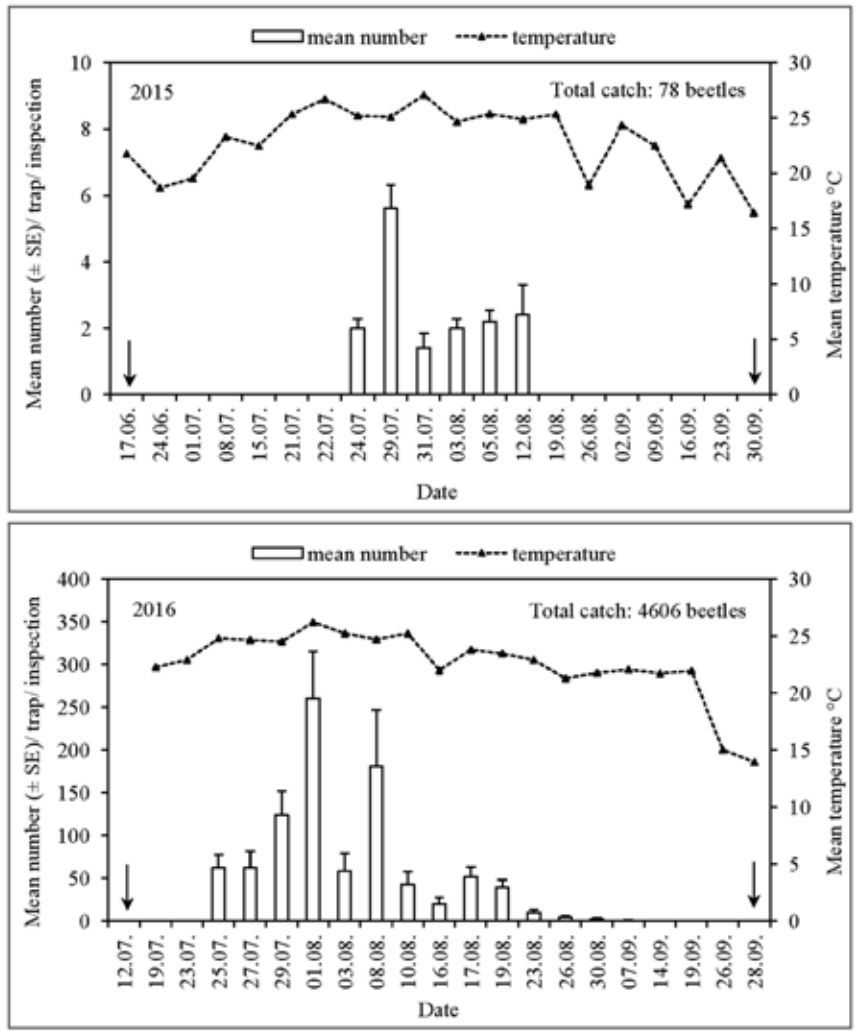

Fig. 1. Mean captures of the Western corn rootworm, *Diabrotica virgifera virgifera*, in Knezha in baited traps, 2015-2016; Five replications. For each year, the arrows (↓) show the dates of the beginning and end of investigation

the first time in 2001 (Ivanova 2002b) and later in 2014 (Toshova et al. 2017). Although the pest has been caught in the regions of Pleven, Sofia and Plovdiv districts (MAF 2015), to the best of our knowledge, there are no published records about its occurrence in the region of Lozitsa Village, and cities of Sofia and Plovdiv, respectively. During our study the earliest capture of *D. v. virgifera* adults was registered in Sofia in the period of 15-22 July 2015, and the last captures – again in Sofia, in the middle of September. In Knezha, the first adults of the pest were recorded at the end of July, which coincides with the results from the same locality in 2014 obtained, using coloured unbaited traps (Toshova et al. 2017). According to the periodical bulletin of plant protection in Bulgaria, *D. v. virgifera* adults appear at the end of June (BFSA 2016), but no details are available. Ivanova (2002a, 2002b) reported data about the appearance of the pest in July – August in Bulgaria, and captures may be found until the end of September, and even the beginning of October (Ivanova, personal communication). In 2015, in the district of Kyustendil, where the highest population density of the pest in Bulgaria was registered for the period 2010-2015, the seasonal flight was recorded from 15 July to 9 September (MAF 2015), which coincides with the activity patterns recorded by us (current study, Toshova et al. 2017).

Similar flight periods of *D. v. virgifera* have been reported for other European countries. In southern Poland, Bereś & Sionek (2010, 2012) found out that the adults appear from the second half of July until the second half of September – late October. In Serbia, the *D. v. virgifera* adults emerge from the end of June, and are present in the field until the first frosts (Sivčev et al. 2012). Igrc Barčić et al. (2003) reported that in Croatia the adults of the pest emerge from the soil in the period of the middle of June – middle of July, and are present in the field until the second half of October. According to Borzykh et

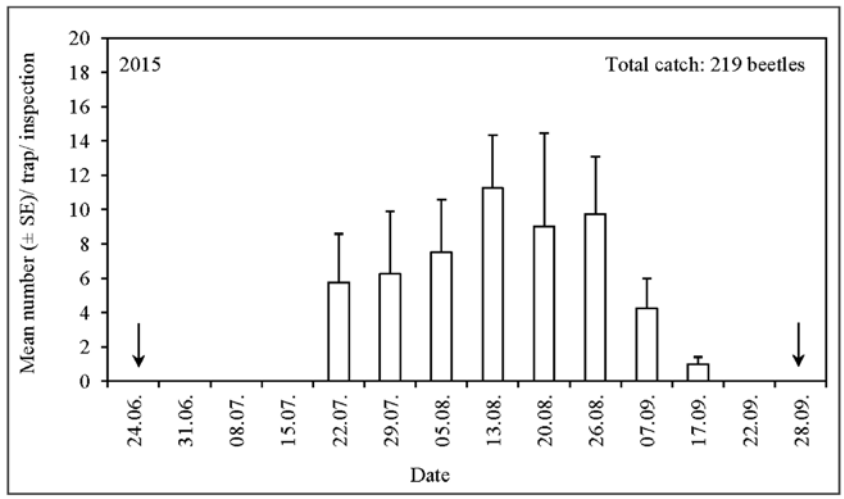

Fig. 2. Mean captures of the Western corn rootworm, *Diabrotica virgifera virgifera*, in Sofia in baited traps, 2015; Four replications. For the legend, see Fig. 1

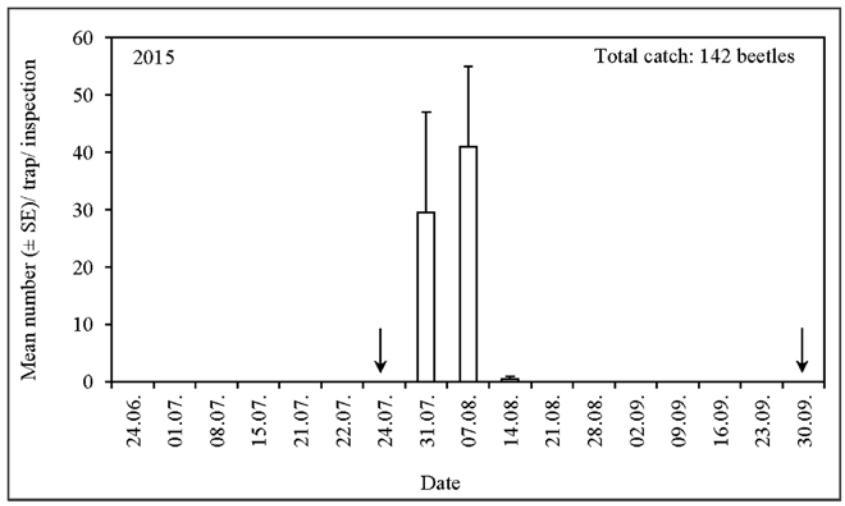

Fig. 3. Mean captures of the Western corn rootworm, *Diabrotica virgifera virgifera*, in Lozitsa village in baited traps, 2015; Two replications. For the legend, see Fig. 1

al. (2013) and Voineac et al. (2015), in Ukraine the adults occur from the end of June - early July until the end of September – the beginning of October.

In 2013-2015, the total captures of *D. v. virgifera* adults in the pheromone and yellow unbaited traps in the observed territory (about 10% of the total area with maize) of Bulgaria varies between 9,000 and 14,000 beetles caught (MAF 2015). In 2016, using five KLP+ traps baited with the dual (pheromone and floral) lures, we recorded more than 4,600 males and females in Knezha, which demonstrates the high potential of the combination of a non-saturable high-capture capacity KLP+ trap with dual lures in detection of the pest occurrence and in monitoring programmes.

Various factors can affect the timing of adult emergence and the abundance of the Western corn rootworm, among which are weather and geographic conditions, edaphic factors, natural mortality factors, period of the maize planting, level of plant density, availability of alternative larval hosts, maize hybrids, natural enemies, and measures for pest control applied (Kuhlmann & van der Burgt 1998, Isard et al. 1999, Keszthelyi 2006, Toepfer & Kuhlmann 2006, Árendás et al. 2009, Ciobanu et al. 2009, Meinke et al. 2009, Grozea et al. 2010a, 2011, Karić & Festić 2011).

The shorter period of the activity of *D. v. virgifera* beetles in Knezha in 2015 in comparison to 2016 could be explained by the higher humidity in August 2015. We found a significant negative correlation between the captures and the air humidity in August 2015. However, in 2016 the soil temperature and air temperature influenced positively the captures in the traps. Similarly, Grozea et al. (2011) found positive

Table 1. Relationships between total captures of the Western corn rootworm, *Diabrotica virgifera virgifera*, and climatic factors in Knezha, in 2015 and 2016. r_s – Spearman's rank correlation coefficient; Sig. – statistical significance; * – P < 0.05 and ** – P < 0.01; '–' – no captures

Climatic factors	2015						2016					
	July		August		September		July		August		September	
	r_s	Sig.	r_s	Sig.	r_s	Sig.	r_s	Sig.	r_s	Sig.	r_s	Sig.
Air T min, (°C)	0.233	0.578	0.205	0.741	–	–	-0.027	0.949	0.636	0.048*	0.707	0.182
Air T max, (°C)	0.546	0.162	0.564	0.322	–	–	0.682	0.062	0.745	0.013*	0.000	1.000
Air T mean (°C)	0.273	0.513	0.359	0.553	–	–	0.627	0.096	0.915	0.000**	0.707	0.182
Soil T, (°C)	0.518	0.188	0.359	0.553	–	–	0.873	0.005**	0.830	0.003**	0.707	0.182
Relative humidity	-0.600	0.116	-0.975	0.005**	–	–	0.123	0.771	-0.612	0.060	-0.707	0.182
Precipitation, mm	0.084	0.844	-0.718	0.172	–	–	-0.429	0.289	-0.356	0.313	-0.544	0.343

correlations between the Western corn rootworm captures in pheromone traps and air and soil temperatures in Romania, and a negative correlation between abundance of the beetles and air humidity. Borzykh et al. (2013) reported that in the climatic conditions of central Ukraine the optimal air and soil temperatures for the appearance of the adults were 19°C and 22°C, respectively.

Conclusions

The high capacity KLP+ traps in combination with two types of lures (pheromone and floral), which attract and capture both males and females of *D. v. virgifera* (i.e. males with pheromone lure, both sexes with floral lure), may be used for various purposes, such as: detection, monitoring of distribution and seasonal activity of the adults of the pest, and providing information about the optimal time for insecticide application.

Our results can contribute to the integrated pest management approach for control of *D. v. virgifera*. For monitoring purposes in Bulgaria, the attractant traps should be used in the maize crops from the middle of July until the middle of September, they should be checked regularly (at least every week) for the presence of adults of the pest, and the lures should be replaced with fresh ones at 5-6-week intervals.

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