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THE BROADENING OF DISTRIBUTION OF THE INVASIVE SPECIES *Diabrotica virgifera virgifera* Leconte IN THE AREA OF MUNTENIA REGION UNDER SPECIFIC CLIMATIC AND TROPHIC CONDITIONS

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Abstract

The spread of invasive species to a new area is influenced and conditioned by a number of drivers that can increase or control (meaning slow down) the speed of invasion. The largest shares in the factors which determine this area of spread are the geographical factors and the trophic and climatic conditions. For that reason, it is intended that immediately after the invasion, when pathways and vectors are determining which facilitated the invasion (early detection), to proceed with a monitoring program in order to develop and establish a management of control of the new pest. The present paper discusses on the possibility of developing the procedures for the surveillance work of maize crops in Southern and South-Eastern Romania to identify, monitor and evaluate the pest potential of Diabrotica virgifera virgifera. The research area included localities from counties in the Southern and South-Eastern Romania, where the monitoring was conducted with specific pheromone traps.

Key words: *invasive species, monitoring, risk assessment*.

INTRODUCTION

In the maize crop from Southern Romania there is about 25 species of insect pests that cause damage considered economically significant. Since 1996 a new invasive species has been registered in the west side of the country (first mention in Nădlac, 1996) and since 1999 this has started to produce significant damage to crops in this area, counties Arad, Timiș and Bihor. It is about on the invasive insect species *Diabrotica virgifera virgifera* LeConte *(Coleoptera: Chrysomelidae)*, the western corn rootworm (WCR). It belongs to a relatively numerous group of species and is considered the main corn pest in USA. Soon after invasion in Europe, one European program for prevention and monitoring had started (Berger, 1996; Vidal et al., 2005). Within the frame of the first meeting (Graz, Austria, 1995) there were established, for the first time, the methods for early detection and monitoring of the species in the invaded countries.

Pheromone traps with specific sex pheromone (Tóth et al., 1996) and the bait based on cucurbitacin were used. Alongside these techniques, it was recommended using yellow glue traps, type Multigard®, supplied by Scentry in Montana, USA. In the 2000-2016 period, the species continues to spread in Europe and western Asia at a rate of 20 km/year, strictly in close dependence on the presence of host plants, especially in the field crops. The current status of WCR in European countries is presented in Table 1.

Discovered and first formal description by LeConte in 1868 from beetles collected near Fort Wallace, Kansas, USA, the species became reported as maize pest in 1909, (Gillette, 1912) approximately 50 years after. From 1909 to 1948, the insect spread eastward at an average rate of about 19 km per year (Metcalf, 1986).

Large-scale applications of soil insecticides were first made in 1949 and more than 700,000 ha were being treated with insecticides by 1954 (Ball and Weekman, 1962).

However, resistance to the soil insecticides was noted as early as 1959, but the insecticide-resistant strain spread eastward at a rate even faster than 112 km/year (Ball and Weekman, 1962; Metcalf, 1983).

In Romania the actual distribution was established by the Phytosanitary Quarantine laboratory team as it is shown in Figure 1 (Manole, 1999).

Table 1. Actual status of *D. virgifera virgifera* in Europe

Country	Year	Locality	Status	Measures
Serbia	1992	Surcin	present	Control
Montenegro	1993	Bijelo Polje	present	Control
Hungary	1995	Csongrád	present	Control
Croatia	1995	Bosnjaci	present	Monitoring
Romania	1996	Nădlac	present	Control
Bosnia-Herzegovina	1997	Tuzla-Posavina	present	Monitoring
Bulgaria	1998	Bregovo	present	Monitoring
Italy	1998	Marco Polo di Tessera (Venice)	eradicated	Monitoring
Slovakia	2000	Lucenec	present	Monitoring
Switzerland	2000	Lugano/Agno	eradicated	Monitoring
Ukraine	2001	Zakarpatya	present	Monitoring
Austria	2002	Deutsch Jahrndorf	present	Control
France	2002	Roissy/Le Bourget/Orly	eradicated	Monitoring
Czech Republic	2002	Cejc	present	Monitoring
UK	2003	London	eradicated	Monitoring
Netherland	2003	Amsterdam	eradicated	Monitoring
Belgium	2003	Brussels	eradicated	Monitoring
Slovenia	2003	Pomurje	present	Monitoring
Poland	2005	Dukla	present	Monitoring
Germany	2007	Baden-Württemberg	eradicated	Monitoring
Belarus	2009	Brest	eradicated	Monitoring
Greece	2009	Salonic	present	Monitoring
Albania	2010	Gjegjan	present	Monitoring
Russia	2011	Matveev Kurgan	present	Monitoring

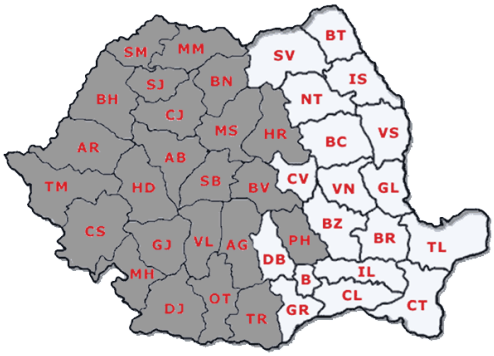

Figure 1. The spreading of *D. virgifera virgifera* in Romania in 1999

The scope of this paper is related to the possibility of developing a procedure for the surveillance of some crops in Southern and South-Eastern Romania and to identify, monitor and establish the pest potential of *D. virgifera virgifera*.

MATERIALS AND METHODS

The monitoring of adult populations of WCR practiced by European countries has allowed a rapid detection and establishing of the spread of this invasive pest since the insect has been first observed in Serbia. As it was shown in Table 1, the monitoring activity is still important in countries with or without isolated infestations, as well as in countries where WCR population is still spreading, and this is the situation in Romania. Our research seeks to establish the habitat, and to early detect the presence of species of WCR in counties from southern Romania, where in the well-known Romanian Plains (Bărăgan, Big Island of Brăila and the Danube plain) the corn is the most cultivated agricultural plant. The survey corn area included localities from the counties Dâmbovita, Teleorman, Giurgiu, Călăraşi and Constanţa (Ciocăneşti, Drăgăneşti-Vlaşca, Naipu, Fundulea, Amzacea), where a number of 1-4 pheromone traps were placed, according to the monitored area. The traps with sex pheromone (3- (4-methoxyphenyl)-2propenal) were bought from the company Csal♀m♂N®, a trademark of the Institute of Plant Protection MTA ATK, Budapest, Hungary, which are used species within the communication system chemical to lure males (Figure 2). Throughout the investigation period, the mean temperature and precipitations (liter/m^2) were registered with a digital field device.

Figure 2. KLPfero + pheromone trap in corn crop

RESULTS AND DISCUSSIONS

Distribution and rate of spreading of *D. virgifera virgifera* was correlated with some environmental factors, and in every analysis of the rate and speed of invasion, those must be taken into consideration. First of all, it is necessary to establish the trophic guild for this species belonging to the largest tribe of Luperini (Wilcox, 1965; Wilcox, 1972).

After many years of the investigation in the natural habitat from North America, it seems that the WCR species, beside of their chemical attraction on maize volatile emissions, (E)-β-farnesene, (E)-β-caryophyllene), they were influenced by the relative humidity (RH%) existing in the field. Taking into account this aspect, there could be an explanation for the low densities of adults of the pest in the south counties of Romania (Ialomița, Brăila, Galați, Constanța, Călărași), despite of the large surfaces of maize. As it is shown in Figure 3, in the observed areas, the climatic conditions especially in the summer months, July and August, of the year 2016, were characterized by much reduced rainfalls and very high values of temperatures. The dry conditions are not favorable for feeding and development larvae or adult.

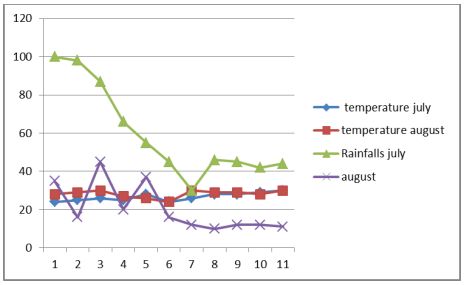

Figure 3. Average temperature (°C) and rainfalls (mm) in the field of samples collection

The WCR, *D. virgifera virgifera* as a member of the *virgifera* subdivision of the largest genus Diabrotica (Smith, 1966), is a specialist on the Poaceae plants and ancestrally probably it reached maturity only on grasses, such as *Zea* spp., *Setaria* spp., and couch grasses in several genera (Clark and Hibbard, 2004; Oyediran et al., 2004; Moeser, 2003; Manole, 1999). Another important aspect related to WCR spreading and distribution must be the cultivated surface of maize. In Romania this crop is the first culture as importance. In 2015, Romania has the most area of maize (2.52 mil ha) in Europe (INS, 2016). In October 2003, the European Union introduced emergency measures to prevent the spread of WCR (Commission Decision 2003/766/EC) and also the Central and Eastern European countries of Bosnia-Herzegovina, Bulgaria, Croatia, Hungary, Romania, Serbia and Slovak Republic have initiated a new regional activity for integrated pest management for WCR as an FAO Trust Fund Project (GTFS/RER/017/ITA) with government of Italy like donor (Berger, 1996). Our investigation started from that point out, because one of the intriguing facts related to the tropho-dynamic module of the species, the abundance of food resources in the south of Romania does not seem to be the main factor for the spread and distribution of species.

Previous research (Manole, 1999) found WCR only in the county Sibiu but not in the counties from southern Romania. The monitoring program concerning the invasion of WCR in Romania started in 1996, soon after the first pest detection in Nădlac, using the same techniques as described in 1995 (Kiss, 2003). Although not the major area of emphasis, the continuation of in-country monitoring was included in this national research project. The monitoring of adult populations has allowed a rapid detection and establishes the spread of this invasive pest inside the country territory. Monitoring is still important for isolated infestations as well as where WCR populations are still spreading. Thus, in these areas, the monitoring has served as a means for detection, and in some instances, containment and control; in the case of new appearances outside the regular spread line, for eradication purposes. The monitoring data could provide information necessary for making quarantine and other regulatory decisions. In terms of thermal and hydrological data in 2016 (especially during the summer months, July and August), they indicated a dry climate, with temperatures reached 35-38°C, frequently in the shade. For this reason and because of the delay in placement of traps at the beginning of the flight of WCR adult (early June, overlapping flowering period and silk from corn), in the area of Fundulea and Amzacea, WCR adults were not trapped although the symptoms of attack were present (Table 2).

Table 2. The presence of WCR adults in the monitored areas during July-August in 2016

Locality	Number of adults	
	♀	♂
Cornățelu	3	46
Naipu	-	20
Drăgănești-Vlașca	-	5
Fundulea	-	-
Amzacea	-	-

As it is shown in Table 2, the presence of WCR adults were established in three out of five collecting points, despite the fact that symptoms of larvae attacks appeared in all locations (Figure 4).

Figure 4. Characteristic "gooseneck" symptom of WCR attack

The pheromonal traps used, Csal♀m♂N®, KLPfero + containing cucurbitacin bait, were able to capture both sexes. Our trapping data showed that the flight activity of males was much intense than of the females, which prefered to feed on the ear corn silk (Table 2). The presence of the adult males in the traps could be, also the effect of ending of the mating period and preparing to hibernate. In the Figure 5, the number of trapped insects appears to confirm a higher density of the pest in the maize crop from Dâmbovița County, probably due to the better climatic situation in the field, especially RH%. Another factor that could be taken into consideration must be the behavior of the adult related to so called 'alternative host'.

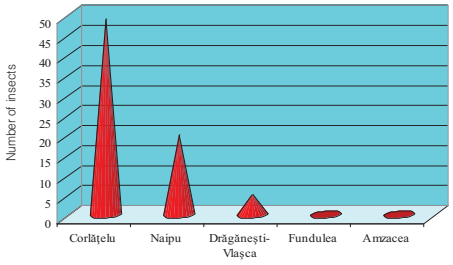

Figure 5. The abundance of WCR adult in monitored areas

Females of *D. virgifera virgifera* do not lay their eggs on a host plant such as maize, for instance, the eggs are laid in the soil in the late autumn, and the larvae emerge the following spring. Host location is then carried out underground by the neonate larvae. The time between egg hatch and larval establishment is one of the more vulnerable times in the life cycle of these insects (Toepfer and Kuhlmann, 2005). If host establishment is delayed for as little as 24 h, survival to the adult stage is significantly reduced (Branson, 1989). Therefore, timing of egg hatch and root availability is so critical. Root toughness may also be a limiting factor. In the light of previous investigations (Abe, 2000; Andersen, 1987; Branson and Krysan, 1981) their data suggest that WCR larvae not only prefer newly developed roots, as was already known but of 41 grasses and 27 broadleaf species were evaluated in early studies giving insight into 'grasses only' as larval hosts (Branson and Ortman, 1967). To evaluate the food conversion efficiency as WCR food resources for alternative host plants must take into consideration the chemical-olfactive behavior of WCR to other important plant volatiles such as cucurbitacin which, for majority of other species this compound was repellent. The corn crop in the field in Dâmbovița County was located near some important surfaces rich in spontaneous vegetation and that could provide an explanation on the higher population density than in Ialomița and Călărași counties for example.

CONCLUSIONS

The trapping data in this study had confirmed the presence of the species *D. virgifera virgifera* in maize crops from Teleorman, Giurgiu and Dâmbovița Counties situated in the South side of Romania.

It was noted the first registration of the pest presence in the County Dâmbovița as result of extending of the corn crop in this zone. Although the area of maize crop has increased in the counties in Southern Romania, no investigation on *D. virgifera virgifera* has been carried out so far, by the plant quarantine service to establish the presence and density of the pest.

In large areas of corn growing, (i.e. The Big Island of Brăila) that are the greatest areas of monoculture corn and the places where the

chemical treatments to seeds and in vegetation are strongly carried out, besides signaling the presence and monitoring, is necessary to determine the resistance of the species to plant protection products used.

Given the spread of *D. virgifera virgifera* in Romania, related to the specific hosts such as maize, soya or pumpkin crops, it will be necessary to continue monitoring activity in 2017.

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