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RESEARCH ON THE WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* LECONTE, COLEOPTERA: CHRYSOMELIDAE) IN CROATIA (1994-2003)

Jasminka IGRC BARČIĆ, Renata BAŽOK, Milan MACELJSKI

The Institute for Agricultural Zoology Agricultural Faculty, University, Zagreb, Svetošimunska 25, Croatia, E-mail: igrc@agr.hr

Accepted: December 15, 2003.

Ten years of research work conducted in the Department for Agricultural Zoology of the Agriculture faculty in Zagreb concerning the western corn rootworm (WCR) resulted in 45 scientific and professional papers published in foreign or national publications and meeting proceedings. All of these publications are reviewed in this paper. The development of the WCR in Croatia and Europe confirms the first statement that this insect pest will develop in Europe even faster than in the USA. This paper is intended to support new research on the WCR, which is underway in many countries of Europe.

***Diabrotica virgifera virgifera*, western corn rootworm, WCR, research, Croatia**

IGRC BARČIĆ, J., BAŽOK, R., MACELJSKI, M., Istraživanje kukuruzne zlatice (*Diabrotica virgifera virgifera* Le Conte) u Hrvatskoj (1994. - 2003). *Zavod za poljoprivrednu zoologiju Agronomskog fakulteta Sveučilišta Zagreb, Svetošimunska 25, Hrvatska*, E-mail: igrc@agr.hr - *Entomol. Croat.* 2003. Vol.7. Num. 1-2: 63 - 83.

Desetogodišnja istraživanja znanstvenika Zavoda za poljoprivrednu zoologiju Agronomskog fakulteta u Zagrebu prikazana su u 45 objavljenih znanstvenih i stručnih radova o kukuruznoj zlatici. Radovi su objavljeni u inozemnim i domaćim publikacijama i na skupovima. Svi ti radovi prikazani su u ovom preglednom radu te navedeni u popisu radova. Razvoj kukuruzne zlatice u Europi potvrdio je prvu prognozu da će se štetnik dobro aklimatizirati u Hrvatskoj i Europi i postati još važnijim štetnikom nego što je sada u SAD. Stoga se ovim preglednim radom želi podržati istraživanja koja se sada provode u mnogim europskim zemljama.

***Diabrotica virgifera virgifera*, kukuruzna zlatica, istraživanja, Hrvatska**

Introduction

Western corn rootworm (WCR) was until the last decade of the past century present and a pest solely in North America. In certain areas of North America, especially

in the so called Corn Belt, along with European corn borer, *Ostrinia nubilalis* (Hübner), it is the most significant corn pest, annually causing losses up to USD 1 billion, including control costs (METCALF and RHODES, 1982, quot. KRYSAN and BRANSON, 1983).

Western corn rootworm belongs to the family Chrysomelidae, sub-family Galerucinae. The Diabroticina was, before the western corn rootworm was introduced to Europe, spread only in the Nearctic Subregion. It is of tropical origin, found mostly in Brazil and Mexico. WILCOX, 1972 (quot. KRYSAN and BRANSON, 1983), differentiates three groups of the Diabroticina: *fucata* with 298 breeds, *virgifera* with 28 breeds and *signifera* with 11 breeds. Twenty-one breeds from the first two groups are corn pests.

In the USA, damage to corn is caused by the following three species:

Diabrotica barberi Smith and Lawrence (northern corn rootworm (NCR)) is spread in southern parts of the USA.

Diabrotica virgifera zea Krysan and Smith (Mexican corn rootworm (MCR)) is spread in southernmost parts of the USA, but lately it has reached Kansas.

Diabrotica virgifera virgifera LeConte (western corn rootworm (WCR)) had been known in the USA for a long time, but became a major corn pest only in the late 40's of the past century. It then started spreading from the Rocky Mountains eastwards, thus reaching all the way to the Atlantic Ocean, becoming a major corn pest.

In the European references on pests, WCR is not rarely mentioned. In the book on agricultural pests MACELJSKI and IGRC (1991) briefly acknowledge *Diabrotica* genus, as a potential threat to Europe.

In the summer of 1994, we learned from a Belgrade daily newspaper of the appearance of WCR in Serbia, soon substantiated by an expert note (BČA & BERGER, 1994).

Realizing the vast significance of the presence of this pest in Europe, experts of the Department for Agricultural Zoology, University of Zagreb, immediately began to study this pest, and continue to do so. Due to many results published in the local scientific and expert journals, which are little known abroad, and partly also in foreign publications, which are again not read by Croatian experts, the decision was made to present all these published studies in forty-five papers in the present review. This was done, in order to compile the most important results on a single publication, thus making them more easily available.

The Period Before the Pest was Discovered in Croatia – 1994

Learning in 1994 that in 1992 a small area planted by corn was discovered to be heavily infested with WCR near the Belgrade airport in Surčin, we concluded that it was highly likely that the pest had already been introduced to Croatia. Our priority task was to inform Croatian colleagues and the public about the pest in question and establish the possibilities of its spread, as well as its importance for both Croatia and Europe.

An extensive bibliography was collected on the pest. The result of studying this was a review published on WCR (MACELJSKI & IGRC BARČIĆ, 1993– actually published towards the end of 1994.). The paper presents a systematic placing and spread of the pest; description, biology and ecology; harmfulness; with a particularly extensive chapter on integrated protection against the pest, separated as per alternative methods, and chemical control – protection against larvae and control of grown beetles, as well as decision-making threshold. A bibliography with 40 entries was also included. The conclusion states: “Although western corn rootworms of *Diabrotica* genus have not been identified in Croatia yet, their presence and fast spread in Srijem indicates that *Diabrotica virgifera virgifera* will soon become a corn pest in our midst as well. That is why we have presented the most important data on this species, in order to be as prepared as possible to undertake integrated measures of corn protection against this pest in Europe”. The conclusion mentions European measures because this paper constitutes the first comprehensive review on WCR published in Europe at the time when the pest was present only on a limited area in Serbia.

The second priority was to establish the possibility of its acclimatization in Croatia (and Europe) and estimate its development. This was done in a separate paper also published towards the end of 1994 (MACELJSKI & IGRC BARČIĆ, 1994).

By studying the WCR data from North American references, conditions were established governing the appearance and increased of WCR in the USA. Especially analyzed were the agrotechnical and climatic conditions, and dynamics of individual corn phenophases. Also identified were thermal conditions on which the said pest depends. It was established that its population in the USA is mostly dependent on crop rotation, i.e. on repeated corn planting, and the degree of area planted to corn. It was furtherly established that rootworm eggs die if minimal winter temperatures in the soil at the depth of 10 cm are below -8°C and that rootworm mostly spreads (within USA) where the average annual temperatures are around 13°C , but that it is also present in the states where the average annual temperature is 9°C , and even in Montana where it is only 6.8°C . In areas where it has spread the most, the annual precipitation level is

1000-1350 mm, but the pest is also numerous where 1000 mm, or even 430-510 mm, of yearly precipitation occurs. It is obvious that WCR is rather flexible in its requirements for heat (eurithermal species) and moisture.

When a comparison was made concerning production conditions for corn in the USA with those in our country, it turned out that they match. Corn is often a single crop in Croatia, with a high share in some respective areas. Phenophases of plant development match the appearance of individual stages of the pest. Rootworm eggs may survive winter because, within a ten-year period, temperatures below -8°C were recorded only once in Croatia (Križevci). Average annual temperatures in northwestern Croatia and Slavonia are above 10°C , while the precipitation level is between 700 and 1000 mm.

Based on American data on thermal threshold of larvae development in the soil (12.7°C) and thermal constant (176.5 degree/day), i.e. for the establishment of 50% of larvae from the eggs (thermal threshold 11.2°C and thermal constant 353.8 degree/day), and comparing the data with our climatic conditions, we have established and graphically presented the estimation of dates when the first larvae are likely to appear, as well as the appearance of 50% of larvae from the eggs in northwestern Croatia and Slavonia.

As a result of this, paper we established a more accurate choice of the date for a curative application of insecticides to the established plants, facilitated the selection of the date to begin monitoring of the pest and estimated damage caused by the larvae.

We have established the possibility of the acclimatization of the pest in Croatia, suggested co-operation with Hungary and other countries on the monitoring of the pest, and established the necessity to study the pest, especially its control.

The most important conclusion by all means was that the western corn rootworm will be propagating and spreading in Europe even faster than in the USA, and that monitoring of the pest and the undertaking of its control should start immediately. If the said recommendation had been accepted on European level, it would have been possible to slow the alarmingly fast spread of WCR in Europe which now has occurred.

A shortened version of this paper was presented on the first international meeting on western corn rootworm in Graz in March 1995 (MACELJSKI & IGRC BARČIĆ, 1995). This is why it is not at all commendable that some French, German and other scientists who have come up with the same conclusions we previously made, although using other criteria, failed to quote us. Our conclusions published back in 1994 were entirely confirmed by the subsequent development of WCR in Europe. We should mention that another paper was published later on the acclimatization possibility of the

rootworm, using new criteria (IGRC BARČIĆ & MACELJSKI, 1998), and confirming our initial conclusions. It goes without saying that in this paper we have mentioned all the authors of similar articles.

Towards the end of 1994, we contacted Hungarian experts (G. PRINCZINGER, I. EKE), requesting international action against WCR. Our common proposals were adopted: the existing working group for European corn borer– IWGO (International Working Group Ostrinia) of IOBC headed by H. BERGER included western corn rootworm as part of the programme activity. After that, EPPO and FAO joined the IWGO's working group.

The IWGO organized meetings in Graz in, 1995 and Gödöllő in, 1995; together with EPPO in Zagreb, 1996; and together with EPPO and FAO in Gödöllő, 1997; Rogaška Slatina, 1998; Paris, 1999; Stuttgart, 2000; Venice, 2001; and Belgrade, 2002; and Engelberg, 2004, while in 1999 the final meeting of IWGO and FAO on the FAO project was held in Zagreb (Bežanec).

Owing to regular contacts and agreements within the said group, WCR became the only pest in the world whose monitoring proceeded using the same methodology in most countries and whose spread was identified in detail each year. The threatened Eastern European states were especially active in this sense. On the other hand, although Western European experts were present at the meetings and well informed about the issue, they made the mistake of concentrating exclusively on research, without efficiently assisting the monitoring in their countries. Therefore, while in Eastern Europe each year's spread was well known and there were no surprises, in Switzerland, and especially in Italy, the pest was identified in Lombardia only after several years of its existence. This was mentioned in the paper by MACELJSKI, 2002. Only, in 2003 did, the EU Commission for Plant Health decide to undertake emergency measures (the same ones whose implementation we had proposed much earlier) in order to prevent further spreading of WCR in the EU. Today's situation concerning the spread of western corn rootworm in Europe per years of pest discovery is shown in Picture 1.

The First Year of Monitoring and Study 1995

The first meeting of IWGO was held in Graz on 20 and 21 March 1995. It was attended by experts from 11 European states, EPPO representatives, and experts from the USA who introduced the participants to this North American pest in some detail. We should point out that one of these experts, Prof. C. Richard EDWARDS, PhD, was offering expert assistance in all actions involving WCR in Europe, and managed to

achieve a FAO project ensuring free supply of baits over several years, and, through his frequent visits to Croatia, -directly assisted monitoring and studies in the country.



Picture 1. Spread of the WCR across Europe
Širenje kukuruzne zlatice po Europi

At this first meeting, apart from the experts from Serbia who informed the group about the first occurrence of the pest in Europe (near Belgrade), and American experts, only the experts from the Faculty of Agriculture in Zagreb had a presentation on the results of analyzing the possibility of western corn rootworm acclimatization and its harmfulness to Croatia, concluding that the species will become even more harmful in Europe than in the States (MACELJSKI & IGRC BARČIĆ, 1995). The need to co-operate with EPPO and FAO was agreed upon, as well as a common monitoring system. Already before that (on 6/03/1995), upon our proposal, the Ministry of Agriculture and Forestry (MAF) passed an «Order on the Measures of Preventing the Introduction of Western Corn Rootworm», making it obligatory for corn growers to participate in the monitoring.

In Croatia, the monitoring was performed using 150 baits of low attractive cucurbitacines, really acting as a feeding arrestant, rather than attractant. Cucurbitacines were obtained from USA, because better attractors were not available at the time. In spite of that, a WCR was caught in Bošnjaci near Županja.

Approximately at the same time, we were informed that a specimen was caught in Hungary. Towards the end of the year, colleagues and the public were informed of the presence of the pest in Croatia, accompanied by numerous data on it (IGRC BARČIĆ & MACELJSKI, 1995).

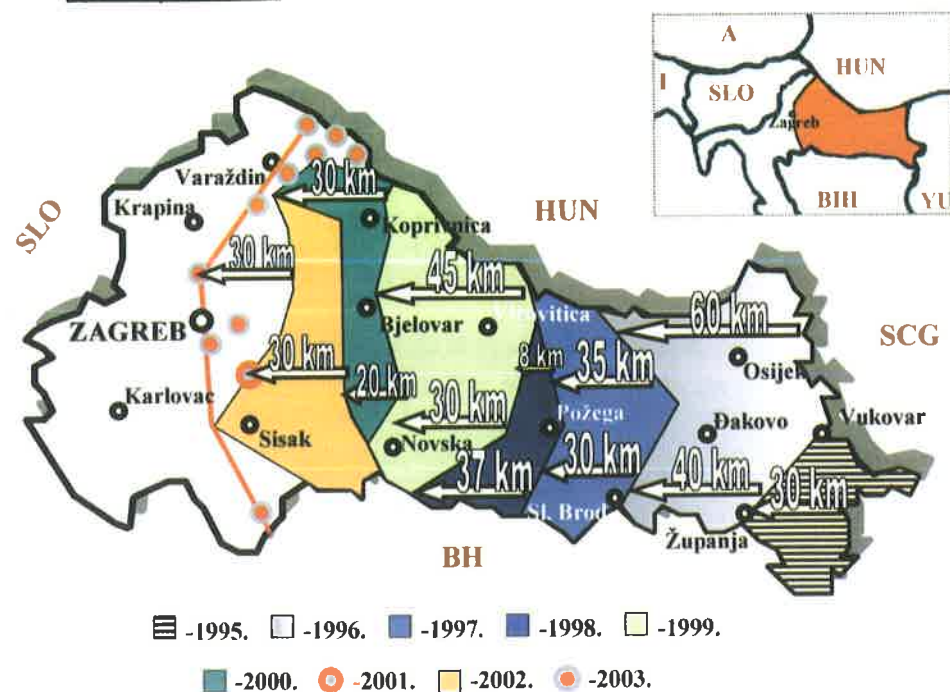
The second working group meeting was held on 8th November, 1995 in Gödöllő, Hungary with about 80 participants from 10 countries. IGRC BARČIĆ & MACELJSKI (1995 a) presented the state of WCR in Croatia. Three hundred cucurbitacine baits and 200 yellow sticky boards were positioned within certain areas of the country. A single male specimen was caught using cucurbitacine bait in Bošnjaci, 28 km to the west of border with Serbia. The paper noted that the appearance of adult rootworm forms would be synchronized with the pollinating of hybrids raised in eastern Slavonia, which would be favourable for the said pest.

It was agreed at the meeting that Croatia, together with Hungary, should take part in the FAO project, and that the same and hence comparable kind of monitoring be performed, using pheromones, which are, -as shown by research in Hungary, much more attractive than cucurbitacine and yellow sticky boards.

Spreading of Western corn rootworm 1996-2003

Each following year, the Department for Agricultural Zoology of the Agricultural Faculty in Zagreb, supported by MAF, -organizes and performs the monitoring of WCR in Croatia. Together with other authors, the Department's experts are, -through special lectures, -informing on the spread in Europe at international IWGO meetings and elsewhere (ŽLOF, 1996; EDWARDS et al., 1997; IGRC BARČIĆ et al., 1997; IGRC BARČIĆ & DOBRINČIĆ, 1998; IGRC BARČIĆ et al., 1999; IGRC BARČIĆ et al., 2000; IGRC BARČIĆ et al., 2001; DOBRINČIĆ et al., 2002; IGRC BARČIĆ et al., 2003). The Croatian colleagues and public are also being informed on rootworm spread (IGRC BARČIĆ, 1996, 1997; MACELJSKI & IGRC BARČIĆ, 1998; IGRC BARČIĆ & DOBRINČIĆ, 2000; IGRC BARČIĆ, 2001; IGRC BARČIĆ et al., 2001 a; IGRC BARČIĆ et al., 2003; IGRC BARČIĆ & DOBRINČIĆ, 2003).

The number of monitoring locations for western corn rootworms ranged between 120 and 140. The spread of western corn rootworm in Croatia in the 1995-2003 period is shown in Picture 2.



Picture 2. The spread of WCR in Croatia, 1995 - 2003
 Širenje kukuruzne zlatice Hrvatskom od 1995 do 2003

In Croatia, the WCR beetles have been spreading about 40 km from east to west each year. We should point out that the capture of a single male does not mean that the population has spread in the said area. Namely, males can fly quite far, especially helped by the wind, but they cannot establish a new population. That is why males were often found much more westwards than the actual spread of the population consisting of both sexes.

Monitoring was also used to identify the pest's population density in the already infested areas. Occasionally, it would expand 2 - 6 times annually. Data on population expansion in the observation spots and within affected counties are shown in Fig. 1. Figure 3 shows catch increases on 10 permanent observation spots from 1997 - 2003. Table 1 shows data on the estimate of the first appearance, as well as the appearance of 50% of rootworm larvae, males and females (DOBRINČIĆ et al., 2003).

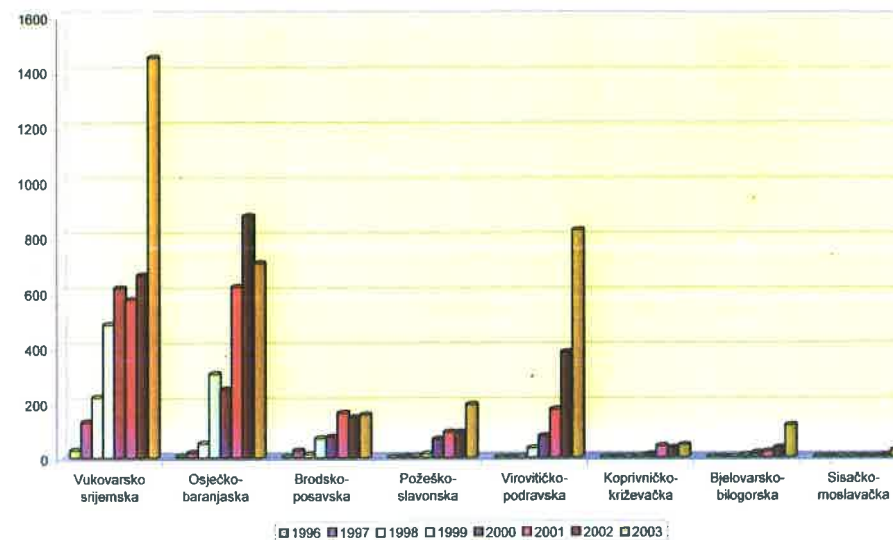
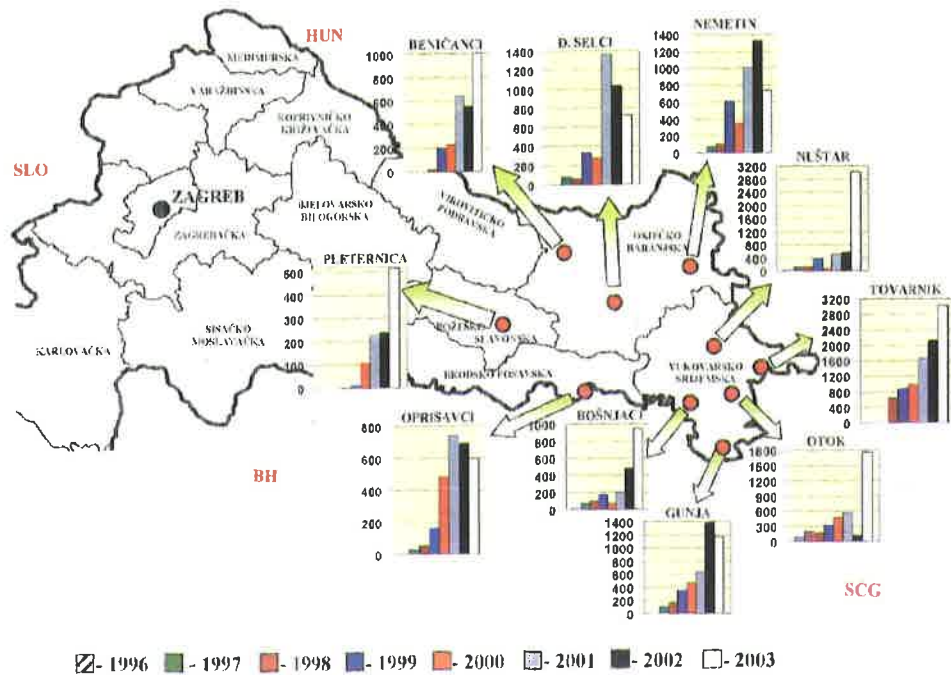


Fig. 1. Number of beetles caught per monitoring site in different counties, 1996 - 2003
 Broj uhvaćenih zlatica po opažračkom mjestu u pojedinim županijama od 1996 - 2003

Table 1. The forecast of the appearance of the different WCR stages, 1996 - 2003
 Prognoza pojave kukuruzne zlatice u periodu 1996 - 2003. god.

YEAR GODINA	FIRST APPEARANCE PRVA POJAVA			THE APPEARANCE OF 50% POJAVA 50%		
	LARVAE LIČINKE	MALES MUŽJACI	FEMALES ŽENKE	LARVAE LIČINKE	MALES MUŽJACI	FEMALES ŽENKE
1996	28.05.	02.07.	05.07.	10.06.	15.07.	17.07.
1997	09.06.	11.07.	13.07.	19.06.	22.07.	24.07.
1998	04.06.	05.07.	07.07.	12.06.	15.07.	18.07.
1999	30.05.	29.06.	02.07.	07.06.	07.07.	09.07.
2000	15.05.	15.06.	17.06.	29.05.	27.06.	29.06.
2001	26.05.	02.07.	05.07.	07.06.	12.07.	15.07.
2002	22.05.	22.06.	23.06.	01.06.,	27.06.,	29.06.
2003	17.05.	13.06.	17.06.	31.05.	24.06.	25.06.



Picture 3. Beetles catches at permanent monitoring sites, 1995 - 2003
 Ulov zlatica na stalnim opažackim mjestima, 1996 - 2003

The time of adult emergence from the soil into the emergence cages is shown in figure 2 and confirms the estimation method accuracy (DOBRINČIĆ et al., 2003).

Although it was expected, not a single case of WCR passive movement by transportation means was ever recorded in Croatia. The pest was never discovered in this way, not even near the Zagreb international airport, which was under regular surveillance. However, such cases were identified near Venice and Milan airports (Italy), the railway station in Lugano (Switzerland), and by some other Western European airports.

We thought it was possible in the very beginning of WCR appearance, in the 1995- 1997 period, that a single international project could temporarily 'slow down the spread of the pest across Croatia, as the shortest way from the infested focus area near Belgrade westwards. In exchange for some compensation, the farmers within the belt around 50 km wide to the west of the infested area should be asked not to replant corn, or use soil insecticides where they do so. Through consultations with our MAF, we came to the conclusion that the passing of a regulation on the prohibition of replanting

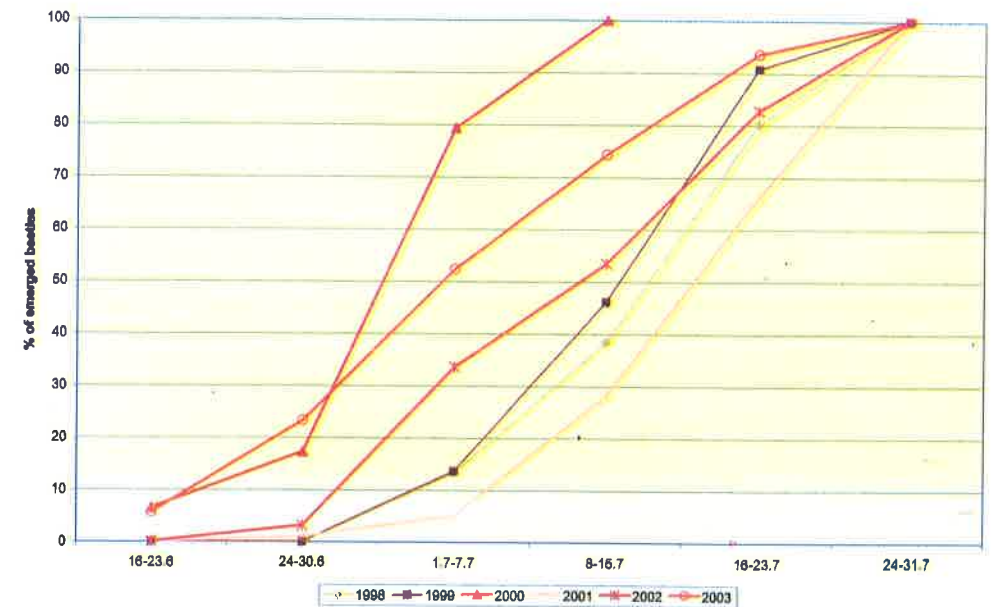


Fig.2. The dynamics of adults emergence from the soil
 Dinamika izlaska kornjaša iz tla

corn would not bear results without compensation. That is why we gave abandoned the idea. Since the Western states showed no interest in financing the project, rootworm spreading continued unhindered in their direction. It was especially difficult to accept a total lack of understanding on the part of international organizations: EPPO, FAO and EU. If EPPO had taken measures the same as in the 50's for the isolation of the fall webworm (*Hyphantria cunea* Drury), maybe the WCR would still have not been present in Italy, Switzerland, France and Belgium (MACELJSKI, 2002).

Research Associated with Monitoring

Parallely with the monitoring, investigations were conducted on the attraction of different bait types, some of which were synthesized at our request at the Pliva plant in Zagreb. Also explored was the possibility of limiting pest spread using a special "ring" system, consisting of the distribution of a large number of baits around the place they were WCR was first found.

Through contacts with the renowned American scientist R. L. METCALF, we were advised to explore the chemical compound 4-methoxycinnamaldehyde (MCA) as a possible WCR attractor. As a result of our request, the compound was synthesized two years at the Pliva plant laboratory. The baits in 1995 were placed in Bošnjaci after the discovery of the first pest specimen, but there was no catch observed (IGRC BARČIĆ & MACELJSKI, 1995). In 1996, we explored the attraction of 13 bait variants, including different MCA bait formulations which we prepared ourselves in 16 replications. Adults were caught on all baits, but the most attractive MCA formulation caught approximately the same number of adults as the yellow sticky boards. However, this was, still some twenty times less than caught with the pheromone traps (IGRC BARČIĆ, 1996a). In separate explorations that same year, 97.6% of specimens were caught on the Hungarian pheromone trap, 0.6% on cucurbitacine bait and 1.7% on yellow sticky boards. In all other tests performed on various sites in eastern Slavonia in 1996 and 1997, pheromone traps were always catching 93.8-97.2% of the rootworm beetles, much more than MCA and cucurbitacine baits or yellow sticky boards. However, we should mention that the MCA bait also catches females beetles (MACELJSKI & IGRC BARČIĆ 1997; IGRC BARČIĆ & DOBRINČIĆ, 1997; DOBRINČIĆ & IGRC BARČIĆ, 1997). In 1998, the catch using pheromones was also high: 95% as opposed to 5% of rootworm beetles caught on yellow sticky boards (DOBRINČIĆ & IGRC BARČIĆ, 1999).

During the monitoring, catches were confirming the exploration results, because in small, initial infestations pheromone attraction was some twenty times higher than that of cucurbitacine; the bait which turned out to be somewhat less attractive than yellow sticky boards. (IGRC BARČIĆ & MACELJSKI, 1997). During the 6 years of monitoring WCR, pheromone traps caught 92.3 to 97.99% of the beetles. Yellow sticky boards (Multigard or Pherocon AM type traps) caught 2.01 to 7.7% rootworms (IGRC BARČIĆ & DOBRINČIĆ, 2002 a).

It was also established that year that pheromones kept in a refrigerator for one year have nearly the same attraction for WCR male beetles as fresh pheromones (IGRC BARČIĆ & DOBRINČIĆ, 1997), so that they may be used in the monitoring the following year as well.

Within the FAO TCP 6712 (A) project, the possibility of slowing down rootworm spread was explored by placing the bait system called the "ring". Additional baits were placed around the first most protruding find spot in all four directions in order to reduce the pressure of the pests in spreading within the infested area (EDWARDS et al., 1998). There were four such rings in Croatia, and the explorations lasted two years.

Their impact on pest spread over the area where the ring was placed was not impacted (IGRC BARČIĆ & DOBRINČIĆ, 2002).

The final report of the FAO project was submitted by EDWARDS et al. (1999) at the meeting in Zagreb (The Bežanec Manor). It included the results of the common work on identifying the WCR spread in Croatia, Hungary, Romania and Bosnia and Herzegovina, revealing increase in the population number on permanent observation spots within the old focus area and great damage (up to 70%) caused by the pest in Serbia, as well as the fact that mountains and mountain ranges do not prevent its spread. It was agreed to try and carry on with the project with the support of states who would act as sponsors.

Biological and Ecological Research

After the first explorations of the possibility of western corn rootworm acclimatization in Croatia and Europe (MACELJSKI & IGRC BARČIĆ, 1994), with criteria being minimal soil temperature during winter and average annual values of temperature and precipitation, new explorations were performed (IGRC BARČIĆ & MACELJSKI, 1998). In these explorations, the following five criteria were chosen, four referring to soil conditions at the depth of 10 cm.: 1. Minimal temperature -10°C ; 2. Average temperature in April above 12.8°C ; 3. Average temperature in May and June over 17°C ; and 5. Average temperature in October below 12.8°C . The fourth criterion refers to average air temperature which in the June-August period should be over 17°C . Based on these criteria, taking into account the values for the four selected sites in Croatia (Osijek, Gradište, Bjelovar and Zagreb), it was established that all four sites meet three criteria, while two sites meet four criteria. None of the sites met the second criterion because on all of them the soil temperature was lower than the chosen criterion. It was concluded that the lack of this criterion would only delay the appearance of larvae and that it would not particularly affect the rootworm's biotic potential. The final conclusion of these investigations is that all the areas in Croatia where corn is grown are favourable also for the development and spread of WCR. In the years that followed, the pest development completely confirmed this conclusion.

In her doctor's thesis, DOBRINČIĆ (2001) confirms this conclusion through the analyses of her data. We should point out that numerous research results encompassed by the said thesis have not been published yet, so that only some of them are mentioned here. Thus the possibility of estimating the time of the first appearance of larvae and of the adult forms was established in detail. It was determined that the period when individual stages of larvae may be found in the soil lasts 17-24 days for the first

stage, 18-22 days for the second, and around 29 days for the third. Development of the larvae in the soil lasts around 50 days. It was established that temperature thresholds and thermal constants determined in the USA by Jackson and Elliot (1988) may be used also in Croatia for estimating the first appearance of males and females. Also established was the sexual index which in 1999 was slightly in the favour of males, while in 2000 it was 1.0. Duration of the ovipositional period of rootworms fed various host plants under laboratory conditions was established (21.8-35.5 days) as well as the average number of eggs laid. The largest number of eggs was laid after feeding on corn (135.6), but the eggs were also laid after feeding on soybean leaves and flowers, sunflower, and sugar-beet leaves.

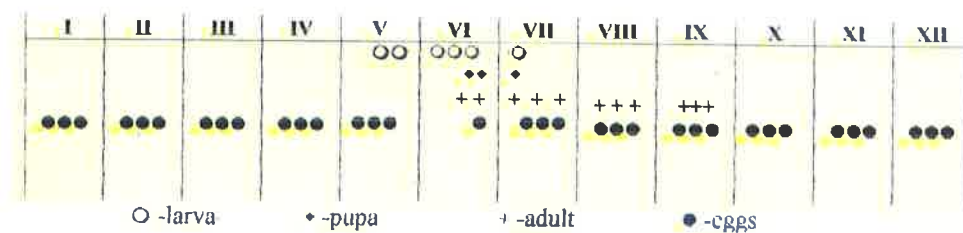
DOBRINČIĆ (2001) established that Hungarian pheromone PAL should be used for detecting infestations, while PALs, catching both sexes, is equally good. These baits caught up to 400 rootworms at the most, because they do not have enough surface to catch more. However, for damage estimations, Multigard traps work better.

Based on the total beetle catch in Croatia for 1997 and 1998, IGRC BARČIĆ & DOBRINČIĆ (2002 a) have shown the dynamics of their flight, and also that of their captures at a selected site (Tovarnik). The yearly WCR increase factor is estimated for each year separately. Increase in the number of beetles in the 1997 - 2001 period ranged from 1.7 to 3.6 times with the average of near 3 times (IGRC BARČIĆ, DOBRINČIĆ, 2002 a).

Also explored was the phenology of WCR and the results obtained were compared to the estimates published earlier. It was established that the adult forms emerge from the soil between 15 June and 14 July with the average being around 30 June. The females emerge some 3-4 days after the males. The period of their emergence from the ground lasts 27 to 36 days. Maximum flight occurs in the 2nd and 3rd decade of July and the 1st decade of August, depending on the meteorological circumstances. Adults are present in the fields until the 2nd decade of October at the latest. In the areas of the first infestation the population has increased so much since 1995 that in 2000 40 beetles/trap/day were caught, thus reaching the highest possible catch with the baits used. This was why damage was envisioned for 2001. (DOBRINČIĆ & IGRC BARČIĆ, 2001).

Phenological studies done in the course of 2000 (DOBRINČIĆ & IGRC BARČIĆ, 2001 a) were continued as well in the years that followed (DOBRINČIĆ et al., 2003), as shown in the pest development phenogram in Picture 4.

Based on several-year's of study, the graphs of adult emergence from the ground are shown, as well as dynamics of their catch per research years (DOBRINČIĆ et al., 2003). Data on the dynamics of adult catch have been supplemented by data from 2003 and the results are shown in figure 3.



Picture 4. Phenogram of the development of WCR (1998 - 2003)
Fenogram razvoja kukuruzne zlatice 1998 - 2003

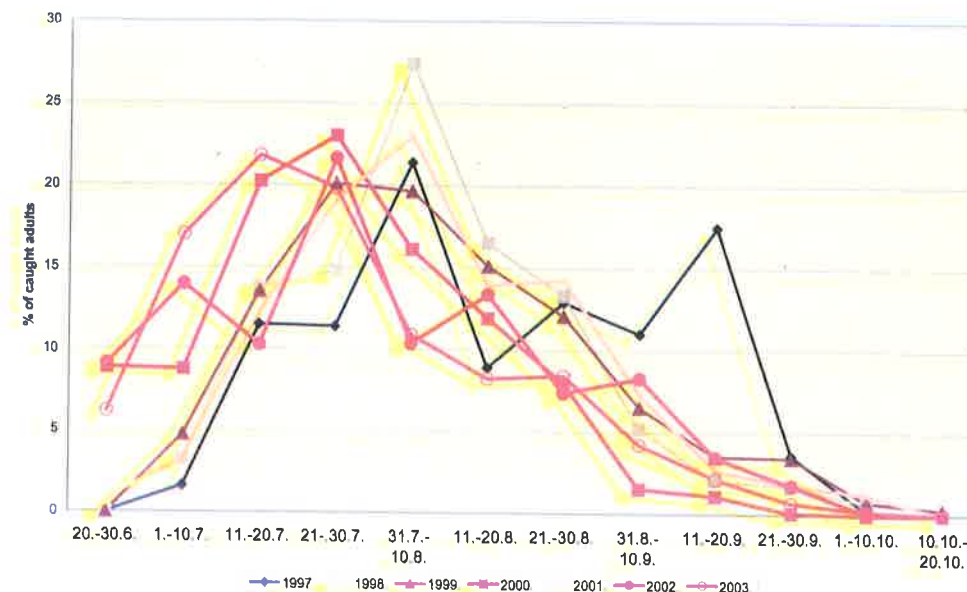


Fig. 3. The dynamics of the flight of WCR in Croatia, 1997 - 2003
Dinamika ulova kukuruzne zlatice u Hrvatskoj 1997 - 2003.

KISS et al. (2001), KISS et al. (2002) and KISS et al. (2003) show the results of investigations on the impact of crop rotation on the appearance of rootworms published in Croatia, Hungary and Serbia using the same methodology. Investigations looked at the impact of crop rotation on the appearance of WCR by constantly planting corn on a rather wide surface, while on smaller plots in 6 replications corn planting was interchanged with either repeated corn or the cultivation of stubble cereal, soybean and

sunflower. On all the surfaces there were cages monitoring the number of adults emerging from the ground, while the traps of the Pherocone AM type registered their number on the crops. In a test performed in Croatia, on the large surface of repeated corn planting, 208 rootworms were caught in cages and 801 by the baits, while on the narrow plot of repeated plantings the number was much lower (34 i.e. 369), while the catch was very low on other crops (0 or 1 in cages, i.e. 11 to 47 by baits). The studies continue and the results will be published in the future.

Exploring the Possibility of Chemical Control

Very extensive explorations of the possibility for chemical protection of corn against WCR were performed during the 1997-2003 period. These explorations were conducted solely in the easternmost parts of Croatia (Otok, Tovarnik, Baranja, etc.) where the infestation was the highest. Apart from checking the efficiency of methods and insecticides known in the USA for soil treatment during planting, as well as on the already established plants, the possibility of protection using the more cost-effective and environmentally tolerable method of seed treatment was also tested. Some of the latest means were explored and satisfactory solutions found (DOBRINČIĆ, 2001), but all results of these research are not yet published.

Identifying Damage Caused by Western corn rootworm

The first damage caused by WCR larvae, consisting in the loss of 15-20% of the yield, was observed in Croatia in 1999 in a continuous corn planting in Tovarnik next to the border with Serbia (IGRC BARČIĆ et al., 1999). There was no damage recorded on other sites that year.

In 2000, on the same location, damage was recorded in the amount of 5 to 25% with root damage based on Iowa State University (ISU) table of 3 to 3.5. The appearance of "goose necking" was recorded for some plants (IGRC BARČIĆ et al., 2000).

At the Tovarnik site, the root infestation in 2001 was the same as in 1999, and lower than in 2000. However, a heavier infestation was identified in Baranja where many plants were found lying down. In these fields, the established root damage ranged from 3.5 to 4.0 (after ISU). Increased silk damage caused by the adult forms was also identified (IGRC BARČIĆ et al., 2001 a).

In 1998, some studies were designed to explore the relationship between the number of WCR larvae and root damage. The studies were performed under conditions of

natural infestation. They were conducted in 1998 on 6 fields of continuous corn plantings in eastern Croatia, and in 1999 and in 2000 in Tovarnik. On all surfaces the number of eggs in the soil was identified: in 1998 they were not found, in 1999 there were 3 and in 2000 there were 5 eggs per sample. The number of beetles emerging from the ground per plant were, in 1999, 17, and in 2000, 48 per plant. Corn damage was determined per the ISU table. Damage done to the root was in 1998; 1.86-2.59, in 1999, 2.38-2.90 and in 2000, 2.83-5.66. The correlation coefficient between the number of beetles coming out of the ground and root damage was very high: $r^2 = 0.924$. The regression line is described with $y = 2.2 + 0.031x$. The results of the three-year study showed that damage caused by the larvae is consistent with that in the USA. (DOBRINČIĆ et al., 2001; DOBRINČIĆ et al., 2002).

In 2002, significant WCR larval damage was occasionally identified. In Baranja, on a field of 1.5 ha, the yield was reduced by 85% (DOBRINČIĆ et al., 2002). In that field, the corn was replanted once. The average was around 40 rootworms per plant. Twenty-eight percent of the plants were lying on the ground and had no ears, while 68% of the plants were bent ("goose necked") and had small ears. Even the small number of upright plants had smaller ears. Silk damage was identified on 50% of ears, and the ears often had less than 50% grains. Plant roots were considerably damaged, but due to a humid summer many regenerated, which is why the loss was not 100%. The measurements from this field, and also on others, showed that the yield of the plants showing symptoms of "goose necking" was in the humid year of 2002 10 to 30% lower than that of the unaffected plants. What was found to be important was the observation of large numbers of WCR beetles on sunflower and their feeding on the flowers. In 2002, high damage, ranging from 20 to 40% of the yield, was also recorded on some other fields in Baranja (IGRC BARČIĆ et al., 2003). The damage was brought to the attention of colleagues and the public at the 47th Plant Protection Seminar in Opatija (IGRC BARČIĆ & DOBRINČIĆ, 2003).

Education

At the very beginning of WCR appearance, the greatest problems were posed by some experts who, after the find of a single rootworm specimen in 1995, were advocating biologically insupportable attitudes of the kind "one is as good as none", while some went so far as to express their doubts in public that the rootworm was caught at all, on TV Zagreb. Some farmers refused to install the baits because they felt that they would attract the pest to their fields by doing so (MACELJSKI & IGRC BARČIĆ, 1997).

A serious problem was also the reaction of some states that were importing corn seed: out of fear of the pest being transferred via the seed, they wanted to prohibit seed imports from Croatia. However we proved that the pest cannot be transferred via the seed because the adult form of WCRs dies in October at the latest, and reappears only the next June, while the eggs are laid in the ground. Only when this was proven and confirmed by EPPO did the problems cease.

In order to let the farmers know as much about the pest as possible, several thousand posters and leaflets were printed in 1995, including colour photos of the pest and the corresponding text. Throughout the entire 1995-2003 period, information was spread through yearly meetings with agronomists, about one hundred radio shows, numerous TV shows, new posters and leaflets, articles in both professional and popular publications, lectures, immediate contacts with farmers, etc.

After the first major damage was identified in Croatia, the Institute for Agricultural Zoology introduced the "Days of Western Corn Rootworm" where agronomists and peasants were introduced to the pest and damage caused by. These Days were held in 2002 in Topolje and Tovarnik (IGRC BARČIĆ et al., 2003), and in 2003 in Kneževo.

In 2001 began the FAO project entitled "Participatory approach as a management tool for western corn rootworm (*Diabrotica virgifera virgifera* LeConte), in short - FFS, featuring, apart from Croatia, also Hungary, Bulgaria, Slovak Republic, Serbia and Bosnia and Herzegovina. Project leader in Croatia is the Department for Agricultural Zoology of the Faculty of Agriculture in Zagreb. Similar programs take place in around 40 other countries, while their purpose is to educate farmers on integrated production and protection of plants of various crops. In Croatia, work is being done individually with 10 farmers, monitoring the appearance and development of WCR on crops raised in different systems of crop rotation (DOBRINČIĆ et al., 2003).

Conclusion

Numerous studies performed, as well as numerous papers published associated with western corn rootworm, point to the fact that the experts of the Department for Agricultural Zoology of the Faculty of Agriculture in Zagreb have conducted timely research and provided useful information on the present and future significance of western corn rootworm for Croatia. Although a large number of published papers is associated with the monitoring of the pest, significant are also the papers referring to the study of its biology and ecology, the possibility of its control and damage preven-

tion. Many papers were collaborations with our foreign colleagues, showing that we are an equal part of the team of scientists studying western corn rootworm in Europe.

The authors from the Department for Agricultural Zoology of the Faculty of Agriculture in Zagreb have in the 1994-2003 period published 45 scientific and professional papers on western corn rootworm. The most important results of the studies shown in these papers are contained in the present review. However, there are many yet unpublished results, especially those contained in the first doctoral's thesis on western corn rootworm ever written in Europe by R. Dobrinčić.

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