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European corn borer and its parasites overwintering abundance and damages on different corn FAO maturity groups

Pojava kukuruznog moljca i njegovih parazita nakon prezimljenja i štete na hibridima različitih FAO grupa

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Abstract

European corn borer (ECB) is one of the most significant maize pests in the world and also in Croatia. ECB causes yield reduction from 2 to 25%, even more in years favorable for its development. According to estimations, these losses are around 7%. About 90% of the hybrids had some resistance to whorl-leaf feeding (first-generation ECB) and 75% had some resistance to sheath and sheath-collar feeding (second-generation ECB). Along with resistance, modern maize hybrids possess certain level of tolerance. The main aim of this paper was to determine moth eclosion of the overwintering generation and presence of the parasites of ECB larvae during the overwintering as well as to estimate population density of ECB on maize growing area in Croatia. Also, the aim was to establish the differences among maize FAO maturity groups in damage caused by ECB larvae. Estimated overwintering population was over 8,000 moths/ha i.e. more than 4 million larvae of first generation. During the overwintering four different parasites attacked the larvae. Two species belong to the order Hymenoptera (*Cotesia marginiventris* Cresson and *Eriborus terebrans* Gravenhorst) and two species belong to the order Diptera (*Ramonda spathulata* Fallén and *Lydella thompsoni* Herting). One caterpillar predator species *Paragymnomerus spiricornis* Spinola (Hymenopetra) overwinters in maize stalks as well. The highest attack of the first ECB generation was recorded on FAO maturity group 500. The damage from second ECB generation was the highest on FAO groups 400 and 500. The maize of the higher FAO groups has high and robust stems with large number of big leaves. That intensive vegetative growth is a biological characteristic that attracts first generation of ECB to intensifying egg lying. High population level of the first generation may lead to high level of second ECB

generation which ultimately caused yield reduced on the hybrids of longer vegetation period (medium-late FAO maturity groups).

Keywords: damages, enclosion, European corn borer, hybrids, maize, maturity groups, parasites, population density, resistance, yield

Sažetak

Kukuruzni moljac (*Ostrinia nubilalis* Hübner) jedan je od najznačajnijih štetnika kukuruza u svijetu i kod nas. Kukuruzni moljac uzrokuje sniženje prinosa od 2 do 25%, a u godinama povoljnim za njegov razvoj i više. Prema procjenama ti su gubici prosječno oko 7%. Otprilike 90% hibrida kukuruza pokazuje određen stupanj otpornosti kod ishrane moljca listovima (prva generacija kukuruznog moljca), a 75% hibrida pokazuje određenu otpornost na ishranu moljca u pazuscima listova i stapkama klipova (druga generacija kukuruznog moljca). Uz otpornost, moderni hibridi kukuruza posjeduju visoku razinu tolerancije na štete. Cilj ovoga rada bio je utvrditi prvu pojavu leptira (prezimljujuća generacija), prisutnost parazita gusjenica kukuruznog moljca te procijeniti visinu populacije kukuruznog moljca u uzgojnom području kukuruza u Hrvatskoj. Također, cilj rada bio je utvrditi razlike u oštećenjima između različitih FAO grupa uzrokovane ishranom gusjenica kukuruznog moljca. Procijenjen potencijal zaraze bio je oko 8.000 leptira prve generacije, odnosno više od 4 milijuna gusjenica prve generacije kukuruznog moljca. Tijekom prezimljenja četiri različite vrste parazita napadaju gusjenice kukuruznog moljca. Dvije vrste pripadaju redu Hymenoptera (*Cotesia marginiventris* Cresson i *Eriborus terebrans* Gravenhorst), a dvije vrste pripadaju redu Diptera (*Ramonda spathulata* Fallén i *Lydella thompsoni* Herting). Jedna vrsta predatora *Paragymnomerus spiricornis* Spinola (Hymenoptera) također prezimljuje u stabljikama kukuruza. FAO grupa 500 pokazuje veću razinu oštećenja prve generacije moljca. Oštećenja od druge generacije bila su najveća na kukuruzu FAO grupa 400 i 500. Kukuruz viših vegetacijskih grupa ima visoke i robusne stabljike s velikim brojem krupnijih listova te je upravo taj intenzivan vegetativni rast biološko svojstvo koje privlači leptire prve generacije na intenzivnije odlaganje jaja. Velika brojnost prve generacije moljca može uvjetovati i velika oštećenja na klipovima od druge generacije kukuruznog moljca što u konačnici utječe na smanjene prinose hibrida viših vegetacijskih grupa.

Ključne riječi: grupe dozrijevanja kukuruz, gustoća populacije, hibridi, kukuruzni moljac, paraziti, pojava, prinos, rezistentnost, štete

Introduction

High maize yield losses everywhere in the world are caused by the European corn borer ECB (*Ostrinia nubilalis* Hübner). According to Maceljski (2002) in Croatia it appears in all areas where maize is grown. It is a polyphagous and, alongside maize, it attacks hemp, millet, sorghum, pepper, chrysanthemums and many weeds. Recently, the damage has increased due to neglecting many farmlands and abandoning severed maize stalks. Research performed in Eastern Slavonia (Ivezić

and Raspudić, 1998) has shown that the average infestation rate between 1992 and 1996 was 64%, and earlier research (Ivezić, 1976) has estimated an average infestation rate of 37%. Raspudić et al. (2010) have also recorded that the ECB attack up to 90% of growing maize. There are many factors that influence the appearance and intensity of the ECB attack: relative air humidity (Royer and McNeil, 1991), soil water regime (Traore et al., 2000; Hunt et al., 2001; Cakir, 2004), nitrogen fertilisation (Haq and Alvi, 1982; Szulc et al., 2008).

During different growth stages the maize plant's sensitivity to ECB attacks varies, which reflects in the yield loss (Lynch et al., 1980). According to Maceljski (2002), in Croatia the ECB causes yield loss ranging from 2 up to 25%. The numbers can be even higher during favorable years for the insect's growth and development. It is estimated that yield losses are about 7% in average (Jarvis et al., 1986). Although the losses are high, control measures against the ECB are not carried out, mostly due to the fact that it never entirely destroys the crops.

Moreover, there are many difficulties in controlling this pest. In order to decrease the damage caused by ECB, it is necessary to employ various non-pesticide based control strategies including appropriate agro-technical methods that can minimize the pest attack, mechanical control and sowing tolerant varieties (Igrc Barčić, 2007). Agro-technical methods are based on crop rotation, which reduces the pest potential. Mechanical control is, for now, the most important and should be applied on the whole area where maize is grown. These imply destroying severed maize stalks where the ECB overwinters. Presumably, yield losses would be much higher if modern maize hybrids did not have some degree of resistance to ECB (Barry and Darrah, 1991). Alongside above mentioned control strategies, biological, biotechnical and chemical control could be applied as well. Biological control including release of natural enemies in Croatia is not applicable due to the high expenses. On smaller maize fields, which are not so common in Croatia, combination of attractants and insecticides could be used as protection measure. Moreover, according to Igrc Barčić (2007), chemical control, such as foliar application of pesticides or seed dressing, do not give satisfactory results when it comes to the ECB. In addition to that, foliar application of pesticides in vegetation shall be carried out by high clearance tractor, which Croatian farmers do not possess. Other possibility could be aerial application of the pesticide that is not allowed in Croatia (Igrc Barčić, 2007; Bažok, 2017).

Resilience to the ECB of the commercial maize hybrids is nowadays a common feature. About 90% of 400 maize hybrids on market have shown a certain degree of resistance in vegetative phases of development (Barry and Darrah, 1994). Alongside resistance, modern maize hybrids are tolerant to a great damage degree caused by the ECB. Tolerance is the ability of a maize plant to withstand a certain population density of the insect without economic loss of yield or quality (Guthrie and Barry, 1989, Barry and Darrah, 1994). The development of tolerant maize hybrids with a strong, robust stalk contributes immensely to reducing yield loss as a consequence of the damage caused by the ECB (Raspudić et al., 1999).

The aim of this study was to estimate: (I) the appearance, overwintering population level of the ECB, presence of parasites in maize stalks; (II) the differences among hybrids of various FAO maturity groups in terms of intensity of attack in the

vegetative maize growing stage (1st ECB generation) and on maize cob (2nd ECB generation); and (III) the differences in yield.

Materials and methods

Research site

The research took place during spring and summer of 2017 at the location Šašinovečki Lug, Zagreb County (45°51′00″ N, 16°10′01″ E), Northwestern Croatia.

Moth eclosion and population level of the overwintering ECB

Samples of severed maize stalk (maize hybrid Bc 282) were collected on an unplugged maize field. On 23th March 2017 twenty random samples were collected from 15 rows. A total number of 300 maize plants were collected. The collected plants were 100 cm tall. At the Department of Agricultural Zoology Faculty of Agriculture in Zagreb all the plants were examined for shot holes from larval feeding. Maize plants were cut into 20 cm pieces and placed into entomological cages. Entomological cages were used for the purpose of rearing ECB larvae and parasites which overwinter in maize stalks. The moth eclosion out of the stalk was monitored up to May 29th 2017. Finally the quantity and gender of the eclosed moths has been established. The eclosion of the parasites from the stalks was monitored in the same period and the parasites were collected at the end of monitoring (i.e. on May 29 2017). For the identification of the parasites the following identification keys were used: Vecht and Carpenter (1990), Goulet and Huber (1993), Tschorsnig (1997), and Scudder and Cannings (2006).

Estimating the ECB larval attack

At the same location 32 maize hybrids from four FAO maturity groups (300, 400, 500 and 600), eight hybrids per each group, have been sown using experimental four-row pneumetic seeding machine. All hybrids were sown by permuted block randomization scheme on 3rd May 2017 as a part of the AGRO-DROUGHT-ADAPT project. Each hybrid was planted in 4 rows and 4 replicates. Each row was 4 m in length, distance between rows were 75 cm, and distance between plants in a row was 20 cm, depth of the seeding was 3 cm. In total, there were 128 maize plots planted. The intensity of ECB attack was estimated on 28th June 2017 on two inner rows of every replicate. Damages of 1st ECB generation on the plants (distinctive leaf holes, shot holes on stalks) were identified and recorded as the percent of the plants attacked by ECB. In September all hybrids have been harvested. Ten maize cobs were randomly selected from each replicate for examination. The damages from 2nd ECB generation have been recorded as the percent of cobs infested by larvae. On 10th October yield for each FAO maturity group have been recorded after harvesting each plot on investigation area. Maize yield have been standardized on 14% moisture and was expresed in tones per hectare.

Data analysis

The data on the percent (%) of damaged maize plants and percent (%) of damaged cobs on hybrids, and maize yields (t/ha) were submitted to two-way variance analysis (ANOVA) in order to determine the difference in the intensity of the ECB attack (1st and 2nd ECB generation) and to establishe difference in yield among different FAO maturity groups. Due to the heterogeneity of the data in some cases, the data were transformed by using the √(x+0.5) transformation. Averages were compared by Tukey's honestly significant range test. All differences were considered statistically significant at P ¼ 0.05. Statistical evaluation of data was performed by the statistical software ARM 9® GDM software, Revision 9.2014.7 (Gylling Data Management Inc., 2015).

Results

Eclosion and population level of the overwintering ECB

After hibernation, ECB larvae developed into moths, whose eclosion from the stalk was monitored. The first moth was recorded on 1st May 2017. In total 32 ECB moths developed from overwintering larvae. Male moths were the first to emerge out of the stalk (protandry). The total number of adult males was 14 (44%), whereas the total number of female moths was 18 (56%). According to these numbers it was estimated that in one hectare of unploughed corn approximately 8,000 moths would overwinter (75,000 corn plants per hectare). This number of moths could produce more than 4 million larvae of first generation (approx.. 500 eggs per female moth; Youngman and Day, 2009).

Apart from the ECB, 13 additional insects developed from corn stalks, three of which belong to the order of Diptera, family Tachinidae (Figure 1) and ten of which belong to the order of Hymenoptera, families Vespidae, Brachonidae and Ichneumonidae (Figure 2).

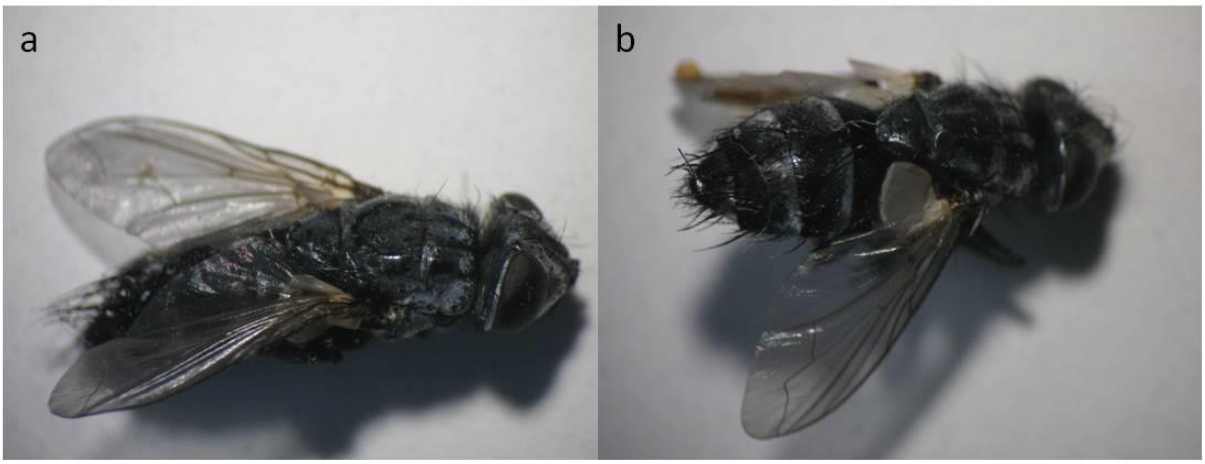
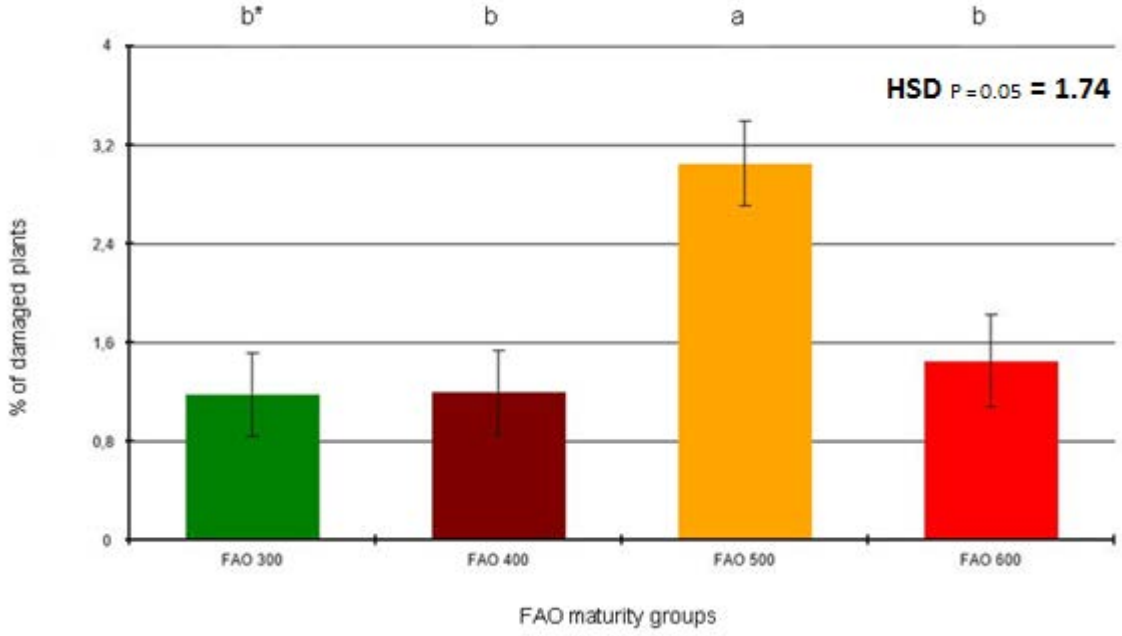


Figure 1. Diptera species developed from corn stalks: a) *Ramonda spathulata* (Fallén, 1820); b) *Lydella thompsoni* Herting, 1959

Figure 2. Hymenoptera species developed from corn stalks: a) Paragymnomerus spiricornis (Spinola, 1808); b) Cotesia marginiventris (Cresson); c) Eriborus terebrans (Gravenhorst, 1829)

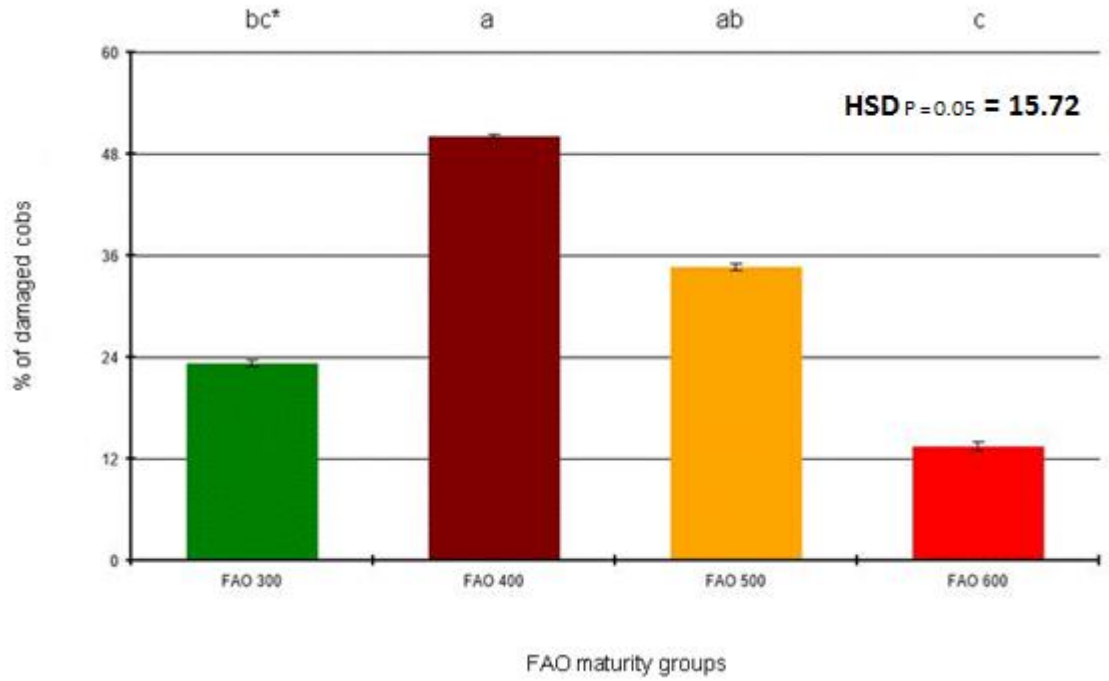
The ECB attack

The intensity of the attack of the 1st ECB generation on maize hybrids stalk from different FAO maturity groups was shown on Figure 3. The highest attack intensity was recorded on stalks of FAO 500 maize hybrids (p=0.0206), which significantly differs from other groups. Figure 4 shows the differences in cob damages between different maize FAO groups which represent 2nd ECB generation attack. The damage from second ECB generation was the highest on the FAO 400 group followed by FAO 500 group (P=0.002). Maize yield have been recorded for each FAO maturity group on investigation area, standardized on 14% moisture and expresed in tones per hectare presented on Figure 5. There was no significant difference in yield between four FAO maturity groups.



*small letters refer to differences among FAO groups

Figure 3. Differences in maize stalk damage on different FAO maturity groups



*small letters refer to differences among FAO groups

Figure 4. Differences in maize cob damage on different FAO maturity groups

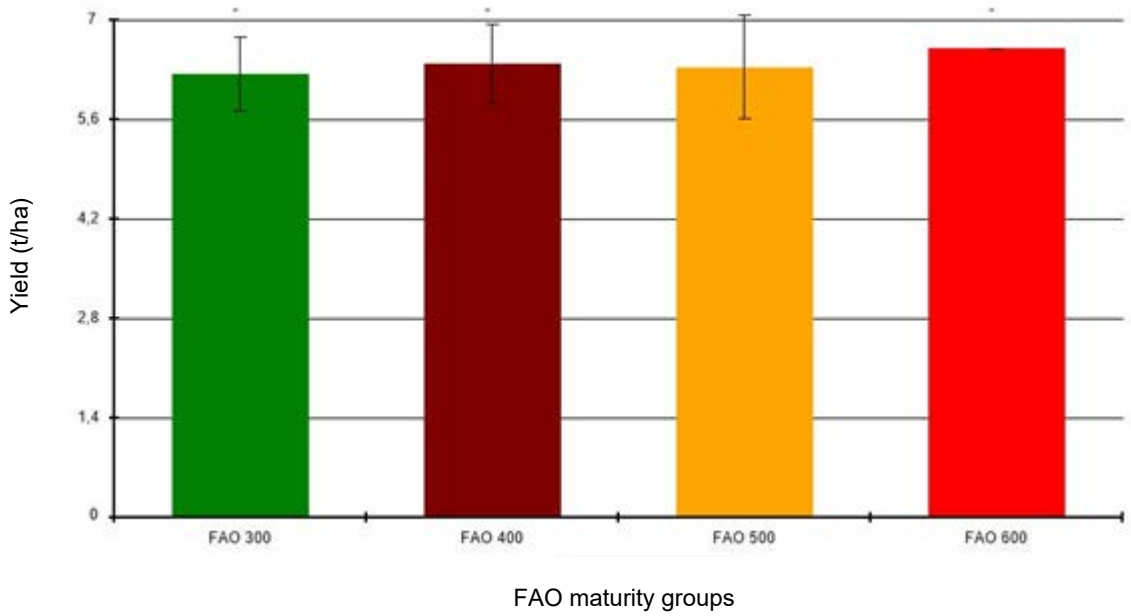


Figure 5. Maize yield on different FAO maturity groups

Discussion

The first phase of this research was monitoring the ECB eclosion from the randomly selected not ploghed maize stalks on the location Šašinovečki Lug (Croatia). The first eclosed moth was recorded on 1st May 2017 (121th day of the year), which is earlier than it was recorded in research conducted between 1988 and 1990 by Kraljević Župić (1993). According to Kraljević Župić (1993), the first moths were recorded on entomological lamps in location Sinj on the 152nd day of the year, whereas in entomological cages with severed maize stalks they appeared somewhat earlier, between the 137th and 147th day of the year. The appearance of the first ECB generation in the field depends on the temperature and relative air moisture (Maceljski, 2002). First eclosion according to Maceljski (2002) usually took place in the middle of May, although the majority of moths appear in June. Deviation in this research can be explained by the fact that the moths in cages were recorded as soon as they emerged from the cocoon, while several days must pass in order to catch the moths in a trap. Additionally, climatic condition influence the eclosion and as it has been found by Bažok et al. (2009) in a very warm year, in 2003, the maximum of ECB moth appearance on pheromones on localities close to the investigation site, was in middle May.

There were 32 moths recorded in cages with a gender ratio of 44 males: 56 females, which is in accordance with the research by Fadamiro et al. (1999) who also recorded a lower number of males compared to females. Taking into consideration the potential of a female moth to lay 500-600 eggs (Youngman and Day, 2009), it can be estimated that the infestation potential goes to 4 million larvae of first generation per hectare.

Apart from the ECB, 13 individuals of other insects have been recorded to enclosed of the severed maize stalks. Three of them belong to the order of Diptera and 10 from the order of Hymenoptera. The determination of the order Diptera by using standard identification keys (Goulet and Huber, 1993; Tschorsnig, 1997) estimated that the individuals belong to the family Tachinidae (*Ramonda spathulata* Fallén and *Lydella thompsoni* Herting, 1959). The determination of the order Hymenoptera by using standard identification keys (Vecht and Carpenter, 1990; Scudder and Cannings, 2006) showed that the individuals from this order belong to the species *Paragymnomerus spiricornis* (Spinola, 1808) (family Vespidae), *Cotesia marginiventris* (Cresson) (family Brachonidae) and *Eriborus terebrans* (Gravenhorst, 1829) (family Ichneumonidae). Both fly species (*Ramonda spathulata* (Fallen, 1820) and *Lydella thompsonil* (Herting, 1959)) and two hymenopteras (*Cotesia marginiventris* (Cresson) and *Eriborus terebrans* (Gravenhorst, 1829)) are recorded to be endoparasitoids to the ECB and are very common in Europe. *Paragymnomerus* is a palearctic genus of solitary (mason) potter wasps (Carpenter, 1986). Most solitary (mason) wasps is predators. They hunt various species of insects (predominantly caterpillars) as food for their larvae, which develop usually in hollow stalks and other crevices (Dvořák and Straka, 2007). Due to the described feeding habitat, *Paragymnomerus spiricornis* (Spinola, 1808) could be predators of ECB caterpillars as well.

The literature in Croatia is listing all parasitoid species found in this research as native and they are well known to parasitize ECB larvae. However, there is no

systematic investigation on ECB parasites in Croatia and in this sense it could be considered that this is the first scientific evidence of the parasitism of those species on ECB in Croatia.

The fly *L. thompsoni* (Herting, 1959) is one of 24 species of parasitoids imported to the USA from Europe in period from 1920 to 1938 (Mahr, 1999). Species of the order Hymenoptera, *C. marginiventris* (Cresson) and *E. terebrans* (Gravenhorst, 1829), are parasitoids of the ECB as well. The ichneumonid wasp *E. terebrans* (Gravenhorst, 1829) and the fly *L. thompsoni* (Herting, 1959) were imported to the USA as a project of biological control of the ECB. About 140.000 wasps and flies were collected in Europe and Asia (where they are native) and released in 13 American states from Vermont to Virginia and as far west as Indiana and Michigan in period from 1927 to 1940 (Shelton, 2017). Species *L. thompsoni* (Herting, 1959) and *E. terebrans* (Gravenhorst, 1829) have established, increased their population and became a wide-spread and effective biological agent in USA. It is an interesting fact that *L. thompsoni* (Herting, 1959) has also spread to area in which it has never been released (Winnie and Chiang, 1982; Landis and Haas, 1992; Ma et al., 1992). Although none of these species are not listed as a significant contributor to the ECB regulation, in the USA the recorded parasitation of the second generation of the ECB is up to 75% and is considered to be the main contributor to the ECB population control (Winnie and Chiang, 1982; Landis and Haas, 1992; Ma et al., 1992; Mahr, 1999). The parasitoids' development, identified in this research, is in synchrony with the European corn borer's development. In this research finding ten parasites on 300 stalks means that 0.033 parasites are present in one stalk, i.e. around 2,500 parasites/ ha.

In the second phase of this research, differences in the intensity of attack of the first generation of the ECB on the 32 maize hybrids belonging to four FAO groups as well as attack of second ECB generation on maize cob were estimated. After analysis for damage intensity, the hybrids of FAO 500 maturity group showed a significantly higher damage level caused by the ECB (Figure 3). The damage from second ECB generation was the highest on FAO maturity groups 400 and 500 (Figure 4). It can be assumed that high population level of the first generation also leads to high level of second ECB generation which ultimately caused reduced yields of the hybrids of longer vegetation period (medium-late FAO maturity groups). Maize hybrids with longer vegetation (medium-late FAO maturity groups) have tall and robust stalks with a large number of thick leaves. It can be assumed that in this research this intensive vegetative development is the biological characteristic which has attracted more first generation moths (overwintering generation) to lay more eggs.

Results of investigations related to differences in FAO groups in the term of ECB attack carried out in Croatia are contradictory one from each other and do not show neither the importance of the hybrids nor tolerance to the ECB.

Ivezić et al. (1997) have recorded attack rates of the ECB according to FAO maturity groups as follow: 34.25% of damage for FAO 100; 45.83% of damage for FAO 200; 56.25% of damage for FAO 300; 66.01% of damage for FAO 400; 65.14% of damage for FAO 500; 71.76% of damage for FAO 600 and 55.57% of damage for FAO 700. Augustinović et al. (2005) have recorded the lowest intensity of ECB attack on FAO 600 on the Križevci site, on maize from FAO 400 group the lowest damage rate was

recorded on locations of the Agricultural Institute Osijek and on locations “Belje” PIK Karanac. The authors (Augustinović et al., 2005) have recorded the highest intensity of ECB attack on the maize from the FAO 400 group at the location Križevci site, for FAO 300 group at the Agricultural Institute Osijek, and for FAO 600 group at the location “Belje” PIK Karanac. Raspudić et al. (2010) have studied the differences in damage caused by the ECB on FAO 400 to FAO 600 maturity groups. That studie has not shown statistically relevant differences. In this research maize yield recorded for four FAO maturity group have not sown significant differences (Figure 5) as well. Previous research conducted on yield in maize hybrids of different FAO maturity groups shown that the significantly highest yield should be expected for the FAO 500 and 600 maturity groups (Hegyi et al., 2007; Vulchinkov et al., 2013; Đurović et al., 2014). It can be assumed that significantly higher ECB attacks (both generations) on medium-late FAO maturity groups have resulted in yield reduction and the yield was lower than expected for these FAO groups and did not differ from the yield of early to medium FAO maturity groups.

The European corn borer is a permanent pest in Croatian maize fields. There is a need to determine the damage caused by the ECB in order to evaluate tolerance of the maize hybrids available on the Croatian market. In order to give a precise evaluation of differences between maize hybrids in terms of intensity of attack, expanded research of the ECB potential to cause the damage is needed as well as artificial infestation for the precise evaluation of yield reduction.

Conclusions

The first moth generation enclosed earlier (May 1) than suggested in earlier research. Female moths are dominant in the population after overwintering. The estimated infestation potential was 8,000 first generation moths thus estimate more than 4 million first generation larvae.

Apart from the ECB, many parasitoids overwinter in severed maize stalks. Species *Lydella thompsoni* (Herting, 1959) (Diptera) and *Eriborus terebrans* (Gravenhorst, 1829) (Hymenoptera) are identified as an important factor to the ECB abundance. In spite the general knowledge on their hosts and presence in the whole Europe (including Croatia) this is the first evidence that they are attacking ECB in Croatia. The parasitoids’ development is in synchrony with the moth’s development.

The FAO group 500 shown higher damage caused by the first ECB generation. The damage cause by the second ECB generation was the highest on FAO groups 400 and 500.

Higher population level of the first ECB generation may lead to higher level of second ECB generation which ultimately caused reduced yields of the hybrids of longer vegetation period (medium-late FAO maturity groups).

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