Rhagoletis completa (Diptera; Tephritidae) distribution, flight dynamics and influence on walnut kernel quality in the continental Croatia

Rhagoletis completa (Diptera; Tephritidae) - rasprostranjenost, dinamika leta i utjecaj na kakvoću jezgre oraha u kontinentalnoj Hrvatskoj

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Rhagoletis completa (DIPTERA; TEPHRITIDAE) DISTRIBUTION, FLIGHT DYNAMICS AND INFLUENCE ON WALNUT KERNEL QUALITY IN THE CONTINENTAL CROATIA

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SUMMARY

Walnut husk fly (WHF), Rhagoletis completa Cresson 1929 is an invasive species spreading quickly and damaging walnuts in Croatia and neighbouring countries. We researched distribution of this pest in the continental part of Croatia, flight dynamics in Međimurje County and its influence on quality of walnut kernels. CSALOMON® PALz traps were used for monitoring the spread and flight dynamics of R. completa. Weight and the protein content of kernels and the presence of mycotoxin contamination were measured. Walnut husk fly was found in six counties (Istria County: pest reconfirmation, Zagreb County, The City of Zagreb, Varaždin County, Međimurje County and Koprivnica-Križevci County). The presence of the fly was not confirmed on one site in Koprivnica-Križevci County (locality Ferdinandovac) and in the eastern part of Croatia (Vukovar-Srijem County: Vinkovci locality). The flight dynamics showed rapid increase in number of adults only a year after the introduction into new area. The weight of infested kernels was 5.81% lower compared to not infested. Protein content was 14.04% in infested kernels and 17.31% in not infested kernels. There was no difference in mycotoxins levels. Additional researches on mycotoxin levels in stored nuts, ovipositional preferences of walnut husk fly and protection measures against this pest are suggested.

Key-words: walnut husk fly, damages, infested kernels, mycotoxin contamination, nutritional value, spread

INTRODUCTION

Walnut husk fly (WHF) (*Rhagoletis completa* (Diptera; Tephritidae)) is an alien phytophagous species of Diptera in Europe originating from North America (Skuhravá et al., 2010). Prinicipal host plants are *Juglans* spp. (EPPO, 2011) but in laboratory assays it was recorded that females can lay eggs on apples (*Malus spp*.), nectarine (*Prunus persica*), tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum annuum* L.) (Kasana and Aliniazee, 1995).

Occurrence of WHF was first reported in Europe in southern Switzerland (Merz, 1991) and northeastern Italy (Duso, 1991). Since then this pest has spread to other European countries and has been recorded in Slovenia (Seljak and Žežlina, 1999), Germany (EPPO, 2004), Croatia (Budinščak et al., 2005), Austria (Lethmayer, 2008), Hungary (Tuba et al., 2012), France and Albania (Skuhravá et al., 2010).

WHF has only one generation per year with adults flying from July until September (EPPO, 2011). Larvae feeding in the husk tissues, caused shell staining, darkened and moldy kernels, husks failing to split properly

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and kernel weight loss. High infestations cause premature nut fall and separation of husk and shell difficult adding to the harvesting costs (Hamersky, 1996; Duso and Dal Lago, 2006). Damage caused by insects attacking husks can expose susceptible tissues to colonization by toxigenic fungi with subsequent mycotoxin formation (Boutrif, 1998). Fungi can grow on walnuts under favourable conditions of temperature and humidity (e. g. *Aspergillus flavus* Johann Heinrich Friedrich 1809 and *Aspergillus parasiticus* Speare 1912) which are the major aflatoxin-producing fungi) (Boutrif, 1998). It can therefore be assumed that the damages caused by WHF could increase the concentration of aflatoxins in infested walnuts.

In Croatia, walnut (*Juglans regia* L.) is produced on an area of 3 178 ha and the largest production area is in the continental part of the country in three Croatian counties (The City of Zagreb: 528 ha, Osijek-Baranja County: 467 ha and Koprivnica-Križevci County: 301 ha) (PAAFRD, 2013).

WHF was first recorded in Croatia in 2004 in Istria County and Primorje-Gorski Kotar County (western part of Croatia) (Budinščak et al., 2005). In the last few years increased damages on walnuts have been observed and reported from various parts of the country which has prompted the detailed research of spread and distribution of this pest in the continental part of Croatia where the majority of walnut producers are situated. The lack of data on population dynamics of WHF, influences on nutritional value of nuts and presence of mycotoxin contamination (Duso, 1991; Duso and Dal Lago, 2006; Skuhravá et al., 2010) has further stimulated our research as these data could be valuable when assessing the impact of spread of this invasive species as well as impact on walnut production.

Aims of this research were:

a) distribution of WHF in continental part of Croatia; b) flight dynamics of WHF; c) comparison of the quality of infested and not infested walnut fruits by measuring the weight and the nutritional value of nuts (proteins) and the presence of mycotoxin contamination (aflatoxins).

MATERIAL AND METHODS

Distribution and flight dynamics of WHF in Croatia

From the beginning of July until the end of September 2013, CSALOMON® PALz traps (Plant Protection Institute, MTA ATK, Budapest, Hungary) were used for defining the distribution of WHF in the continental part of Croatia.

These traps attract primarily by fluorescent yellow coloring of its sticky surface, however, catches are significantly increased by the addition of the synthetic food attractant (ammonium salt). The trap catches both males and females of target species. The traps were used to confirm the presence or absence of pest in new locations so they were set up at the beginning of August (when the population peak is expected) in seven Croatian counties (locality Poreč will be used as a reference location) at a total of 12 sites (11 unsprayed solitary walnut trees, and 1 commercial walnut orchard Prelog) (as shown in Table 1). The traps were hung on 2 m of height, on *J. regia*.

Table 1. Monitoring localities of walnut husk fly (*Rhagoletis completa*) with counties and coordinates in Croatia in 2013

Tablica 1. Mjesta istraživanja orahove muhe (Rhagoletis completa) s prikazom županija i navedenim koordinatama u Hrvatskoj 2013.

Locality *Lokalitet*	County *Županija*	Coordinates *Koordinate*
1-Poreč (reference location)	Istria County	45° 15′ 57″ N 13° 36′ 7″ E
2-Gonjeva	Zagreb County	45° 43′ 60″ N 15° 41′ 40″ E
3-Jastrebarsko		45° 40′ 5″ N 15° 38′ 29″ E
4-Tupčina		45° 42′ 36″ N 15° 25′ 57″ E
5-Zagreb	The City of Zagreb	45° 49′ 13″ N 15° 59′ 21″ E
6-Lepoglava	Varaždin County	46° 12′ 39″ N 16° 3′ 4″ E
7-Nedelišće	Međimurje County	46° 23′ 0″ N 16° 23′ 0″ E
8-Prelog		46° 20′ 0″ N 16° 37′ 0″ E
9-Ferdinandovac	Koprivnica-Križevci County	46° 3′ 8″ N 17° 11′ 41″ E
10-Kozarevac		45° 54′ 46″ N 17° 6′ 23″ E
11-Velika Mučna		46° 7′ 44″ N 16° 43′ 12″ E
12-Vinkovci	Vukovar-Srijem County	45° 6′ 15″ N 19° 2′ 33″ E

The same traps were used to determine the flight dynamics of WHF in Međimurje County. The monitoring of the flight dynamic started at the beginning of July (traps were placed on 5th July) and lasted till the end of September during the years 2011, 2012 and 2013 on the same locality Šenkovec (46° 25′ 0″ N 16° 25′ 0″ E). The traps were hanged each year on the same two solitary, unsprayed walnut trees (*J. regia*) (one trap per tree) and replaced every six weeks.

Walnut fruits analyses

The quality of walnut fruits was evaluated on 60 infested and 60 not infested nuts with WHF, collected in commercial walnut orchard Prelog by measuring the weight and protein content of kernels and the presence of mycotoxin contamination (aflatoxins). Analysis of proteins and aflatoxins was performed in kernel samples equal in weight (60 g) in the referral laboratory for food control. International Standard for Food and Feed Products (ISO: 1871:2009) was used for protein analysis, while the analysis of aflatoxins was performed using laboratory methodology OK-M/13, Issue 2/2012.

RESULTS AND DISCUSSION

Distribution of WHF in Croatia

WHF was found in six out of seven counties in which the traps were operated. The pest was reconfirmed in Istarska county (locality 1-Poreč), where WHF was first recorded in Croatia in 2004. The pest has successfully expanded its range from the far western part of the country (Istria County and Primorje-Gorski Kotar County) into the northwestern part of the continental Croatia and Hungarian border (Figure 1).

It can be assumed that the pest has spread naturally since 2004 but also new separate introductions from other countries, e.g. Slovenia where the pest is also present (Seljak and Žežlina, 1999) cannot be excluded. Adult flight and the transport of infected fruit are the major means of movement and dispersal to previously uninfected areas (EPPO, 2011). Duso and Del Lago (2006) suggest that the colonization of Slovenia and Croatia by WHF from North-eastern Italy has been faster because of the presence of unsprayed walnuts in agroecosystems.

The WHF has been first recorded in Međimurje County in 2011 and in neighbouring Hungary in 2012 (Voigt et al., 2012) so it can be assumed that the pest has spread (probably naturally) from Croatia to Hungary.

The results show first records of this pest in Zagreb County (locality 2-Gonjeva, 3-Jastrebarsko and 4-Tupčina), The City of Zagreb (locality 5-Zagreb), Varaždin County (locality 6-Lepoglava), Međimurje County (locality 7-Nedelišće and 8-Prelog) and Koprivnica-Križevci County (locality 10-Kozarevac and 11-Velika Mučna). The presence of pests was neither confirmed on one site in Koprivničko-križevačka County (locality 9-Ferdinandovac) and in the eastern part of Croatia in Vukovar-Srijem County (locality 12-Vinkovci) (Figure 1) nor in the southern part of Hungary (County Baranya) (Voigt and Tóth, 2013), which does not exclude very high probability that the pest will reach these uninfected regions in the following years.

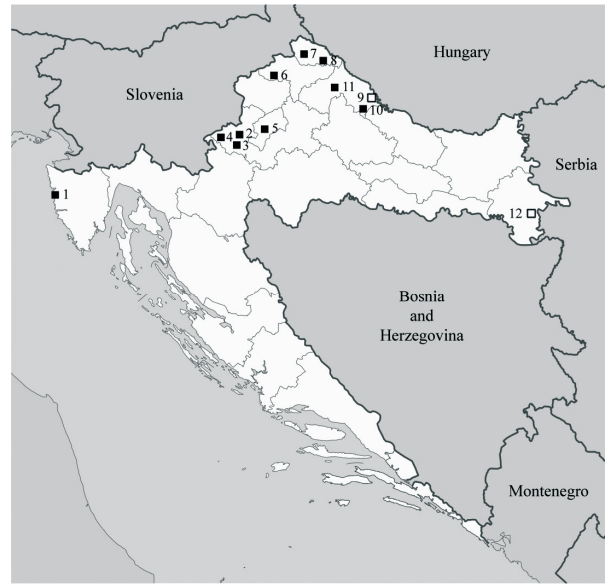

Figure 1. Monitoring localities of walnut husk fly (*Rhagoletis completa*) in Croatia [1-Poreč, 2-Gonjeva, 3-Jastrebarsko, 4-Tupčina, 5-Zagreb; 6-Lepoglava, 7-Nedelišće, 8-Prelog, 9-Ferdinandovac (pest not confirmed), 10-Kozarevac, 11-Velika Mučna and 12-Vinkovci (pest not confirmed)]

Slika 1. Mjesta istraživanja orahove muhe (Rhagoletis completa) u Hrvatskoj [1-Poreč, 2-Gonjeva, 3-Jastrebarsko, 4-Tupčina, 5-Zagreb, 6-Lepoglava, 7-Nedelišće, 8-Prelog, 9-Ferdinandovac (štetnik nije potvrđen), 10-Kozarevac, 11-Velika Mučna i 12-Vinkovci (štetnik nije potvrđen)]

Flight dynamics of WHF

The flight dynamics with number of specimens caught in 2011, 2012 and 2013 is shown in Table 2. It has to be pointed out that the adults trapped on 1st August 2011 are first records in Međimurje County. Total numbers of trapped adults show rapid increase from the first record in 2011 (24 adults) to subsequent years (947 adults in 2012 and 670 adults in 2013) (as shown in Table 2). In 2011 flight occurred on 1st August and lasted till 23rd September, with a peak on 5th September. In the next year (2012) it occurred on 25th July and lasted till 19th September, with a peak on 7th August. In 2013 flight appeared on 11th July and lasted till 25th September, with a peak on 27th August.

Differences in dates of first catches and flight peaks can be attributed to different weather conditions during the emergence and adult flight. The distribution of flight activities (peaks in August and September) were slightly different to similar researches with flight activity ceasing in September (Miklavc et al., 2009).

Damages and negative influences on nut production in Croatia can be expected in following years having in mind that this is an invasive pest which has few natural enemies in its new habitat (Laznik and Trdan, 2013) and that one member of the family *Tephritidae* (*Ceratitis capitata* Wiedemann, 1824) has already become an economic pest in Europe (Skuhravá et al., 2010).

Table 2. Flight dynamics of walnut husk fly (*Rhagoletis completa*) at location Šenkovec in Međimuje County in 2011, 2012 and 2013

Tablica 2. Dinamika leta orahove muhe (Rhagoletis completa) na lokalitetu Šenkovec u Međimurskoj županiji 2011., 2012. i 2013.

Date *Datum*	Number of caught specimens (2011) *Broj ulovljenih primjeraka (2011.)*	Number of caught specimens (2012) *Broj ulovljenih primjeraka (2012.)*	Number of caught specimens (2013) *Broj ulovljenih primjeraka (2013.)*
11th July			1
18th July			3
25th July		69	2
31th July		128	
1st August	3		19
7th August		214	
8th August	5		30
13th August		133	
16th August		185	85
27th August			191
30th August		211	
3rd September			152
5th September	11		
7th September		6	
11th September			167
18th September			18
19th September		1	
23th September	5		
25th September			2
Σ	24	947	670

Quality of walnut fruits

Total weight of 60 infested kernels amounted to 146 g (average value = 2.43 g per kernel), while the total weight of 60 not infested kernels amounted to 155 g (average value = 2.58 g per kernel), thereby this value was 5.81% lower in infested kernels compared to not infested kernels. Analysis of the nutritional value of walnuts estimated by the measurement of protein content amounted to 14.04% in infested kernels while this value amounted 17.31% in not infested kernels (as shown in Table 3).

Duso and Del Lago (2006) report significantly reduced weight of kernels in infected nuts and no references on reduced protein content could be found. Our preliminary research has shown reduced weight and protein content in infested nuts but we strongly suggest additional research with larger samples. Considering the fact that walnuts are valuable source of proteins, fats, and dietary fibers (Banel and Hu, 2009) the reduction in protein content could seriously influence their nutritional value.

Table 3. Protein values in infested and not infested kernels (60 g)

Tablica 3. Proteinske vrijednosti u napadnutim i nenapadnutim jezgrama oraha (60 g)

Proteins (N x 6.25) *Proteini (N x 6,25)* (%)	Infested kernels *Napadnute jezgre oraha*	Not infested kernels *Nenapadnute jezgre oraha*
	14.04	17.31

Analysis of mycotoxin contamination showed that in both samples (infested and not infested) the values of aflatoxins are in compliance with the limit of quantification (LOQ) (as shown in Table 4). Although the shell provides a physical barrier, it is not entirely homogeneous and can be penetrated at the suture and at the stem end, where the structure is less dense, so the fungal spores may enter it (Mahoney et al., 2000). We suggest further research of mycotoxins as their levels can be higher in stored nuts (Molyneux et al., 2007).

Table 4. Amounts of mycotoxins in infested and not infested kernels

Tablica 4. Količine mikotoksina u napadnutim i nenapadnutim jezgrama oraha

Mycotoxin (μg/kg) *Mikotoksin (μg/kg)*	Infested kernels *Napadnute jezgre oraha*	Not infested kernels *Nenapadnute jezgre oraha*
aflatoxin B1	<0.9*	<0.9*
aflatoxin B2	<0.3*	<0.3*
aflatoxin G1	<1.0*	<1.0*
aflatoxin G2	<0.7*	<0.7*

*limit of quantification (LOQ); **granica kvantifikacije (GK)*

CONCLUSION

Lots of factors have positively influenced the spread of WHF in Croatia. After the initial introduction the population density grows rapidly (as shown in Table 2) increasing damages on walnuts. Regarding the rapid increase in population density and negative influence on walnut production, protective measures against WHF should be considered in Croatia. Međimurje County is an important apple producing region in Croatia so we strongly suggest additional field research on ovipositional preferences of WHF and damages on other hosts especially on economically important species (apples, nectarines) in Croatia.

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Rhagoletis completa (Diptera; Tephritidae) - RASPROSTRANJENOST, DINAMIKA LETA I UTJECAJ NA KAKVOĆU JEZGRE ORAHA U KONTINENTALNOJ HRVATSKOJ

SAŽETAK

Orahova muha, Rhagoletis completa Cresson 1929, invazivna je vrsta koja se brzo širi i oštećuje plodove oraha u Hrvatskoj i susjednim zemljama. U radu je istraživana rasprostranjenost štetnika u kontinentalnome dijelu Hrvatske, dinamika leta u Međimurskoj županiji te njegov utjecaj na kakvoću jezgre oraha. Za praćenje širenja i dinamike leta R. completa korištene su lovke CSALOMON® PALz. Mjereni su težina, sadržaj proteina i količina mikotoksina u uzorcima jezgre oraha. Orahova muha utvrđena je u šest županija (Istarskoj: štetnik ponovno potvrđen, Zagrebačkoj, Gradu Zagrebu, Varaždinskoj, Međimurskoj i Koprivničko-križevačkoj). Prisutnost štetnika nije utvrđena na jednom lokalitetu u Koprivničko-križevačkoj županiji (Ferdinandovac) i u istočnim dijelovima Hrvatske (Vukovarsko-srijemska županija: lokalitet Vinkovci). Praćenjem dinamike leta utvrđeno je brzo širenje i povećanje broja odraslih muha samo godinu dana od prvog utvrđivanja. Težina napadnutih jezgri oraha bila je za 5,81% niža u usporedbi s nenapadnutim jezgrama oraha. Sadržaj proteina iznosio je 14,04% u napadnutim i 17,31% u nenapadnutim jezgrama oraha. U uzorcima nije bilo razlika u razini mikotoksina. Preporučuju se dodatna istraživanja razine mikotoksina u uskladištenim plodovima oraha, preferencije odlaganja jaja ženki i mjera zaštite.

Ključne riječi: orahova muha, štete, napadnute jezgre oraha, količina mikotoksina, hranjiva vrijednost, širenje

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