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Slow establishment of *Rhagoletis cingulata* in Croatia

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Abstract

Following the first finding of *Rhagoletis cingulata* (Loew) (Diptera Tephritidae) in Croatia in 2005, a surveys conducted in 2008, 2009 and 2013 in fifteen counties in Croatia confirmed the presence of pest in four counties. Under this survey program, the pest was found at two locations in 2008, one locations in 2009 and fourteen locations in 2013. During the three surveys, very low number of adults of *R. cingulata* were caught on Rebell® amarillo traps during the period from the last week of July until first week of September. Fruits infested with larvae of *R. cingulata* were found only during 2013 in two samples of *Prunus cerasus* L. (sour cherry). The results of the surveys during three years on Croatian territory showed slow establishment of *R. cingulata* in central Dalmatia; the fly is present at very low levels and no economic damage was recorded. The status of *R. cingulata* in Croatia is thus, present and locally established at low population levels in central Dalmatia.

Key words: *Rhagoletis cingulata*, low establishment, detection survey, central Dalmatia, *Rhagoletis cerasi*.

Introduction

The eastern cherry fruit fly, *Rhagoletis cingulata* (Loew) (Diptera Tephritidae) is native to eastern North America from southern Ontario to northern Florida and west to Iowa and Mississippi (Bush, 1966, CABI, 2016). It is also present in Mexico with restricted distribution (EFSA, 2014). In its native range in North America, *R. cingulata* primarly infest the fruits of *Prunus serotina* Ehrhart (black cherry), rarely *Prunus pennsylvanica* L. (fire cherry) and *Prunus virginiana* L. (chokecherry), and also infest fruits of non-native *Prunus avium* L. (sweet cherry) and *Prunus cerasus* L. (sour cherry) trees (Teixeira *et al.*, 2007; 2009).

After its first detection in Europe in 1983 until today, it is reported that *R. cingulata* has been introduced and spread into many European countries (figure 1). It was first detected in Switzerland, in Ticino, in 1983 (Merz, 1991) and further research reported that the fly is widely established in southern Switzerland (Boller and Mani, 1994; EPPO, 2010a). In Germany, the fly was detected in 1993 and further research reported high abundance in some areas and wide distribution in almost all important cherry-growing regions of Germany (Lampe *et al*., 2005; Vogt, 2007; Vogt *et al.*, 2008). *R. cingulata* was detected in the Netherlands in 2001 and by 2003, the fly was widespread in the natural environment along the coastal areas and in the central part of the Netherlands (EPPO, 2004). *R. cingulata* was detected in Belgium in 2004 in the Brussels and Wallonia regions (Baugnée, 2006; EPPO, 2010b), but the fly has not become established (EPPO, 2014a). The fly was detected in Hungary in 2006 in Fejér county (Szeoke, 2006), in eastern Slovenia in 2007 (EPPO, 2007; Bjeliš and Seljak, 2008), in Austria in 2007 near Vienna and Steiermark (Egatner *et al*., 2010), in Poland in 2009 near Skierniniewice (EFSA, 2014), in France in 2010 in Alpes-Côte d'Azur and Aquitaine provinces and during 2012 in Languedoc-Roussillon province (EPPO, 2013). The fly was detected in the Czech Republic in 2014 in the South Bohemian and South Moravian regions (EPPO, 2014b). Major cultivated host plants of *R. cingulata* in Europe are *P. avium* and *P. cerasus*, while the most important wild and ornamental host plant is *P. serotina* (black cherry). Minor hosts are also reported to be *Prunus mahaleb* L. (Mahaleb cherry) and *P. virginiana* (EFSA, 2014).

R. cingulata was detected for the first time in Croatia in 2005 in the city of Split in central Dalmatia when two adult flies were captured in McPhail traps baited with dry ammonium bicarbonate plugs placed in a mixed fruit orchard and during 2006 the fly was detected again at the same location (Bjeliš, 2007). Fly identification based on molecular methods was confirmed by Agroscope, Wädenswill in Switzerland (Bjeliš, 2008). Potentialy, the main hosts in Croatia are *P. cerasus*, *P. avium* and *P. serotina*.

Figure 1. Years of first detection and current distribution of *R. cingulata* in Europe.

After its first discovery in 2005, surveys that were conducted during 2008, 2009 and 2013 have aimed to produce the following data: what is the current distribution of *R. cingulata* in Croatia; whether the *R. cingulata* has become established and inflicts economic damage.

Materials and methods

Detection and monitoring sites

The surveys were implemented in traditional growing areas of *P. cerasus* and *P. avium* trees. In 2008, the survey was conducted in 12 counties at 32 managed orchard sites (6 sweet cherry, 11 sour cherry and 15 mixed orchards of sweet and sour cherry trees); in 2009, the survey was conducted in 13 counties at 34 managed orchard sites (of which 7 sour cherry, 2 sweet cherry and 25 mixed orchards of sweet and sour cherry trees); and during 2013, the survey was conducted in 8 counties at 46 sites with only sour cherry trees of which 8 were neglected orchards and 38 managed orchards.

Traps and trapping period

Yellow sticky traps of type Rebell® amarillo (Andermatt Biocontrol AG, Switzerland) were used together with ammonium bicarbonate or ammonium acetate dispensers. Traps were suspended on south-facing parts of canopies in trees bearing fruits during the first week of June until late September. Traps were visually inspected weekly and replaced every 4 weeks and ammonium dispensers were refilled. During 2008, 93 traps were placed, 102 during 2009 and 138 during 2013. During these surveys, the numbers of *Rhagoletis cerasi* L. (Diptera Tephritidae) specimens caught on the traps were not recorded because the objective of the survey was only the detection of *R. cingulata.* During visual inspections, *R. cerasi* specimens were removed from the traps and the presence of other species at the observation site was only noticed. Adult specimens that were suspected to be *R. cingulata* were separated from the sticky boards using the medical petroleum ether (Gram-Mol d.o.o., Croatia) and the glue that was on the body of the specimens were melted by immersing individuals in dichloromethane (Gram-Mol d.o.o., Croatia) turning them on all sides until the specimens were completely clean.

Fruit sampling

In all three survey years, fruit sampling was carried out when *P. cerasus* fruits were fully ripe. Collection of samples in south Croatia were done during the first half of July and in northern and north-eastern Croatia in the second half of July. During sampling, fruits showing symptoms of infestation were selectively sampled. The sample size varied between sites, but one sample consisted normally of 150 to 200 randomly-picked fruits from several trees in the orchard. Samples (n = 112) were transferred to the laboratory and placed on wire mesh that was fixed above the trays with water. After completing development, fully developed larvae left the fruit and fell into the water. The larvae were collected, killed over water vapour and transferred to a tube with 70% ethanol for further processing and identification.

Identification

Identifications of collected adult flies and larvae were made using a binocular Olympus SZX7 microscope. Identification of adults was carried out on the basis of morphological characteristics using an identification key for *R. cingulata* adults (White and Elson-Harris, 1992). Larvae identification was carried out using a key for third instar larvae based on morphological characteristics of anterior spiracles and number of tubules (White and Elson-Harris, 1992).

Results and discussion

Following the first detection of *R. cingulata* in Croatia in 2005, its spread to new uninfected areas was expected, especially taking into account the reports from other invaded countries in Europe. During 2008, 2009 and 2013, surveys were conducted in 15 counties (figure 2). The current distribution of *R. cingulata* in Croatia is limited to the coastal area of the central Dalmatia region, where the fly was detected in 3 counties and in the northern Croatia region, where the fly was detected in only one county (figure 3). During 2008 and 2009, the fly was detected only at 3 sites, but results from 2013, when the fly was detected at 14 sites, shows that the fly has been spreading to the new uninfected areas (figure 4). Besides *R. cingulata* has spread along the coast and the neighbouring island of Brač of Split-Dalmatia county, and also to the Šibenik-Knin and Dubrovnik-Neretva counties in central Dalmatia region. It was detected for the first time at 3 sites in Križevci-Koprivnica county in the northern Croatia region (figure 3).

The number of *R. cingulata* specimens captured by traps in Croatia was very low in 2008 (n = 2), 2009 (n = 1) and 2013 (n = 16), which is also contrary to expectations due to reports by other European countries where trap captures range from a few hundred in Slovenia to several thousand captured adults specimens of *R. cingulata* in the Netherlands (EPPO, 2004; 2007). Sampling of infested fruits of *P. cerasus* and larval collection and identification confirms the dominant presence of *R. cerasi* over the *R. cingulata* (figure 5). *R. cerasi* is present in Croatia in all areas where sour and sweet cherries are growing (Bjeliš, 2005) and during the surveys, the presence of *R. cerasi* adults and larvae was regularly confirmed (figure 2 and 3). During 2013, *R. cingulata* larvae were only found in 2 fruit samples of *P. cerasus* at the same location in a neglected small orchard in Tugare village (N43°28'26.0832" E16°38'5.7192", 245 m asl) in Split-Dalmatia county, 30 km inland from the site of the first findings in 2005.

Although only a small number of individuals were captured (n = 19), the pest was found in 34% of all survey sites during 2013 (figure 4). The results of trap inspections (figure 6) have confirmed the presence of adults of *R. cingulata* since the last week of July to the first week of September (figure 7), while the flight period of *R. cingulata* in Germany lasts from mid-June till mid-August (Lampe *et al*., 2005, Wogt *et al*., 2008), and in its native area in the USA from June to September (data from Michigan), depending on the different farm-

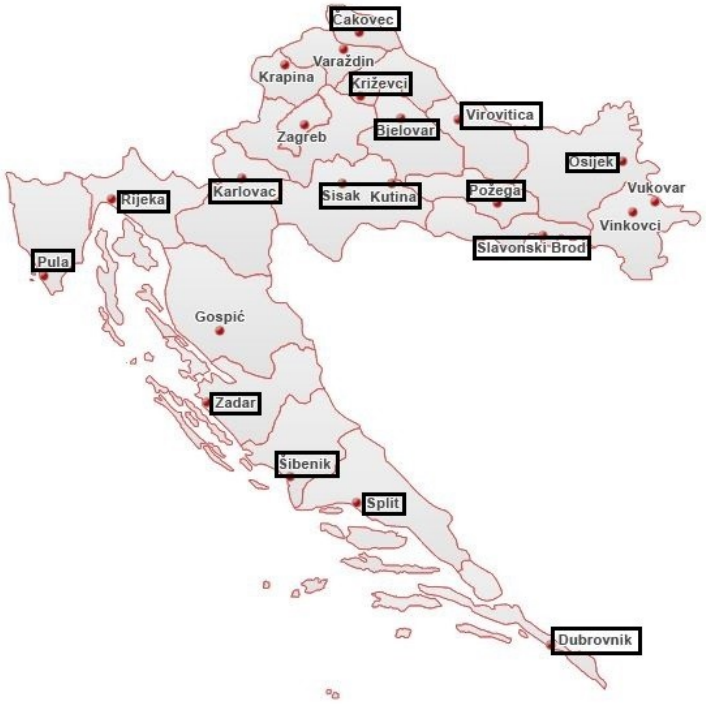

Figure 2. Counties of Croatia. Detection surveys for *R. cingulata* were conducted in 2008, 2009 and 2013 in counties inside rectangle only.

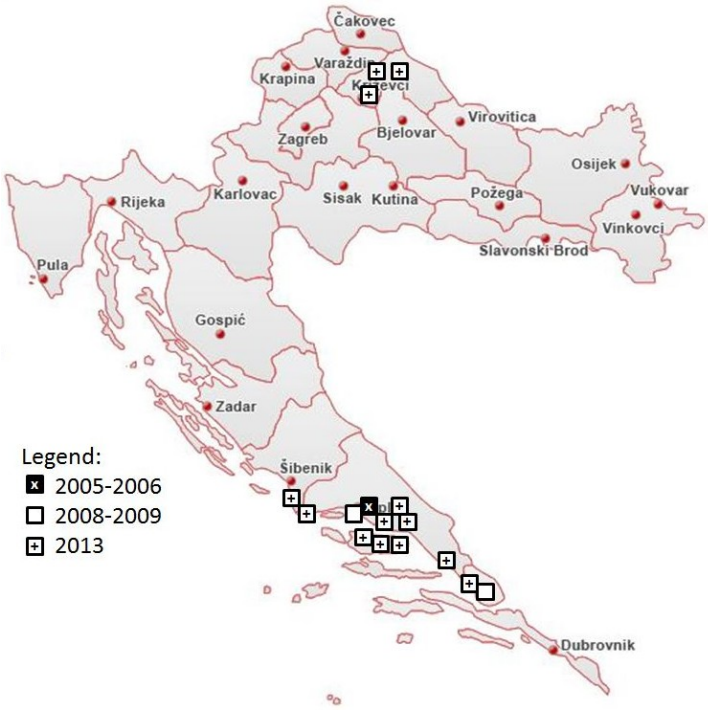

Figure 3. Locations of historical detections and outbreaks of *R. cingulata* in Croatia from the first finding in 2005 to 2013.

ing systems (Teixeira *et al.*, 2007). Therefore, in relation to the latitude, the flight period of *R. cingulata* adult in central Dalmatia can be described as late and extended. Due to the fact that the during 3 years of survey program in Croatia only 19 adult flies were captured, it could be assumed that during the period since its introduction *R. cingulata* has been adjusting to the different environmental conditions. Results from the central Dalmatia region in 2013 show that *R. cingulata* was spreading to other uninfested areas with a very low population. Nevertheless, it is not to be expected that *R. cingulata* will increase to a large established popula-

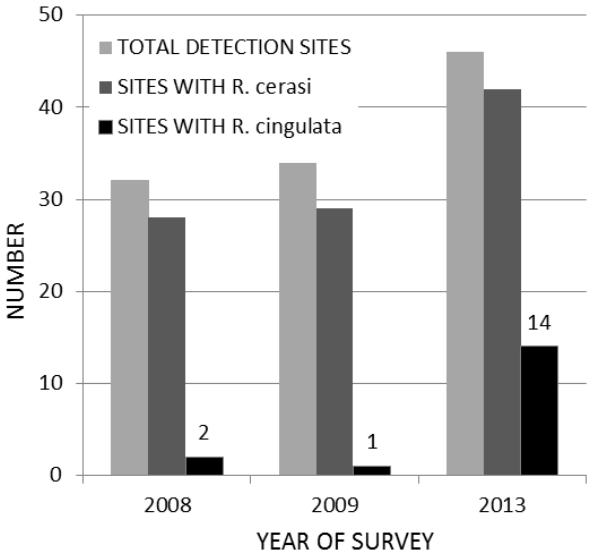

Figure 4. Number of monitoring sites with *R. cingulata* and *R. cerasi* adult fly detections during surveys in 2008, 2009 and 2013.

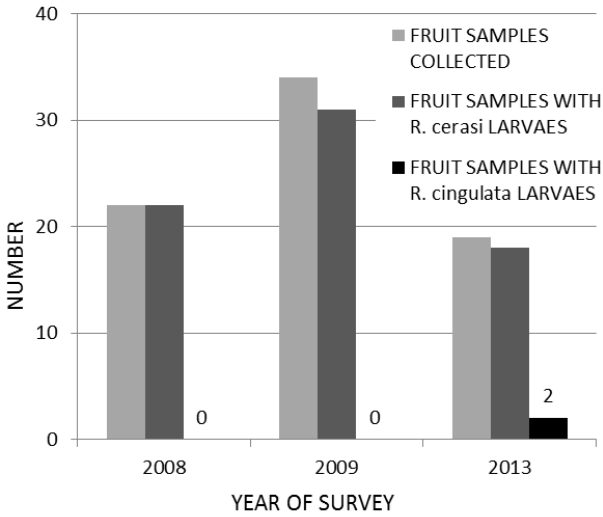

Figure 5. Samples of *P. cerasus* fruits with *R. cingulata* and *R. cerasi* larvae detections during surveys in 2008, 2009 and 2013.

tions that will cause significant economic damage in the near future. However, the unexpectedly low fly abundance that was found throughout three years of surveys in Croatia and the finding of larvae in only two fruit samples only in 2013, suggest a slow spread and adaptation, possible due to the unfavourable climatic conditions in the central Dalmatia region.

Even though the fly is also found in the northern USA, which is more temperate than Croatia, with very cold winters and warm and humid summers, the native area of *R. cingulata* distribution in the USA (Bush, 1966) is dominantly classified as a humid subtropical climate (Cfa) and zone humid continental climate (Dsb) as from the Köppen-Geiger climate classification. The area of

Figure 6. *R. cingulata* and *R. cerasi* flies caught on REBELL® amarillo trap (see difference in the blackish bands on the distal margin of the wing, *R. cingulata* circle, *R. cerasi* rectangle).

central Dalmatia region where the *R. cingulata* was first noticed, as well as all of the recent detections, fall into a warm and dry Mediterranean climate (Csa) (Peel *et al.*, 2007) that could significantly affect the biological cycle, mainly the overwintering, because of high and adverse temperature during winter diapause, and possibly the behaviour and reproduction of adults during spring and summer due to the prevailing dry and hot conditions. The area of recent detection during 2013 of *R. cingulata* in Križevci-Koprivnica county in northern Croatia belongs to a much more favourable humid subtropical climate zone (Cfa), which is the same climatic zone as the one in the native area of *R. cingulata*. Comparing the distribution of the fly in Križevci-Koprivnica county to the same climate and geographically relatively close area in Slovenia, where the pest has become established (Seljak and Bjeliš, 2008; Bjeliš *et al*., 2008), one can expect a higher level of establishment and economic damage in this county and its surrounding area in the near future due to the much more favourable climate conditions for *R. cingulata* than in central Dalmatia (Bjeliš *et al*., 2014).

Conclusions

Current *R. cingulata* distribution data collected in Croatia since its first detection until today indicate that, despite the fact that the pest was introduced long ago into areas of southern Croatia, it is dominated by the particular local environmental conditions, which appear to

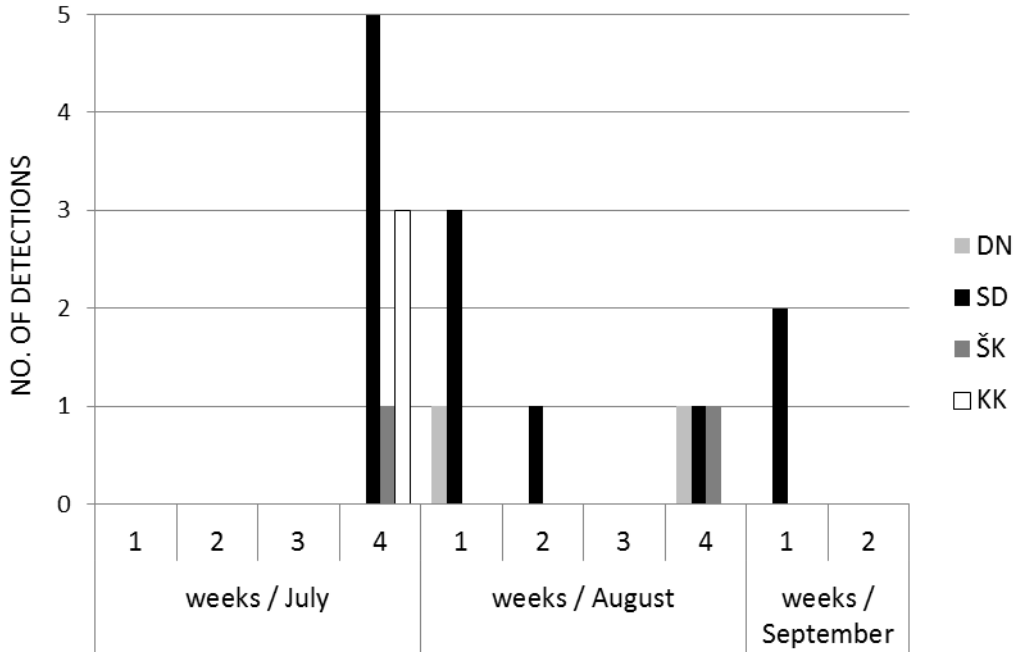

Figure 7. Cumulative seasonal captures of *R. cingulata* on REBELL® amarillo traps in Croatia during surveys in 2008, 2009, and 2013. DN: Dubrovnik-Neretva county; SD: Split-Dalmacija county; ŠK: Šibenik-Knin county; KK: Križevci-Koprivnica county.

adversely affect *R. cingulata* population, but do not affect its further spread along the coast and to climatically less marginal regions. The current status of the *R. cingulata* can be categorised as present with few occurrences in the northern Croatia region and established locally based on breeding populations in the central Dalmatia region in the south of Croatia.

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