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Rapid Communication

First report of the American eastern cherry fruit fly *Rhagoletis cingulata* (Loew) (Diptera: Tephritidae) in Lithuania

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Abstract

In the last decade, the American cherry fruit *Rhagoletis cingulata* has become an important pest of cherry crops in many temperate regions of Europe. Native to North America, this fruit fly species was introduced in Europe in the 1980s. It was first described in Switzerland in 1983, and since then populations have been detected in the Netherlands, Hungary, and Germany. The risk this widespread invasive fly poses to native cherry crops is considerable. Here we show, using molecular markers, that *R. cingulata* is present in Lithuania, and that this region is the most northerly eastern region where populations of this invasive fly are found to date. Moreover, it is also shown that the native cherry fruit fly *R. cerasi* and the invasive fly share at least one parasitoid species in the region, which could contribute to population suppression of the invasive pest. This work shows that this invasive fruit fly is expanding farther east, and systematic monitoring in the western part of the East European Plain should be performed to prevent damage by this pest species.

Key words: invasive species, parasitoid, pest species, *Prunus serotina*

Introduction

The eastern cherry fruit fly, *Rhagoletis cingulata* (Loew, 1862) (Diptera: Tephritidae) is an agricultural pest of cherries native to eastern North America (Bush 1966, 1969), that is also found in some restricted regions of northern Mexico (Rull et al. 2011; Smith et al. 2013; Doellman et al. 2019, 2020; Bruzzese et al. 2021). In its native range, the primary host of *R. cingulata* is the black cherry *Prunus serotina* (Ehrhart), but it also attacks *P. avium* L. (sweet cherry), and *P. cerasus* L. (sour cherry), and has been very rarely reported on *P. virginiana* L. (chokecherry) and *P. pensylvanica* L. (fire cherry) (Teixeira et al. 2007, 2009; Tadeo et al. 2015; Saint Jean et al. 2018).

Rhagoletis cingulata was first detected in Europe in the mountainous region of Ticino (Switzerland) in 1983, but continuous monitoring between 1991 and 1993 showed that it had not been established in the region (Merz

1991; Boller and Mani 1994). In the 1990s, *R. cingulata* was detected in the Rhineland region of Germany where it established, especially on late fruiting cherry varieties (Lampe et al. 2005; Vogt et al. 2010; EPPO 2002, 2004a, 2006, 2010a).

Since its first detection in 1983 to date, *R. cingulata* has spread in Switzerland and it has been introduced in many European countries. It is becoming an important quarantine pest in temperate regions and is registered on the EPPO A2 list as a severe pest of cherries. Fly populations have been reported in Italy (EPPO 2017), Switzerland (EPPO 2010c), Austria (EPPO 2010e), Czechia (EPPO 2014b), Slovenia (EPPO 2007), Croatia (Bjeliš et al. 2016), and Montenegro (Radonjić et al. 2019), but are not considered established in these countries. In Hungary (Szeoke 2006), France (EPPO 2010b, 2011, 2013), Germany (EPPO 2002, 2004a, 2006, 2010a), Belgium (Baugnée 2006; EPPO 2010d, 2014a), and the Netherlands (Smit and Dijkstra 2008; EPPO 2004b), the species is present and appears to have established breeding populations since their introduction. Interestingly, no official report has been made of the presence of *R. cingulata* in Poland, but given the size of the country, the frequent cherry commerce through the region, the proximity to infested countries, and the widespread distribution of *P. serotina* (Bijak et al. 2014), it can be assumed that *R. cingulata* is present (Augustinos et al. 2019).

In Europe, *R. cingulata* now has an overlapping ecological niche with the native European cherry fruit fly, *R. cerasi* L. The phenology of *R. cingulata* is similar to *R. cerasi*. It has an univoltine life cycle, and females oviposit into ripening fruits where larvae feed for three to four weeks before pupating and falling to the ground to overwinter. The main difference between the two species is the timing of their peak flight activity, with *R. cingulata* active about two weeks later than *R. cerasi* (Vogt et al. 2010). The wing patterns is also a major distinction between the two species. In *R. cerasi*, the anterior apical band or costal band is extended to vein M, whereas in *R. cingulata* it does not extend to vein M. In addition, *R. cingulata* has a posterior crossband on its wings which is absent from *R. cerasi* (Figure 1D; see Carroll et al. 2002 for more diagnostic characters between the two species). Up to twelve different parasitoid species are known to attack *R. cerasi* (Hoffmeister 1990; 1992), with *Psyttalia rhagoleticola* (Sachtleben, 1934) and *Utetes magnus* (Fischer, 1958), both Hymenoptera in the family Braconidae, also parasitizing *R. cingulata* (Schuler et al. 2016).

Research studies on fruit flies of the genus *Rhagoletis* have been carried out in Lithuania in the last decades focusing on *Rhagoletis alternata* and its control measures (Miseliūnienė et al. 1979; Bartninkaitė et al. 1981; Ryliškienė 1981). Recently, *Rhagoletis batava* has also reached Lithuania (Stalažs 2014) from the east and gained attention as the most important pest of sea buckthorn (Aleknavičius and Būda 2019). *Rhagoletis cerasi* and *Rhagoletis meigenii* are also present in Lithuania, but mainly known from general research

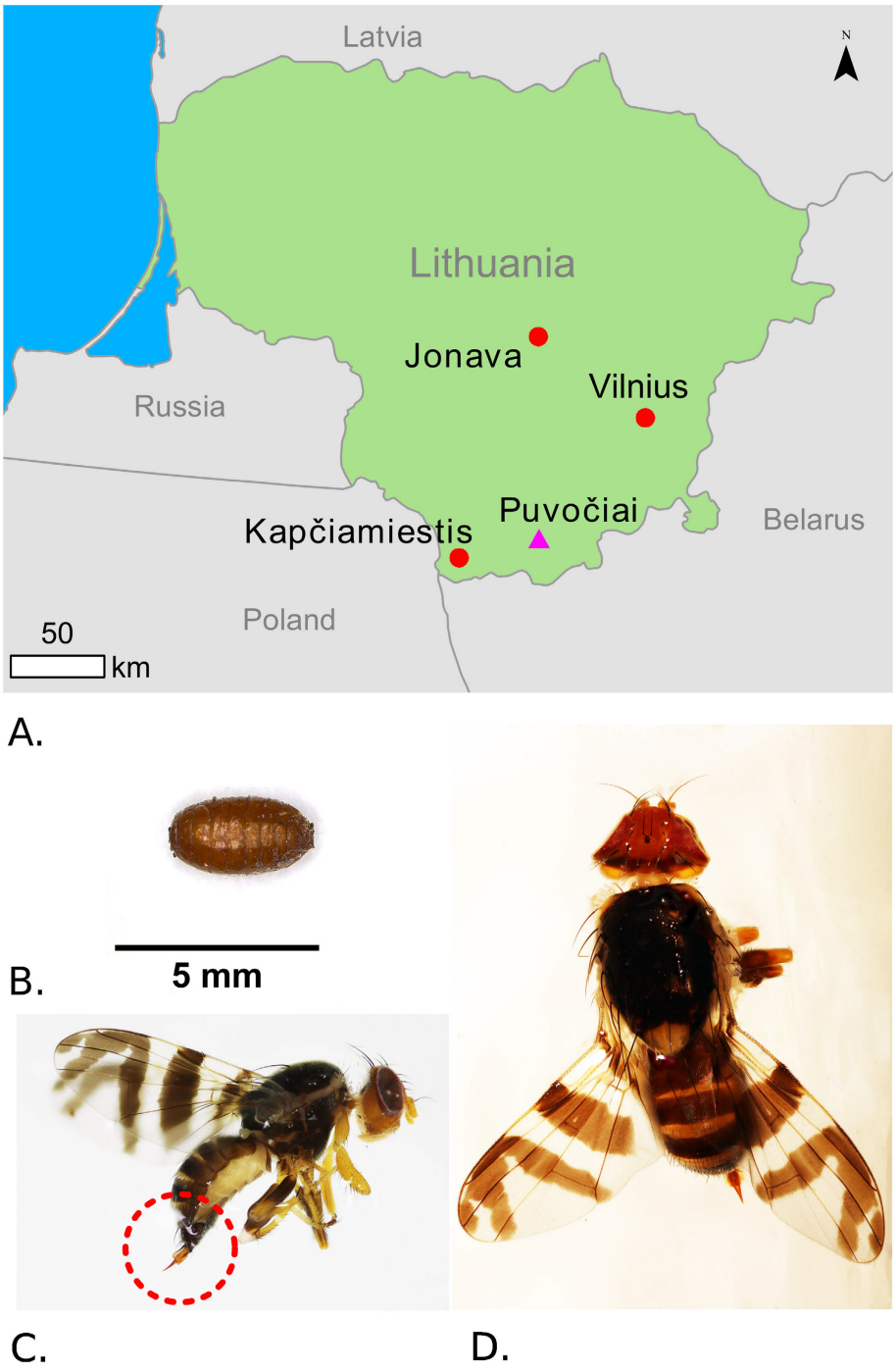

Figure 1. A. Map of the localities where *Rhagoletis* was sampled. In red dots are localities where *P. serotina* was sampled and fly pupae were found, but not genotyped. Lilac triangle is Puvočiai where *Rhagoletis cingulata* was confirmed with molecular screening. B. Pupae collected in 2019. C. Photograph of *Rhagoletis cingulata* with the characteristic wing pattern and the ovipositor, highlighted in the red circle. D. This picture shows *R. cingulata* wing patterns and the ovopositor. Photo by Sigitas Juzėnas.

on Diptera diversity or faunal lists (Pakalniškis et al. 2006; Lutovinovas 2014). Because of the scarcity of research on *Rhagoletis* in Lithuania, the presence of *R. cingulata* in that country may have been overlooked. The focus of the current study is to report the first detection and positive identification of *R. cingulata* in Lithuania, where it is now confirmed as the most northerly eastern infested region in Europe.

Table 1. Table showing the sampled localities, their coordinates, the number of collected pupae, and the collection year.

Site	Latitude	Longitude	Number of pupae	Year
Vilnius municipality	54.687019	25.194399	9	2021
Jonava, Jonava district	55.084324	24.313235	37	2021
Kapčiamiestis, Lazdijai district	54.017399	23.658334	46	2021
Puvočiai, Varėna district	54.110315	24.301387	2	2019
Puvočiai, Varėna district	54.110315	24.301387	25	2020
Puvočiai, Varėna district	54.113202	24.308103	5	2021

Materials and methods

Prunus serotina fruits were first collected in 2019 in Puvočiai village in Marcinkonių eldership, Varėna district municipality, Alytus County, southeastern Lithuania (WGS84: 54.110315; 24.301387 (~ 100 m)). Fly larvae emerged from the fruits and pupated; they were then stored in 100% Ethanol at −20 °C until preparation for DNA extraction. An additional sampling took place in 2020 in Puvočiai, and 2021 in Puvočiai, Vilnius (WGS84: 54.687019; 25.194399 (~ 100 m)), Jonava (WGS84: 55.084324; 24.313235 (~ 100 m)), and Kapčiamiestis (WGS84: 54.017399; 23.658334 (~ 100 m), Figure 1A, Table 1), as well as nine other sites across Lithuania where no pupae were found (data not shown).

DNA extractions of seven pupae were performed with Gentra Puregene Kit (QIAGEN, Venlo, Netherlands) following manufacturer's instructions, and a PCR run was done with standard barcoding primers LCO1490 and HCO2189, for amplification of the mitochondrial cytochrome *c* oxidase subunit I gene COI (Folmer et al. 1994).

Amplification reactions contained a total volume of 20 µl including 1x Buffer (DreamTaq, Thermofisher Scientific, Waltham, Massachusetts), 0.4 µM dNTPs (VWR, Radnor, Pennsylvania), 0.6 µlM of each primer, 0.05 U/µl DreamTaq Polymerase (Thermofisher Scientific, Waltham, Massachusetts) and 2.0 µl template DNA. The PCR conditions used were: an initial denaturation step at 95.0 °C for 3 min, followed by 35 cycles of denaturation at 95.0 °C, for 30 sec, an annealing step at 50.0 °C, for 45 sec, and extension at 72.0 °C, for 75 sec, followed by a final elongation step at 72.0 °C, for 10 min. The PCR products of the COI amplified region were sequenced by Eurofins Genomics Ebersberg, Germany.

The sequences were blasted against NCBI and the highest best hits are reported in Supplementary material Table S1.

Results and discussion

The first suspected *Rhagoletis cingulata* pupae were detected on *P. serotina* in Puvočiai in 2019, which prompted a DNA sequencing-based confirmation for the 2020 collection (Table 1, Table S1). The 25 pupae collected in 2020 showed a dark brown colour characteristic of *R. cingulata* pupae (Figure 1B). This contrasts with the general lighter yellowish coloured *R. cerasi* pupae (Papanastasiou and Papadopoulos 2014) found in the region on various

host cherry trees cultivars, but not on *P. serotina*. The COI sequencing revealed that of the seven sequenced samples, six were *R. cingulata* with the highest hits matching the American samples from the COI sequence SNP panel (Frey et al. 2013). We also found one sequence identified as *Psyttalia rhagoleticola*, which were recently classified as *P. carinia* (Sachtleben, 1934) (Wu et al. 2016), which attacks *R. cerasi* (Table S1; Figure S1; Belokobylskij et al. 2003; Schuler et al. 2016). This suggests that the parasitoid is already attacking *R. cingulata* in Lithuania, possibly on an incidental basis.

During the summer of 2021, a third extensive sampling of *P. serotina* was performed in Puvočiai and twelve other sites in Lithuania (Figure 1A, Table 1). Out of these twelve sites, three were infested with *Rhagoletis*, and in Puvočiai, *R. cingulata* pupae were collected in 2019, 2020 and 2021 which confirms that the population at this locality has been established at least since 2019. In addition to the pupae, several adult flies were also identified by characteristic *R. cingulata* wing patterns (Carroll et al. 2002; Figure 1C, D). Adult specimens are stored at the Museum of Zoology of Vilnius university. We did not confirm with barcoding pupae collected samples from the other three sites (Vilnius, Jonava, Kapčiamiestis) but due to the typical dark coloration of the pupal case it is very likely these are also *R. cingulata*.

In summary, this report identifies the first *R. cingulata* populations established at least since 2019 in Lithuania, currently the most northerly eastern site in Europe. So far, most monitoring in previous studies reported the invasive flies on various European native *Prunus* cherry cultivars. In this study, *R. cingulata* is collected on its native host *P. serotina*. This suggests that Lithuania might be at the forefront of the invasion route. Continuous monitoring in the region and active alerting strategies with neighbouring countries, such as Latvia to the north and Belarus to the east, are needed to prevent the ongoing spread of *R. cingulata*.

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Authors' contribution

Conceptualization: HS, TW; sample collection: SJ, AP; data analysis and interpretation: SH, TW, HS, CS; writing – original draft: TW, SH; writing – review and editing: HS, CS, AP, SJ, TW.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Sequence alignments for the partial COI amplicon for the samples in our study.

Table S1. Table showing the sequence information including sequence length, and percent identity with the species for the accessions found on NCBI.