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Old residents and new arrivals of *Rhagoletis* species in Europe

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Abstract

The genus *Rhagoletis* (Diptera: Tephritidae) comprises more than 65 species distributed throughout Europe, Asia and America, including many species of high economic importance. Currently, there are three *Rhagoletis* species that infest fruits and nuts in Europe. The European cherry fruit fly, *Rhagoletis cerasi* (may have invaded Europe a long time ago from the Caucasian area of West Asia), and two invasive species (recently introduced from North America): the eastern American cherry fruit fly, *R. cingulata*, and the walnut husk fly, *R. completa*. The presence of different *Rhagoletis* species may enhance population dynamics and establish an unpredictable economic risk for several fruit and nut crops in Europe. Despite their excessive economic importance, little is known on population dynamics, genetics and symbiotic associations for making sound pest control decisions in terms of species-specific, environmental friendly pest control methods. To this end, the current paper (a) summarizes recently accumulated genetic and population data for the European *Rhagoletis* species and their association with the endosymbiont *Wolbachia pipientis*, and (b) explores the possibility of using the current knowledge for implementing the innovative biological control methods of sterile insect technique and incompatible insect technique.

Keywords: Fruit flies, Tephritidae, microsatellites, polytene maps, *Wolbachia pipientis*

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Introduction

The genus *Rhagoletis* (Diptera: Tephritidae) consists of at least 65 known species, most of Nearctic and Neotropic and few of Palearctic origin (Bush, 1966; Berlocher & Bush, 1982; Smith & Bush, 1997, 2000). It currently exhibits a wide geographic distribution from New World to Eurasia (White & Elson-Harris, 1992). Most *Rhagoletis* species are considered as major agricultural pests, despite the fact that they have a

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narrower host plant range than tropical tephritids, such as those of the *Anastepha*, *Bactrocera*, *Ceratitis*, *Zeugodacus* and *Dacus* genera (Boller & Prokopy, 1976). Three species of phytophagous *Rhagoletis* are of utmost importance in Europe: the native European cherry fruit fly, *Rhagoletis cerasi* (L.), and two invasive species, the American eastern cherry fruit fly, *R. cingulata* (Loew), and the walnut husk fly, *R. completa* (Cresson), which have been recently introduced from North America and established, mainly, in central Europe. A fourth species, the north American walnut fly, *R. suavis* (Loew), was found for the first time in Germany (2013) in a private garden in Kleinmachnow (Brandenburg). To date, its presence has been confirmed in only two areas (Brandenburg and Berlin), and thus it is considered of minor importance for Germany (EPPO, 2016). Earlier reports of the western American cherry fruit fly, *R. indifferens* (Rhagin), in Europe are now attributed to misidentification of *R. cingulata* samples. Misidentification was driven mainly by the similarities in larval stages between the two species, even though their adults are morphological distinct (EFSA PHL Panel, 2014).

Larvae of *Rhagoletis* species feed on fruit mesocarp and cause extensive damage on cherries and nuts. Mature larvae leave the fruit or nut to pupate in soil, where they overwinter. Even though, *Rhagoletis* fruit flies are primarily univoltine (having one generation per year), some species may have a partial second generation or even exhibit long-life cycles through prolonged pupal dormancy (Moraiti *et al.*, 2014; Moraiti & Papadopoulos, 2017). Despite the huge economic losses regarding fruit and nut production (>80%), in case of unmanaged *Rhagoletis* populations, development of effective and environmentally friendly control methods remains a challenge (Kovanci & Kovanci, 2006; Daniel & Grunder, 2012; Daniel & Baker, 2013; Daniel *et al.*, 2014; Nježić *et al.*, 2017; Verheggen *et al.*, 2017; Florian *et al.*, 2018). In addition, the potential hybridization events among native and invasive *Rhagoletis* species in European habitats could result in unpredictable pest dynamics in the area (Johannesen *et al.*, 2013). In this context, there is an urgent need of consideration of all the available population and genetic data related to the three *Rhagoletis* species in Europe and further explore their endosymbiotic association with *Wolbachia pipientis* in an effort to promote environmentally friendly, species-specific control methods, such as the sterile insect technique (SIT) and the incompatible insect technique (IIT) (Robinson *et al.*, 1999; Zabalou *et al.*, 2004; 2009; Dyck *et al.*, 2005; Apostolaki *et al.*, 2011; Lanzavecchia *et al.*, 2014; Zacharopoulou *et al.*, 2017; Nikolouli *et al.*, 2018).

In the current review, we focus on population genetics, cytogenetics and *Wolbachia* symbiosis of *R. cerasi*, *R. cingulata* and *R. completa*, with all of them currently present in the European region. Genetic and population data can provide insight into population structuring, origin of invasions, population expansion patterns and possible hybridization events that can subsequently lead to incipient speciation and unpredictable pest dynamics. On the other hand, data on symbiosis, together with the genetic and population data, can be exploited for the enhancement of SIT and other related techniques such as IIT.

Origin and dispersion

Rhagoletis cerasi is believed to have originated in the Caucasian area of western Asia, and until recently it was considered to be widespread throughout Europe and the temperate regions of the Middle and Near East, and Russia (White & Elson-Harris, 1992; fig. 1). In 2016, *R. cerasi* was detected into Ontario, Canada, and in 2017 in Niagara County in New York State, USA, demonstrating the entry of this pest in North America (Barringer, 2018). *Rhagoletis cerasi* mainly infests fruits of the genus *Prunus* spp., such as those of *Prunus avium*, *P. cerasus*, *P. serotina* and *P. mahaleb*. Wild growing cherries (*Prunus* spp.) and *Lonicera* spp (*Lonicera xylosteum*, *L. tatarica*) can serve as reservoirs or secondary hosts (White & Elson-Harris, 1992). To date, sweet and sour cherry producing areas of Europe are under a serious threat since fruit infestation can reach up to 100%, if no insecticide control is applied, while the tolerance level of the market for damaged fruits is less than 2% (Daniel & Grunder, 2012). Moreover, *R. cerasi* populations express extensive geographic variability in life-history traits (i.e. life span, reproduction) and diapause traits, as a result of local adaptation to the ecological heterogeneity of their European habitats (Vallo *et al.*, 1976; Papanastasiou *et al.*, 2011; Moraiti *et al.*, 2012*a*, 2014, 2017). In addition, *R. cerasi* can plastically respond to unpredictable environmental variation of their local habitats through diapause bet-hedging strategies ensuring persistence in European orchards (Moraiti *et al.*, 2014; Moraiti & Papadopoulos, 2017). Since the biology and ecology of the European cherry fruit fly populations are extensively studied in European habitats compared to those of the other two currently introduced *Rhagoletis* species (Boller & Bush, 1974; Boller & Prokopy, 1976; Papanastasiou *et al.*, 2011; Daniel & Grunder, 2012; Moraiti *et al.*, 2012*a*, *b*, 2014, 2017; Moraiti & Papadopoulos, 2017), *R. cerasi* could serve as a model species for predicting the adaptation patterns of the two new residents in European orchards.

Rhagoletis cingulata is another cherry-infesting *Rhagoletis* species that has been established over the last few decades in several central European countries. This species is endemic in North America, distributed from Southeastern Canada to eastern USA (Florida and Texas) (Bush, 1966; Smith & Bush, 1997; Rull *et al.*, 2011; Yee *et al.*, 2014) and Mexico (Rull *et al.*, 2011; Yee *et al.*, 2014). It was reported for the first time in Europe in 1983 in Switzerland, even though it was initially reported as *R. indifferens*. In Germany, the first specimens were caught in 1999 in Rheinland-Pfalz. Since 2004, the number of insects caught in cherry-growing areas increased considerably and progressively and frequently been detected in other parts of the country. Although it is widely distributed in the Netherlands and Hungary, its distribution remains relatively restricted in Austria, Belgium, Croatia, Poland and Slovenia (fig. 1) (Egartner *et al.*, 2010; EFSA PHL Panel, 2014). *Rhagoletis cingulata* attacks all cultivated and wild cherries but is particularly damaging to late-maturing varieties, especially sour cherries of the widely planted 'Schattenmorellen' variety in Germany (EFSA PHL Panel, 2014). It can also attack and complete its life cycle in *P. serotina* (black cherry), *P. mahaleb* (St. Lucie cherry) and *P. virginiana* (choke cherry), which are considered secondary hosts (Glasgow, 1933; Johannesen *et al.*, 2013). However, *P. serotina* is the main host of *R. cingulata* in the Netherlands (EFSA PHL Panel, 2014). Additionally to cherries, *P. salinica* (Japanese plum) and *Pyrus communis* (European pear) are major hosts of *R. cingulata*.

Finally, *R. completa* (that has recently invaded Europe), is also considered native to North America (Bush, 1966; Smith & Bush, 1997). Its current distribution includes North-eastern Mexico and South-western USA (Texas) up to Kansas (Central USA) (Bush & Smith, 1998; Rull *et al.*, 2012; Yee *et al.*, 2014).

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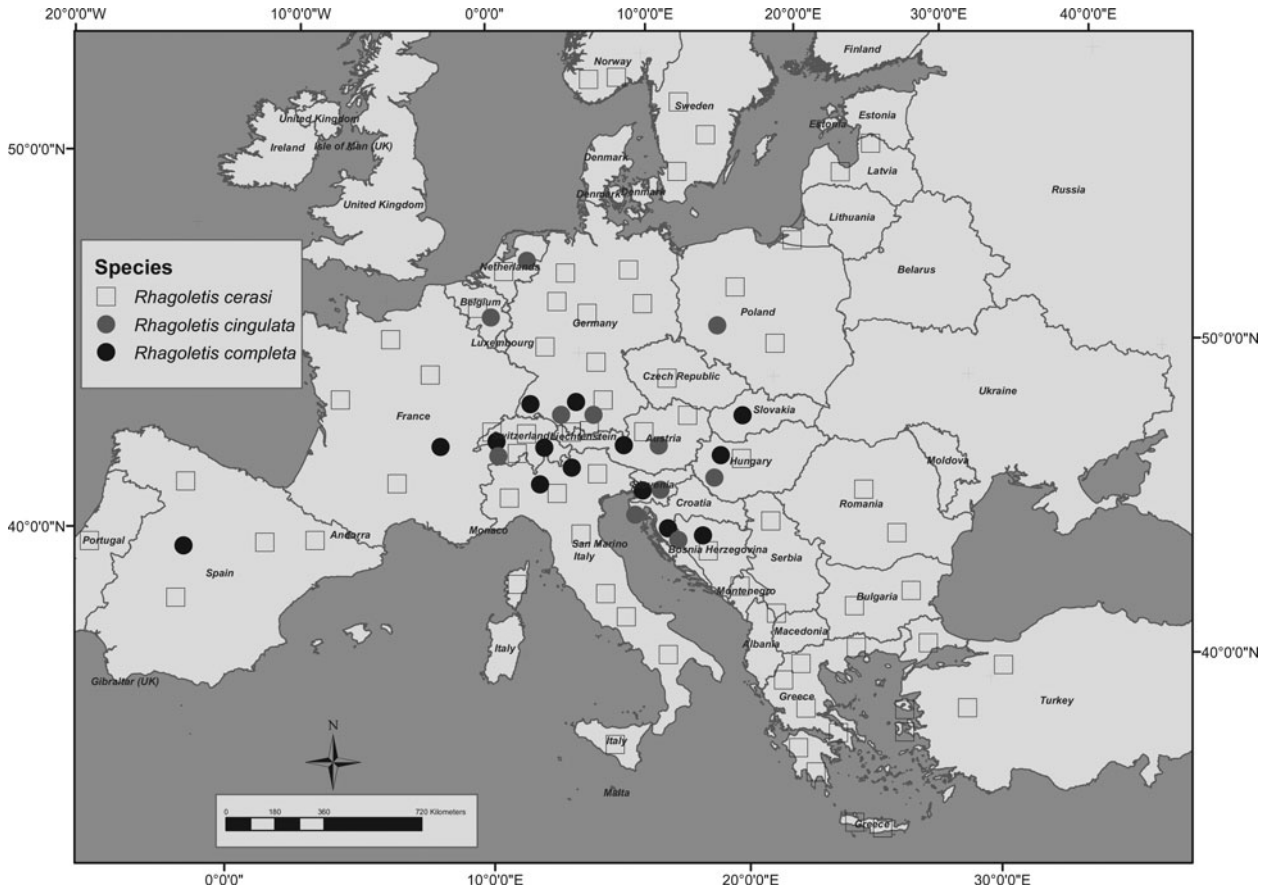

Fig. 1. Geographic distribution of *R. cerasi*, *R. cingulata* and *R. completa* in Europe. Marks show indicative areas where the species occur.

Regarding Europe, it was initially identified in Switzerland (1988) and Italy (1991), from where it spread to eight additional countries: Bosnia and Herzegovina and Croatia where it is regularly observed as well as in Austria, France, Germany, Hungary, Spain and Slovenia with restricted or occasional occurrence (fig. 1) (Duso & Dal Lago, 2006; Ostojic *et al*., 2014; Verheggen *et al*., 2017). In 2018, it was detected in Slovakia (Kozánek *et al*., 2018). According to Aluja *et al*. (2011), *R. completa* can be considered as an example of an alien species that settled first in the Mediterranean Basin and then invaded several central European countries by crossing the Alps. *Rhagoletis completa* attacks several species of walnuts (*Juglans* spp.) such as *Juglans nigra*, *J. hindsii* and *J. californica* and *J. regia* in North America. *Juglans regia* is the only economically significant host in Europe, particularly across cultivars with large and heavy fruits (Bush, 1966; Guillén *et al*., 2011). In unmanaged orchards, 100% of walnut trees can be infested causing losses in walnut yields of up to 80% (Verheggen *et al*., 2017).

Genetic relationships among the three *Rhagoletis* species

Rhagoletis cingulata belongs to the *cingulata* group, *R. completa* to the *suavis* group and *R. cerasi* to the Palearctic *cerasi* group (Bush, 1966; Smith & Bush, 1997, 2000; Smith *et al*., 2005). Studies based on genetic markers such as mitochondrial sequences (mainly COI and COII; cytochrome oxidase I and II genes) and morphological characters suggest the proximity of *suavis* and *cingulata* groups while the *cerasi* group is distantly related (Smith & Bush, 1997, 2000; Smith *et al*., 2005; Ramírez *et al*., 2008; Rull *et al*., 2012; Frey *et al*., 2013). A recent comparison of mtDNA divergence among the *R. suavis*, *R. pomonella* and *R. cingulata* species groups suggested that these taxa do not share a common biogeographic history, diverging in different regions at different times in the past, despite current similarities in geographic distributions in the United States and Mexico (Glover *et al*., 2018).

Microsatellite markers that amplify across taxa can be a very powerful tool for the resolution of closely related species (cryptic or under incipient speciation) since they are quite stable and conserved in close taxa. In fact, microsatellite markers have been used for the resolution of species complexes in Tephritidae, such as that of *Bactrocera correcta* (Bezzi) (Qin *et al*., 2016), *B. dorsalis* (Hendel) (Krosch *et al*., 2013), *B. musae* (Tryon) (Drew *et al*., 2011) and the *Ceratitis* FAR cryptic species complex (De Meyer *et al*., 2015). In *Rhagoletis*, microsatellite markers have been developed for *R. cerasi* (Arthofer *et al*., 2009), *R. completa* (Chen *et al*., 2006), *R. indifferens* (Maxwell *et al*., 2009) and *R. pomonella* (Velez *et al*., 2006) (table 1). Even though microsatellite markers have not been *de novo* developed for *R. cingulata*, Maxwell and colleagues (2009) showed that all 16 microsatellite markers developed in their study for *R. indifferens* produced the expected amplicon in *R. cingulata* (16/16 amplified, 16/16 polymorphic). This result was expected, since the above two taxa are considered sister species, as

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Table 1. Microsatellite markers developed for different *Rhagoletis* species and their cross-amplification in other *Rhagoletis* species.

Marker	Developed for	Reference	*R. cerasi* A	*R. cerasi* B	*R. cingulata* A	*R. cingulata* B	*R. completa* A	*R. completa* B	*R. electromorpha* A	*R. electromorpha* B	*R. suavis* A	*R. suavis* B	*R. pomonella* A	*R. pomonella* B	*R. mendax* A	*R. mendax* B	*R. berberis* A	*R. berberis* B	*R. ribicola* A	*R. ribicola* B	*R. fausta* A	*R. fausta* B	*R. zephyria* A	*R. zephyria* B	*R. basiola* A	*R. basiola* B
RcMic 76-1	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 76-3	*Rcer*	Arthofer *et al.* (2009)			X +^{II}	– P^{II}	+	nt	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 76-7	*Rcer*	Arthofer *et al.* (2009)			+	nt	+	nt	nt	nt	nt	nt	X	–	+	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 79-4	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 82-10	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 82-28	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 82-46	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 82-47	*Rcer*	Arthofer *et al.* (2009)			+	nt	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 83-16	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 83-26	*Rcer*	Arthofer *et al.* (2009)			+	nt	+	nt	nt	nt	nt	nt	+	nt	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 83-44	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 84-35	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
RcMic 84-42	*Rcer*	Arthofer *et al.* (2009)			X	–	X	–	nt	nt	nt	nt	X	–	X	–	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P1	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	+ +^{II}	P P^{II}	nt	nt	+	M	+	M			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P2	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	+ +^{II}	P P^{II}	nt +^{III}	nt P^{III}	+	M	+	M			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P4	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	+	P	nt	nt	+	M	+	M			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P5	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt X^{I}	+	M	nt +^{III}	nt P^{III}	+	X	+	X			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P6	*Rpom*±	Velez *et al.* (2006)	nt X^{I}	nt –^{I}	X	–	nt +^{III}	nt P^{III}	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P9	*Rpom*±	Velez *et al.* (2006)	nt X^{I}	nt –^{I}	X	–	nt	nt	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P11	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	X +^{II}	– P^{II}	nt	nt	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P12	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	X	–	nt	nt	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P13	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt M^{I}	X	–	nt	nt	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P15	*Rpom*±	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	X	–	nt	nt	X	–	X	–			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P7	*Rpom*^{v}	Velez *et al.* (2006)	nt X^{I}	nt –^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P8	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt M^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P10	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt X^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P14	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt M^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P16	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt M^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P17	*Rpom*^{v}	Velez *et al.* (2006)	nt X^{I}	nt –^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P18	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt P^{I}	nt +^{II}	nt P^{II}	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P19	*Rpom*^{v}	Velez *et al.* (2006)	nt +^{I}	nt M^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
P20	*Rpom*^{v}	Velez *et al.* (2006)	nt X^{I}	nt –^{I}	nt	nt	nt	nt	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

Table 1. (*Cont.*)

Marker	Developed for	Reference	*R. cerasi* A	*R. cerasi* B	*R. cingulata* A	*R. cingulata* B	*R. completa* A	*R. completa* B	*R. electromorpha* A	*R. electromorpha* B	*R. suavis* A	*R. suavis* B	*R. pomonella* A	*R. pomonella* B	*R. mendax* A	*R. mendax* B	*R. berberis* A	*R. berberis* B	*R. ribicola* A	*R. ribicola* B	*R. fausta* A	*R. fausta* B	*R. zephyria* A	*R. zephyria* B	*R. basiola* A	*R. basiola* B
WCFF-031	*Rind*	Maxwell *et al.* (2009)	X X[I]	– –[I]	+	P	+	P	nt	nt	nt	nt	+	P	nt	nt	+	M	+*	P*	X	–	+	P	X	–
WCFF-024	*Rind*	Maxwell *et al.* (2009)	+? +[I]	P? M[I]	+	P	X	–	nt	nt	nt	nt	+	P	nt	nt	+	P	+*	P*	+?	P?	+	M	X	–
WCFF-083	*Rind*	Maxwell *et al.* (2009)	X +[I]	– P[II]	+	P	+	P	nt	nt	nt	nt	+	P	nt	nt	+	P	+? *	P? *	+	M	+	P	+	P
WCFF-048	*Rind*	Maxwell *et al.* (2009)	+ +[I]	M P[I]	+	P	+	P	nt	nt	nt	nt	X	–	nt	nt	X	–	+*	P*	+	M	X	–	+	M
WCFF-111	*Rind*	Maxwell *et al.* (2009)	+ +[I]	P P[I]	+	P	+	P	nt	nt	nt	nt	X	–	nt	nt	+	P	+*	M*	+	P	X	–	X	–
WCFF-061B	*Rind*	Maxwell *et al.* (2009)	+ +[I]	P M[I]	+	P	+	M	nt	nt	nt	nt	+	P	nt	nt	+	P	+*	M*	+	P	+	P	X	–
WCFF-084A	*Rind*	Maxwell *et al.* (2009)	+? X[I]	P? –[I]	+	P	+	P	nt	nt	nt	nt	+	P	nt	nt	+	P	+*	P*	+	M	X	–	X	–
WCFF-086A	*Rind*	Maxwell *et al.* (2009)	X +[I]	– M[I]	+	P	+	P	nt	nt	nt	nt	+	P	nt	nt	+?	P?	X*	–	+	P	+	P	X	–
WCFF-049	*Rind*	Maxwell *et al.* (2009)	X X[I]	– –[I]	+	P	X	–	nt	nt	nt	nt	X	–	nt	nt	X	–	X*	–	X	–	X	–	X	–
WCFF-105	*Rind*	Maxwell *et al.* (2009)	+ X[I]	P –[I]	+	P	X	–	nt	nt	nt	nt	+	P	nt	nt	+	M	+*	M*	X	–	+	P	+	M
WCFF-007	*Rind*	Maxwell *et al.* (2009)	X +[I]	– X[I]	+	P	+	P	nt	nt	nt	nt	X	–	nt	nt	X	–	X*	–	+	P	X	–	X	–
WCFF-093	*Rind*	Maxwell *et al.* (2009)	+ +[I]	P P[I]	+	P	+	P	nt	nt	nt	nt	+?	P?	nt	nt	+	P	+*	M*	+	M	+	P	+	M
WCFF-011	*Rind*	Maxwell *et al.* (2009)	X +[I]	– X[I]	+	P	+	P	nt	nt	nt	nt	X	–	nt	nt	+	P	X*	–	+	P	+?	P?	+	P
WCFF-057	*Rind*	Maxwell *et al.* (2009)	+ X[I]	P –[I]	+	P	+	P	nt	nt	nt	nt	+	P	nt	nt	+	P	+*	M*	+?	P?	+	M	+	P
WCFF-065B	*Rind*	Maxwell *et al.* (2009)	X X[I]	– –[I]	+	P	+	M	nt	nt	nt	nt	X	–	nt	nt	+	P	X*	–	+	M	X	–	X	–
WCFF-067	*Rind*	Maxwell *et al.* (2009)	+ +[I]	P P[I]	+	P	+	M	nt	nt	nt	nt	+?	P?	nt	nt	+	P	+*	P*	+	P	+	P	+	P
Rcom mic1	*Rcom*	Chen *et al.* (2006)	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Rcom mic2	*Rcom*	Chen *et al.* (2006)	nt	nt	nt	nt			nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt

Results in brackets refer to the original studies. Subsequent studies dealing with cross-species amplification are indicated with superscript Latin numbers, under the findings of the original study: [I]Augustinos *et al.* (2011); [II]Drosopoulou *et al.* (2011); [III]Chen *et al.* (2006)
Rcer: *R. cerasi*; Rpom: *R. pomonella*; Rind: *R. indifference*; Rcom: *R. completa*.
Column A: Polymerase chain reaction (PCR) amplification and agarose gel electrophoresis; column B: polymorphism analysis through genotyping of a varying number of individuals; ±: polymorphism of cross-amplified markers was tested through PCR, cloning and measure of size of recovered clones; +: amplified; X: not amplified (or poor resolution during genotyping); nt: not tested; –: not done, due to PCR failure; P: polymorphic; M: monomorphic; *for *R. ribicola*: only two individuals tested; *Rpom*[v]: developed for *R. pomonella* by Velez *et al.* (2006), but without reference of testing by them in other species.

verified in other recent independent studies (Drosopoulou *et al.*, 2011; Johannesen *et al.*, 2013). Most of the *R. indifferens* markers amplify also in *R. completa* (13/16 amplified, 10/16 polymorphic) and to a lesser extent in *R. cerasi* (9/16 amplified, 6/16 polymorphic) (Augustinos *et al.*, 2011, 2014). Recently, Johannesen and colleagues (2013) managed to cross-amplify more *R. indifferens* microsatellite markers to *R. cerasi* (13/14 functional, 12/14 polymorphic). Nonetheless, as it is reported therein, the above loci were significantly less polymorphic in *R. cerasi* samples than samples derived from *R. cingulata* and *R. indifferens*, which is frequently reported when microsatellite markers are transferred from one species to another and becomes more evident at genetically distant species. The distant relationship between *R. cerasi* and the other two species is further supported by the reduced transferability of microsatellite markers developed for *R. cerasi* to the other two species since only 3/13 produced the expected amplicon in each species (Arthofer *et al.*, 2009). Moreover, a subset of the *R. pomonella* microsatellite markers developed by Velez *et al.* (2006) were employed for the genotyping of *R. cerasi* (Augustinos *et al.*, 2011, 2014) and *R. cingulata* populations (Drosopoulou *et al.*, 2011). Consistent with the above results and the established taxonomy, many genetic markers performed well in *R. cingulata* and fewer in *R. cerasi.* Although not providing more insight into the genetic relationships of the different *Rhagoletis* species under study, Chen *et al.* (2006, 2010) successfully genotyped *R. completa* samples using three of the *R. pomonella* microsatellite markers, thus further enriching the microsatellite marker 'toolkit' for *Rhagoletis* species. Table 1 summarizes efforts to cross-amplify microsatellite markers in different *Rhagoletis* species. Markers that are polymorphic, nuclear and can be *in situ* localized on chromosomes can support progress in different fields of *Rhagoletis* research. Ongoing and future whole genome sequencing efforts will provide further markers useful for genetic and genomic studies.

Cytogenetic studies and polytene chromosome maps

In general, *Rhagoletis* species consist of six pairs of chromosomes (Bush, 1966; Bush & Boller, 1977; Procunier & Smith, 1993). Specifically, early cytological analyses of *R. cerasi* demonstrated its karyotype to consist of six pairs of chromosomes, including one pair of sex chromosomes (Bush, 1966). More recent cytological analyses have further confirmed the previous karyotype analysis of Kounatidis *et al.* (2008). The same number of chromosomes ($2n = 12$) was also found in the metaphase mitotic complements of *R. completa* (Drosopoulou *et al.*, 2010) and *R. cingulata* (Drosopoulou *et al.*, 2011). On the contrary, *R. meigenii* was found to consist of four pairs of autosomes with the male and female karyotype having X0 and XX, respectively (Bush & Boller, 1977).

In most genera of tephritids, including *Anastrepha*, *Bactrocera* and *Ceratitis*, the sex chromosomes are heteromorphic and easily identified, with the Y chromosome being smaller and dot-like (Bedo, 1986; Zacharopoulou, 1987; Mavragani-Tsipidou *et al.*, 1992; Cevallos & Nation, 2004; Garcia-Martinez *et al.*, 2009; Zacharopoulou *et al.*, 2011*a*, *b*). However, the X and Y chromosomes of *R. cerasi*, *R. cingulata* and *R. completa* are very similar in length. Sex chromosomes are long in the case of *R. cerasi*, and very small, dot-like, in the case of *R. cingulata* and *R. completa* (Procunier & Smith, 1993; Kounatidis *et al.*, 2008; Drosopoulou *et al.*, 2010, 2011). This observation is consistent with the closer phylogenetic relationship of the latter two species and is in accordance with morphological and genetic data, as well as with the established taxonomy (Smith and Bush, 1997, 2000).

Comparative analysis of the polytene chromosomes banding pattern of *R. cerasi*, *R. cingulata* and *R. completa* showed extensive homology of certain polytene regions among these species, with the most extensive chromosome banding pattern conservation recorded between *R. cingulata* and *R. completa* (Drosopoulou *et al.*, 2011). This homology, together with the similarity found in the mitotic karyotype of the two species, is indicative of their close phylogenetic relationship. Based on the polytene and mitotic karyotype analysis, *R. cerasi* seems to be more distantly related to the above species. This observation is also in agreement with the phylogenetic relationships accepted for the species of the *Rhagoletis* genus (Smith and Bush, 1997, 2000).

Another finding, that raises a series of interesting questions, is the presence of multiple asynaptic phenomena both in *R. cerasi* and *R. cingulata*, but not in *R. completa* (Kounatidis *et al.*, 2008; Drosopoulou *et al.*, 2010, 2011). It has been proposed that such asynaptic phenomena may be linked to the presence of endosymbiotic bacteria, such as *Wolbachia*, and evolutionary relationship events of integration of their DNA (in part or whole) into the chromosomal DNA (Kounatidis *et al.*, 2008; Drosopoulou *et al.*, 2010, 2011).

Genetic structuring and population dynamics

Despite the progress in genetic markers development, little is known about these three species regarding the genetic structure and dynamics of their natural populations. In *R. cerasi*, Schwarz *et al.* (2003) found little evidence for host race formation, using allozyme markers for the analysis of natural populations derived from Switzerland and Germany and two different host plants, *L. xylosteum* L. and *P. avium* L. Recently, Augustinos and his colleagues (2011, 2014) used some of the microsatellite markers developed for *R. cerasi* (Arthofer *et al.*, 2009), in addition to several more cross-amplified ones from other *Rhagoletis* species (*R. pomonella* and *R. indifferens*), to perform a population analysis targeting mainly *R. cerasi* populations in Greece, Northern Europe (Germany) and Russia. This analysis revealed at least three to four genetic groups clustering samples from: (a) Germany, (b) mainland Greece and some Greek islands, (c) Eastern Aegean islands and (d) Russia (just one sample).

Regarding *R. cingulata*, recent attempts using microsatellite markers failed to determine any genetic differentiation correlated with the host preference or geographic origin of populations derived from the native area of Michigan (Smith *et al.*, 2014). On the other hand, Johannesen *et al.* (2013), utilizing also microsatellite markers, clearly demonstrated that *R. cingulata* samples from the recently invaded regions of Germany and Hungary are genetically different. Based on these findings, this study raised the possibility of independent invasions of this pest in Europe. The same study also detected signals of hybridization events between *R. cerasi* and *R. cingulata*. This hybridization 'sets the alarm on' regarding the management of cherry pests in the area since it may generate new more aggressive biotypes of pests, such as the 'Lonicera fly' which has emerged from the hybridization of *R. mendax* and *R. zephyria* (Schwarz *et al.*, 2003).

Based on allozyme markers, Berlocher (1984) revealed high polymorphism during the colonization process of *R. completa* in California considering native and introduced populations. Chen and colleagues (2006, 2010) used a set of five

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microsatellite markers (along with allozymes) to analyse natural populations based on new and historical collections. In the first study, they did not observe significant bottlenecks; in fact, in some cases the introduced populations seemed to be more polymorphic than native ones (Chen *et al*., 2006). Since the introduced populations harboured alleles not sampled in the native ones, the need for more extensive sampling in native areas, and the hypothesis of multiple introduction events, were discussed and further addressed in the second study (Chen *et al*., 2010). This study gave insight into *R. completa* natural population genetic structuring and provided important findings, such as: (a) the need for good sampling across the distribution area of the species; (b) the decline of structuring in introduced and native populations over time and (c) greater fluctuation of genetic variability of introduced populations and increase in their genetic variability over time.

Rhagoletis–Wolbachia *associations: dynamic and still unresolved*

Recently, much interest has been shown on studies regarding the intracellular bacterium *Wolbachia* that is found in more than 40% of the terrestrial arthropod species (Werren *et al*., 2008; Zug & Hammerstein, 2012). The presence of *Wolbachia* has been associated with the induction of a variety of host reproductive phenotypes such as feminization, parthenogenesis, male killing and cytoplasmic incompatibility (CI) (for review see Werren *et al*., 2008; Saridaki & Bourtzis, 2010; Mateos *et al*., 2019). For these reasons, *Wolbachia* is considered a key player affecting biological and evolutionary processes as well as a tool for the control of agricultural pests and disease vectors (Werren *et al*., 2008; Saridaki & Bourtzis, 2010; Mateos *et al*., 2019).

In the late 1970s, researchers observed the existence of reproductive incompatibility between European populations of *R. cerasi* derived from north and south Europe (Matolin, 1976; Boller, 1989). Riegler & Stauffer (2002) attributed this incompatibility to the presence of different *Wolbachia* strains. Despite the univoltine life cycle of *R. cerasi* populations and the limited dispersal abilities of adults (Daniel & Grunder, 2012), it has been shown that *Wolbachia* spread in natural host populations is rapid (Bakovic *et al*., 2018). In fact, all natural populations of *R. cerasi* studied so far have been found to be 100% *Wolbachia*-infected (Riegler & Stauffer, 2002; Kounatidis *et al*., 2008; Arthofer *et al*., 2009, 2011; Augustinos *et al*., 2014; Karimi & Darsouei, 2014). Hosts can be infected by one or more distinct strains of *Wolbachia* (table 2), with the European populations of *R. cerasi* were found to be infected by five different *Wolbachia* strains, named *w*Cer1 to *w*Cer5 (Riegler & Stauffer, 2002; Kounatidis *et al*., 2008; Arthofer *et al*., 2009, 2011; Augustinos *et al*., 2014); and *R. cerasi* samples from Iran were found to be infected with a single new strain of *Wolbachia*, named *w*Cer6 (Karimi & Darsouei, 2014). But, the *R. cerasi*–*Wolbachia* interactions seem to be even more complicated than this. Augustinos and colleagues (2014) pointed to the presence of additional uncharacterized *Wolbachia* strains on *R. cerasi* populations from Greece, and raised concerns about the suitability of the currently used diagnostic markers for genotyping. Considering the information mentioned earlier, along with the fact that *Wolbachia* cannot be cultivated in the laboratory, the presence of low-titre infections and/or multiple infections in the same individual, as well as the highly recombinant nature of the bacterial chromosome, make *Wolbachia* characterization in *R. cerasi* samples a very difficult task (Zabalou *et al*., 2004, 2009; Schneider *et al*., 2013; Mateos *et al*., 2019). Allelic intersection analysis provided an additional tool for the characterization of multiple *Wolbachia* infections in *R. cerasi* (Arthofer *et al*., 2011); however, this analysis requires *a priori* knowledge on the *Wolbachia* strains harboured by a species and can be quite laborious. The rapid technological advances in the field of sequencing technologies (such as different types of next generation sequencing and single cell genomics) are expected to shed light on the *Wolbachia* status of *Rhagoletis* species and gradually overcome difficulties related to the presence of multiple and/or low-titre *Wolbachia* infections.

Based on multilocus sequence typing (MLST) and *wsp* gene analysis, all populations of *R. cingulata* studied so far have been 100% infected with the *Wolbachia w*Cin2 strain (table 2), which is identical to *w*Cer2 of *R. cerasi* (Schuler *et al*., 2009, 2013; Drosopoulou *et al*., 2011). In addition, Schuler *et al*. (2013) demonstrated that several European populations of *R. cingulata* are infected also with the *w*Cin1 strain (identical to *w*Cer1 of *R. cerasi*, again based on MLST and *wsp* genes analysis), but not in any individual studied from the USA (table 2). They have also suggested a possible horizontal transmission of *Wolbachia* from *R. cerasi* to *R. cingulata* during the invasion route of this species in Europe. Screening of one wild population of *R. completa* (Drosopoulou *et al*., 2010), originating from Southern Germany, gave no evidence of *Wolbachia* infection. Nonetheless, a more recent study on a population collected from Austria (Schuler *et al*., 2012) showed the presence of low-titre *Wolbachia* infections with strains similar to *w*Cer1, *w*Cer2 and *w*Cer5 (table 2). The authors suggested that such infections are unlikely to induce reproductive phenotypes such as CI and therefore their possible impact in natural populations is unclear. Regarding *R. pomonella*, Schuler *et al*. (2011) screened both host races (apple and hawthorn-infesting species) for *Wolbachia* infections and detected the presence of wPom1 and at least three more uncharacterized strains. Specifically, wPom1 strain found to occur in both host races, whereas different sequence types were found at low frequencies only in apple-infesting *R. pomonella*.

Implementation of SIT and IIT methods

The SIT is a species-specific method of pest population suppression that relies on repetitive releases of mass-produced sterile insects, ideally only males. Sterile males are expected to compete with wild males and mate with wild females, inducing sterility in the wild females (Dyck *et al*., 2005). For more than 30 years, cytogenetics of tephritids have played a catalytic role in the development of key tools in support of SIT, including the characterization of tephritid genetic sexing strains and the support of integrative taxonomic studies for clarifying relationships between closely related species and/or incipient speciation phenomena (Zacharopoulou *et al*., 2017). In fact, polytene chromosomes are an important tool for understanding organization and evolution of chromosomes, mapping of traits of interest, and analysing chromosomal rearrangements (Robinson *et al*., 1999; Zacharopoulou *et al*., 2017). Therefore, the development of polytene chromosome maps for *R. cerasi*, *R. cingulata* and *R. completa* (Kounatidis *et al*., 2008; Drosopoulou *et al*., 2010, 2011) can support SIT approaches in *Rhagoletis* species in general. Apart from cytogenetics tools, microsatellite markers used for identifying the genetic structure and population dynamics of the three *Rhagoletis* species could provide valuable insights into colonization patterns

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Table 2. *Wolbachia* status in *Rhagoletis* species.

Species	Infected	Type of infection	Strains	Percentage	Reference
R. cerasi	+	Multiple	*w*Cer1-5 plus more uncharacterized *w*Cer6	100	Riegler & Stauffer (2002), Zabalou *et al.* (2004), Arthofer *et al.* (2009), Arthofer *et al.* (2011), Augustinos *et al.* (2014), Karimi & Darsouei (2014)
R. cingulata	+	Multiple	At least *w*Cin1 and *w*Cin2	100	Drosopoulou *et al.* (2011), Schuler *et al.* (2009)
R. completa	–	–	–	–	Drosopoulou *et al.* (2010)
	+	Multiple	*w*Cer1, *w*Cer2, *w*Cer5 (low-titre infection)	100	Schuler *et al.* (2012)
R. pomonella	+	Multiple	*w*Pom1 and at least three more uncharacterized	100	O'Neill *et al.* (1992), Schuler *et al.* (2011)

and phylogenetic relationships of this species and into ecological strategies in the field.

The mechanism of *Wolbachia*-induced CI is receiving much attention as a tool for pest and disease control (Mateos *et al.*, 2019; Nikolouli *et al.*, 2018). It is worth noting that the IIT, which is based on the *Wolbachia*-induced CI, had been applied for the population control of *R. cerasi* even before the aetiological agent (*Wolbachia* infections) of the reproductive incompatibilities observed between northern and southern European populations was discovered (Boller *et al.*, 1976; Matolin, 1976; Boller, 1989; Riegler & Stauffer, 2002). In the same context, infected *R. cerasi* populations were used as *Wolbachia* donors to species that do not harbour this bacterium, such as the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Zabalou *et al.*, 2004, 2009) and the olive fly, *B. oleae* (Rossi) (Apostolaki *et al.*, 2011) for the development of IIT-based population control. In diplo-diploid species, CI is commonly expressed as embryonic lethality in crosses between *Wolbachia*-infected males with females, which are either uninfected or they carry a different *Wolbachia* strain.

In principle, the IIT technique based on *Wolbachia* could be used in a way analogous to the SIT; mass-released *Wolbachia*-infected males are expected to suppress a target pest population. However, the application of IIT strictly depends on the availability of an efficient and robust sexing system, otherwise a population suppression strategy may result in population replacement. In the absence of a perfect sexing system, the combination of SIT and IIT has been proposed via the application of low doses of irradiation which can sterilize any accidentally released females while males would be rendered sterile by both *Wolbachia* and irradiation (Bourtzis & Robinson, 2006; Brelsfoard *et al.*, 2009; Bourtzis *et al.*, 2014, 2016; Zhang *et al.*, 2015*a*, *b*, 2016; Mateos *et al.*, 2019). In any case, the development of a population suppression strategy against *Rhagoletis* species via SIT, IIT or a combination of irradiation and *Wolbachia*-based approaches would require a mass rearing system; unfortunately, the fact that these species are univoltine and an artificial diet has yet to be developed hinder, for the time being, any plans towards this direction.

Conclusions

In recent years, there is major concern regarding invasive insect species including their interactions with native fauna. Depending on their genetic and behavioural proximity, alien and native species could interact either through mating or through exchanging genetic material (e.g. symbionts) due to common plant hosts and/or predators and parasitoids. These interactions could lead to new pest dynamics through the development of hybrid species or altered behaviour of existing species, phenomena that Europe has experienced during the last decades following the invasion of new members of the *Rhagoletis* genus. The prediction of these changes and therefore the control of these pests certainly becomes more difficult. It is clear that the clarification of the species identity by using molecular and (cyto)genetic approaches, population analysis and characterization of the symbionts associated with the target pest populations can facilitate control strategies such as SIT, IIT and other genetic methods, which are environment-friendly and species specific. In the current review, the currently available knowledge on the cytogenetics, the population genetic structure and *Wolbachia* infections of *Rhagoletis* pest species in Europe was summarized, highlighting the importance of such knowledge as an essential prerequisite of any population control programme.

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References

Aluja, M., Guillén, L., Rull, J., Höhn, H., Frey, J., Graf, B. & Samietz, J. (2011) Is the alpine divide becoming more permeable to biological invasions? – Insights on the invasion and establishment of the Walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae) in Switzerland. *Bulletin of Entomological Research* **101**, 451–465.

Apostolaki, A., Livadaras, I., Saridaki, A., Chrysargyris, A., Savakis, C. & Bourtzis, K. (2011) Transinfection of the olive fruit fly *Bactrocera oleae* with *Wolbachia*: towards a symbiont-based population control strategy. *Journal of Applied Entomology* **135**, 546–553.

Arthofer, W., Riegler, M., Schneider, D., Krammer, M., Miller, W.J. & Stauffer, C. (2009) Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). *Molecular Ecology* **18**, 3816–3830.

https://doi.org/10.1017/S0007485319000063 Published online by Cambridge University Press

Arthofer, W., Riegler, M., Schuler, H., Schneider, D., Moder, K., Miller, W.J. & Stauffer, C. (2011) Allele intersection analysis: a novel tool for multi locus sequence assignment in multiply infected hosts. *PLoS One* **6**, e22198.

Augustinos, A.A., Asimakopoulou, A.K., Papadopoulos, N.T. & Bourtzis, K. (2011) Cross-amplified microsatellites in the European cherry fly, *Rhagoletis cerasi*: medium polymorphic-highly informative markers. *Bulletin of Entomological Research* **101**, 45–52.

Augustinos, A.A., Asimakopoulou, A.K., Moraiti, C.A., Mavragani-Tsipidou, P., Papadopoulos, N.T. & Bourtzis, K. (2014) Microsatellite and *Wolbachia* analysis in *Rhagoletis cerasi* natural populations: population structuring and multiple infections. *Ecology and Evolution* **4**, 1943–1962.

Bakovic, V., Schebeck, M., Telschow, A., Stauffer, C. & Schuler, H. (2018) Spatial spread of *Wolbachia* in *Rhagoletis cerasi* populations. *Biology Letters* **14**, 20180161.

Barringer, L. (2018) First record of the European cherry fruit fly, *Rhagoletis cerasi* (Linnaeus) (Diptera: Tephritidae), in North America. *Insecta Mundi* **0622**, 1–14.

Bedo, D.G. (1986) Polytene and mitotic chromosome analysis in *Ceratitis capitata* (Diptera; Tephritidae). *Canadian Journal of Genetics and Cytology* **28**, 180–188.

Berlocher, S.H. (1984) Genetic changes coinciding with the colonization of California by the walnut husk fly, *Rhagoletis completa*. *Evolution* **38**, 906–918.

Berlocher, S.H. & Bush, G.L. (1982) An electrophoretic analysis of *Rhagoletis* (Diptera, Tephritidae) Phylogeny. *Systematic Zoology* **31**, 136–155.

Boller, E. (1989) Cytoplasmatic incompatibility in *Rhagoletis cerasi*. pp. 69–74 in Robinson, A.S. & Hooper, G. (*Eds*) *Fruit Flies Their Biology, Natural Enemies and Control*. *3B*. Amsterdam, The Netherlands, Elsevier.

Boller, E.F. & Bush, G.L. (1974) Evidence for genetic variation in populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae) based on physiological parameters and hybridization experiments. *Entomologia Experimentalis et Applicata* **17**, 279–293.

Boller, E.F. & Prokopy, R.J. (1976) Bionomics and management of *Rhagoletis*. *Annual Review of Entomology* **21**, 223–245.

Boller, E.F., Russ, K., Vallo, V. & Bush, G.L. (1976) Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae), their origin and potential use in biological control. *Entomologia Experimentalis et Applicata* **20**, 237–247.

Bourtzis, K. & Robinson, A.S. (2006) Insect pest control using *Wolbachia* and/or radiation. pp. 225–246, vol. **2** in Bourtzis, K. & Miller, T.A. (*Eds*) *Insect Symbiosis*. CRC Press, Boca Raton.

Bourtzis, K., Dobson, S.L., Xi, Z., Rasgon, J.L. & Calvitti, M. (2014) Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. *Acta Tropica* **132S**, 150S–163S.

Bourtzis, K., Lees, R.S., Hendrichs, J. & Vreysen, M.J. (2016) More than one rabbit out of the hat: radiation, transgenic and symbiont-based approaches for sustainable management of mosquito and tsetse fly populations. *Acta Tropica*, **157**, 115–130.

Brelsfoard, C.L., Clair, W.S. & Dobson, S.L. (2009) Integration of irradiation with cytoplasmic incompatibility to facilitate a lymphatic filariasis vector elimination approach. *Parasites and Vectors* **2**, 38.

Bush, G.L. (1966) The taxonomy, cytology, and evolution of the genus *Rhagoletis* in North America (Diptera, Tephritidae). *Bulletin of the Museum of Comparative Zoology* **134**, 431–562.

Bush, G.L. & Boller, E. (1977) The chromosome morphology of the *Rhagoletis cerasi* species complex (Diptera, Tephritidae). *Annals of the Entomological Society of America* **70**, 316–318.

Bush, G.L. & Smith, J. (1998) The genetics and ecology of sympatric speciation: a case study. *Researches on Population Ecology* **40**, 175–187.

Cevallos, V.E. & Nation, J.L. (2004) Chromosomes of the Caribbean fruit fly (Diptera: Tephritidae). *Florida Entomologist* **87**, 361–364.

Chen, Y.H., Opp, S.B., Berlocher, S.H. & Roderick, G.K. (2006) Are bottlenecks associated with colonization? Genetic diversity and diapause variation of native and introduced *Rhagoletis completa* populations. *Oecologia* **149**, 656–67.

Chen, Y.H., Berlocher, S.H., Opp, S.B. & Roderick, G.K. (2010) Post-colonization temporal genetic variation of an introduced fly, *Rhagoletis completa*. *Genetica* **138**, 1059–1075.

Daniel, C. & Baker, B. (2013) Dispersal of *Rhagoletis cerasi* in commercial cherry orchards: efficacy of soil covering nets for cherry fruit fly control. *Insects* **4**, 168–176.

Daniel, C. & Grunder, J. (2012) Integrated management of European cherry fruit fly *Rhagoletis cerasi* (L.): situation in Switzerland and Europe. *Insects* **3**, 956–988.

Daniel, C., Mathis, S. & Feichtinger, G. (2014) A new visual trap for *Rhagoletis cerasi* (L.) (Diptera: Tephritidae). *Insects* **5**, 564–576.

De Meyer, M., Delatte, H., Ekesi, S., Jordaens, K., Kalinova, B., Manrakhan, A., Mwatawala, M., Steck, G., Van Cann, J., Vaníčková, L., Břízová, R. & Virgilio, M. (2015) An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. *ZooKeys* **540**, 405–427.

Drew, R.A.I., Ma, J., Smith, S. & Hughes, J.M. (2011) The taxonomy and phylogenetic relationships of species in the *Bactrocera musae* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Papua New Guinea. *Raffles Bulletin of Zoology* **59**, 145–162.

Drosopoulou, E., Koeppler, K., Kounatidis, I., Nakou, I., Papadopoulos, N.T., Bourtzis, K. & Mavragani-Tsipidou, P. (2010) Genetic and cytogenetic analysis of the walnut-husk fly (Diptera: Tephritidae). *Annals of the Entomological Society of America* **103**, 1003–1011.

Drosopoulou, E., Augustinos, A.A., Nakou, I., Koeppler, K., Kounatidis, I., Vogt, H., Papadopoulos, N.T., Bourtzis, K. & Mavragani-Tsipidou, P. (2011) Genetic and cytogenetic analysis of the American cherry fruit fly, *Rhagoletis cingulata* (Diptera: Tephritidae). *Genetica* **139**, 1449–1464.

Duso, C. & Dal Lago, G. (2006) Life cycle, phenology and economic importance of the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) in northern Italy. *Annales de la Societe Entomologique de France* **42**, 245–254.

Dyck, V.A., Hendrichs, J. & Robinson, A.S. (2005) *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. The Netherlands, Springer.

Egartner, A., Zeisner, N., Hausdorf, H. & Blümel, S. (2010) First record of *Rhagoletis cingulata* (Loew) (Dipt., Tephritidae) in Austria. *EPPO Bulletin* **40**, 158–162.

EFSA PLH Panel (EFSA Panel on Plant Health). (2014) Scientific Opinion on the pest categorisation of *Rhagoletis cingulata* (Loew). *EFSA Journal* **12**(10), 3854.

EPPO. (2016) *Rhagoletis suavis* found in Berlin (DE). Reporting Service 2016/167. https://gd.eppo.int/reporting/article-5912.

Florian, T., Macavei, L.I., Hulujan, I.B., Vasian, I., Totos, S., Gorgan, M., Oltean, I. & Florian, V. (2018) Testing of semio-chemical products in monitoring and control of *Rhagoletis cerasi* L. *AgroLife Scientific Journal* **7**, 61–68.

Frey, J.E., Guillén, L., Frey, B., Samietz, J., Rull, J. & Aluja, M. (2013) Developing diagnostic SNP panels for the identification

of true fruit flies (Diptera: Tephritidae) within the limits of COI-based species delimitation. *BMC Evolutionary Biology* **13**:106 https://doi.org/10.1186/1471-2148-13-106.

Garcia-Martinez, V., Hernandez-Ortiz, E., Zepeta-Cisneros, C.S., Robinson, A.S., Zacharopoulou, A. & Franz, G. (2009) Mitotic and polytene chromosome analysis in the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). *Genome* **52**, 20–30.

Glasgow, H. (1933) The host relations of our cherry fruit flies. *Journal of Economic Entomology* **26**, 431–438.

Glover, M.M., Egan, S.P., Hood, G.R., Rull, J., Aluja, M. & Feder, J.L. (2018) Phylogeography of walnut-infesting *Rhagoletis suavis* (Diptera: Tephritidae) flies. *Insect Systematics and Diversity* **2**, 1–9.

Guillén, L., Aluja, M., Rull, J., Höhn, H., Schwizer, T. & Samietz, J. (2011) Influence of walnut cultivar on infestation by *Rhagoletis completa*: behavioural and management implications. *Entomologia Experimentalis et Applicata* **140**, 207–217.

Johannesen, J., Keyghobadi, N., Schuler, H., Stauffer, C. & Vogt, H. (2013) Invasion genetics of American cherry fruit fly in Europe and signals of hybridization with the European cherry fruit fly. *Entomologia Experimentalis et Applicata*, **147**, 61–72.

Karimi, J. & Darsouei, R. (2014) Presence of the endosymbiont *Wolbachia* among some fruit flies (Diptera: Tephritidae) from Iran: a multilocus sequence typing approach. *Journal of Asia-Pacific Entomology* **17**, 105–112.

Kounatidis, I., Papadopoulos, N., Bourtzis, K. & Mavragani-Tsipidou, P. (2008) Genetic and cytogenetic analysis of the fruit fly *Rhagoletis cerasi* (Diptera: Tephritidae). *Genome* **51**, 479–491.

Kovanci, O.B. & Kovanci, B. (2006) Effect of altitude on seasonal flight activity of *Rhagoletis cerasi* flies (Diptera: Tephritidae). *Bulletin of Entomological Research* **96**, 345–351.

Kozánek, M., Semelbauer, M. & Bartoš, R. (2018) The first finding of walnut husk fly *Rhagoletis completa* (Diptera, Tephritidae) in Slovakia. *Folia Faunistica Slovaca* **22**, 29–31.

Krosch, M.N., Schutze, M.K., Armstrong, K.F., Boontop, Y., Bonkin, L.M., Chapman, T.A., Englezou, A., Cameron, S.L. & Clarke, A.R. (2013) Piecing together an integrative taxonomic puzzle: microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis* s.l. (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. *Systematic Entomology* **38**, 2–13.

Lanzavecchia, S.B., Juri, M., Bonomi, A., Gomulski, L., Scannapieco, A.C., Segura, D.F., Malacrida, A., Cladera, J.L. & Gasperi, G. (2014) Microsatellite markers from the 'South American fruit fly' *Anastrepha fraterculus*: a valuable tool for population genetic analysis and SIT applications. *BMC Genetic*, **15** (Suppl 2), S13.

Mateos, M., Martinez, H., Lanzavecchia, S.B., Conte, C., Guillén, K., Morán-Aceves, B., Toledo, J., Liedo, P., Asimakis, E.D., Doudoumis, V., Kyritsis, G.A., Papadopoulos, N.T., Avgoustinos, A.A., Segura, D.E., Tsiamis, G. & Bourtzis, K. (2019) *Wolbachia pipientis* associated to tephritid fruit fly pests: from basic research to applications. *BioRxiv*, https://doi.org/10.1101/358333 (in press).

Matolin, S. (1976) Mechanism causing incompatibility between different strains of *Rhagoletis cerasi* (Diptera, Tephritidae). *Acta Entomologica Bohemoslovaca* **73**, 289–292.

Mavragani-Tsipidou, P., Karamanlidou, G., Zacharopoulou, A., Koliais, S. & Kastritsis, C. (1992) Mitotic and polytene chromosome analysis in *Dacus oleae* (Diptera: Tephritidae). *Genome* **35**, 373–378.

Maxwell, S.A., Rasic, G. & Keyghobadi, N. (2009) Characterization of microsatellite loci for the western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae). *Molecular Ecology Resources* **9**, 1025–1028.

Moraiti, C.A. & Papadopoulos, N.T. (2017) Obligate annual and successive facultative diapause establish a bet-hedging strategy of *Rhagoletis cerasi* (Diptera: Tephritidae) in seasonally unpredictable environments. *Physiological Entomology* **42**, 225–231.

Moraiti, C.A., Nakas, C.T., Köppler, K. & Papadopoulos, N.T. (2012*a*) Geographical variation in adult life-history traits of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae). *Biological Journal of the Linnean Society* **107**, 137–152.

Moraiti, C.A., Nakas, C.T. & Papadopoulos, N.T. (2012*b*) Prolonged pupal dormancy is associated with significant fitness cost for adults of *Rhagoletis cerasi* (Diptera: Tephritidae). *Journal of Insect Physiology* **58**, 1128–1135.

Moraiti, C.A., Nakas, C.T. & Papadopoulos, N.T. (2014) Diapause termination of *Rhagoletis cerasi* pupae is regulated by local adaptation and phenotypic plasticity: escape in time through bet-hedging strategies. *Journal of Evolutionary Biology* **27**, 43–54.

Moraiti, C.A., Nakas, C.T. & Papadopoulos, N.T. (2017) Local adaptation, intrapopulation adult emergence patterns, sex and prolonged diapause regulate the rate of postwinter development in pupae of the European cherry fruit fly. *Biological Journal of the Linnean Society* **121**, 282–293.

Nikolouli, K., Colinet, H., Renault, D., Enriquez, T., Mouton, L., Gibert, P., Sassu, F., Cáceres, P., Stauffer, C., Pereira, R. & Bourtzis, K. (2018) Sterile insect technique and *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. *Journal of Pest Science* **91**, 489–503.

Nježić, B., Pećanin, D. & Đurić, G. (2017) Improvement of management of *Rhagoletis cerasi* in Bosnia and Herzegovina based on a phenological model. *Proceedings of the Latvian Academy of sciences. Section B* **71**, 229–232.

O'Neill, S.L., Giordano, R., Colbert, A.M.E., Karr, T.L. & Robertson, H.M. (1992) 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proceedings of the National Academy of Sciences of the USA* **89**, 2699–2702.

Ostojic, I., Zovko, M. & Petrovic, D. (2014) First record of walnut husk fly *Rhagoletis completa* (Cresson, 1929) in Bosnia and Herzegovina. *Works of the Faculty of Agriculture University of Sarajevo* **59**, 121–126.

Papanastasiou, S.A., Nestel, D., Diamantidis, A.D., Nakas, C.T. & Papadopoulos, N.T. (2011) Physiological and biological patterns of a highland and a coastal population of the European cherry fruit fly during diapause. *Journal of Insect Physiology* **57**, 83–93.

Procunier, W.S. & Smith, J.J. (1993) Localization of ribosomal DNA in *Rhagoletis pomonella* (Diptera: Tephritidae) by in situ hybridization. *Insect Molecular Biology* **2**, 163–174.

Qin, Y.-J., Buahom, N., Krosch, M.N., Du, Y., Wu, Y., Malacrida, A.R., Deng, Y.-L., Liu, J.-Q., Jiang, X.-L. & Li, Z.-H. (2016) Genetic diversity and population structure in *Bactrocera correcta* (Diptera: Tephritidae) inferred from mtDNA cox1 and microsatellite markers. *Scientific Reports* **6**, 38476.

Ramírez, C.C., Salazar, M., Palma, R.E., Cordero, C. & Meza-Basso, L. (2008) Phylogeographical analysis of Neotropical *Rhagoletis* (Diptera: Tephritidae): did the Andes

uplift contribute to current morphological differences? *Neotropical Entomology* **37**, 651–661.

Riegler, M. & Stauffer, C. (2002) *Wolbachia* infections and superinfections in cytoplasmically incompatible populations of the European cherry fruit fly *Rhagoletis cerasi* (Diptera, Tephritidae). *Molecular Ecology* **11**, 2425–2434.

Robinson, A.S., Franz, G. & Fisher, K. (1999) Genetic sexing strains in the medfly, *Ceratitis capitata:* Development, mass rearing and field application. *Trends in Entomology* **2**, 81–104.

Rull, J., Aluja, M. & Feder, J.L. (2011) Distribution and Basic Biology of Black cherry-infesting *Rhagoletis* (Diptera: Tephritidae) in México. *Annals of the Entomological Society of America* **104**, 202–211.

Rull, J., Tadeo, E., Aluja, M., Guillén, L., Egan, S.P. & Feder, L.J. (2012) Hybridization and sequential components of reproductive isolation between parapatric walnut-infesting sister species *Rhagoletis completa* and *Rhagoletis zoqui*. *Biological Journal of the Linnean Society* **107**, 886–898.

Saridaki, A. & Bourtzis, K. (2010) *Wolbachia*: more than just a bug in insects genitals. *Current Opinion in Microbiology* **13**, 67–72.

Schneider, D.I., Riegler, M., Arthofer, W., Merçot, H., Egan, S.P. & Feder, L.J. (2013) Uncovering *Wolbachia* diversity upon artificial host transfer. *PloS One* **8**, e82402.

Schuler, H., Arthofer, W., Krumböck, S., Köppler, K., Vogt, H., Teixeira, L.A.F., Riegler, M. & Stauffer, C. (2009) The bacterial endosymbiont *Wolbachia* in the invasive cherry fruit fly *Rhagoletis cingulata* (Diptera, Tephritidae). *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* **17**, 99–101.

Schuler, H., Arthofer, W., Riegler, M., Bertheau, C., Krumböck, S., Köppler, K., Vogt, H., Teixeira, L.A.F. & Stauffer, C. (2011) Multiple *Wolbachia* infections in *Rhagoletis pomonella*. *Entomologia Experimentalis et Applicata* **139**, 138–144.

Schuler, H., Arthofer, W., Krumbock, S., Bertheau, C. & Stauffer, C. (2012) *Wolbachia* infection in the Walnut-Husk fly *Rhagoletis completa* Cresson 1929 (Diptera: Tephritidae). *Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte Entomologie* **18**, 243–245.

Schuler, H., Bertheau, C., Egan, S.P., Feder, J.L., Riegler, M., Schlick-Steiner, B.C., Steiner, F.M., Johannesen, J., Kern, P., Tuba, K., Lakatos, F., Köppler, K., Arthofer, W. & Stauffer, C. (2013) Evidence for a recent horizontal transmission and spatial spread of *Wolbachia* from endemic *Rhagoletis cerasi* (Diptera: Tephritidae) to invasive *Rhagoletis cingulata* in Europe. *Molecular Ecology* **22**, 4101–4111.

Schwarz, D., McPheron, B.A., Hartl, G.B., Boller, E.F. & Hoffmeister, T.S. (2003) A second case of genetic host races in *Rhagoletis*? A population genetic comparison of sympatric host populations in the European cherry fruit fly, *Rhagoletis cerasi*. *Entomologia Experimentalis et Applicata* **108**, 11–17.

Smith, J.J. & Bush, G.L. (1997) Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. *Molecular Phylogenetics and Evolution* **7**, 33–43.

Smith, J.J. & Bush, G.L. (2000) Phylogeny of the subtribe Carpomyina (Trypetinae), emphasizing relationships of the genus *Rhagoletis*. pp. 187–217 in Aluja, M. & Norrbom, A.L. (*Eds*) *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*. Boca Raton, FL. CRC Press.

Smith, J.J., Jaycox, M., Smith-Caldas, M.R.B. & Bush, G.L. (2005) Analysis of mitochondrial DNA and morphological characters in the subtribe Carpomyina (Diptera: Tephritidae). *Israel Journal of Entomology* **35-36**, 317–340.

Smith, J.J., Powell, T.H.Q., Teixeira, L., Armstrong, W.O., McClowry, R.J., Isaacs, R., Hood, G.R., Feder, L.J. & Gut, L. (2014) Genetic structure of cherry fruit fly (*Rhagoletis cingulata*) populations across managed, unmanaged, and natural habitats. *Entomologia Experimentalis et Applicata* **150**, 157–165.

Vallo, V., Remund, U. & Boller, E.F. (1976) Storage conditions of stockpiled diapausing pupae of *Rhagoletis cerasi* for obtaining high emergence rates. *Entomophaga* **21**, 251–256.

Velez, S., Taylor, M.S., Noor, M.A.F., Lobo, N.F. & Feder, J.L. (2006) Isolation and characterization of microsatellite loci from the apple maggot fly *Rhagoletis pomonella* (Diptera: Tephritidae). *Molecular Ecology Notes* **6**, 90–92.

Verheggen, F., Verhaeghe, A., Giordanengo, P., Tassus, X. & Escobar-Gutiérrez, A. (2017) Walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae), invades Europe: invasion potential and control strategies. *Applied Entomology and Zoology* **52**, 1–7.

Werren, J.H., Baldo, L. & Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews. Microbiology* **6**, 741–51.

White, I.M. & Elson-Harris, M. (1992) *Fruit Flies of Economic Significance: Their Identification and Bionomics*. Wallingford, Oxfordshire, CABI.

Yee, W.L., Hernández-Ortiz, V., Rull, J., Sinclair, B.J. & Neven, L. G. (2014) Status of *Rhagoletis* (Diptera: Tephritidae) pests in the NAPPO countries. *Journal of Economic Entomology* **107**, 11–28.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C. & Bourtzis, K. (2004) *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 15042–15045.

Zabalou, S., Apostolaki, A., Livadaras, I., Franz, G., Robinson, A. S., Savakis, C. & Bourtzis, K. (2009) Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomologia Experimentalis et Applicata* **132**, 232–240.

Zacharopoulou, A. (1987) Cytogenetic analysis of mitotic and salivary gland chromosomes in the medfly *Ceratitis capitata*. *Genome* **29**, 67–71.

Zacharopoulou, A., Augustinos, A.A., Sayed, W.A.A., Robinson, A.S. & Franz, G. (2011*a*) Mitotic and polytene chromosomes analysis of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Genetica* **139**, 79–90.

Zacharopoulou, A., Sayed, W.A.A., Augustinos, A.A., Yesmin, F., Robinson, A.S. & Franz, G. (2011*b*) Analysis of mitotic and polytene chromosomes and photographic polytene chromosome maps in *Bactrocera cucurbitae* (Diptera: Tephritidae). *Annals of the Entomological Society of America* **104**, 306–318.

Zacharopoulou, A., Augustinos, A.A., Drosopoulou, E., Tsoumani, K.T., Gariou-Papalexiou, A., Franz, G., Mathiopoulos, K.D., Bourtzis, K. & Mavragani-Tsipidou, P. (2017) A review of more than 30 years of cytogenetic studies of Tephritidae in support of sterile insect technique and global trade. *Entomologia Experimentalis et Applicata* **164**, 204–225.

Zhang, D., Lees, R.S., Xi, Z., Gilles, J.R. & Bourtzis, K. (2015*a*) Combining the sterile insect technique with *Wolbachia*-based approaches: II – A safer approach to *Aedes albopictus* population suppression programmes, designed to minimize the consequences of inadvertent female release. *PLoS One* **10**, e0135194.

Zhang, D., Zheng, X., Xi, Z., Bourtzis, K. & Gilles, J.R. (2015*b*) Combining the sterile insect technique with the incompatible insect technique: I – Impact of *Wolbachia* infection on the

fitness of triple and double-infected strains of *Aedes albopictus*. *PLoS One* **10**, e0121126.

Zhang, D., Lees, R.S., Xi, Z., Bourtzis, K. & Gilles, J.R. (2016) Combining the sterile insect technique with the incompatible insect technique: III – Robust mating competitiveness of irradiated triple *Wolbachia*-infected *Aedes albopictus* males under semi-field conditions. *PLoS One* **11**, e0151864.

Zug, R. & Hammerstein, P. (2012) Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PloS One* **7**, e38544.

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