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Occurrence of the non-native species *Ophraella communa* on *Ambrosia artemisiifolia* in north-eastern Italy, with records from Slovenia and Croatia

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Abstract

The ragweed leaf beetle, *Ophraella communa* LeSage (Coleoptera Chrysomelidae), is of North American origin and is associated with the Asteraceae, and especially with the invasive neophyte *Ambrosia artemisiifolia* L., which is native to North and Central America and currently naturalized in many parts of Europe, including Italy. The presence of the beetle in northern Italy and southern Switzerland was first noted in 2013. In 2017 it was first reported for Veneto region (north-eastern Italy) and Slovenia, and in 2018 for Croatia. An extensive survey was carried out in summer and autumn 2017 in Friuli Venezia Giulia, after the first accidental finding of some *O. communa* specimens on *A. artemisiifolia* plants at a site on the High Friulian Plain in July. To determine a possible pathway of *O. communa* entry, sites from the nearby eastern Veneto, western Slovenia and north-western Croatia were also inspected. *O. communa* was detected on *A. artemisiifolia* in 70 out of the 77 sampled sites in Friuli Venezia Giulia (54 sites), Veneto (5 sites), Slovenia (7 sites) and Croatia (4 sites). The distribution of *O. communa* on the Friulian and Veneto plains seems to be continuous. In 20% of cases, *A. artemisiifolia* defoliated plants or completely dried plants, lacking reproductive structures and with stem tissues seriously damaged were observed. In some cases, *O. communa* was found on other three Asteraceae, namely *Xanthium orientale italicum* (Moretti) Greuter, *Erigeron canadensis* (L.) Cronquist and *Helianthus annuus* L. The pathway of colonization by the beetle in the study area seems to be from west to east. These are the first records of the beetle for Friuli Venezia Giulia. The beetle seems to spread very rapidly across large areas and is very capable of effective control of the invasive weed, *A. artemisiifolia*.

Key words: invasive exotic species, biological control, distribution, Europe, ragweed, crop weed, pollen allergy.

Introduction

Ophraella communa LeSage (Coleoptera Chrysomelidae) is an oligophagous beetle native to North America (LeSage, 1986; Futuyma, 1990) that feeds on various taxa of the Asteraceae, with common ragweed, *Ambrosia artemisiifolia* L., as the main host (Yamazaki *et al*., 2000; Cao *et al*., 2011; Cardarelli *et al*., 2018; Kim and Lee, 2019). This annual herb, native to North and Central America (Lorenzi and Jeffery, 1987), is currently naturalized in many areas of Asia, Australia and Europe. It is considered as an invasive ruderal and crop weed, abundant in both open and disturbed habitats, with negative impacts on agriculture, human health and biodiversity (Makra *et al*., 2015; Schindler *et al*., 2016; Gentili *et al*., 2017; Montagnani *et al*., 2017). Specifically, its wind-dispersed pollen is one of the primary causes of allergenic problems in sensitive people, manifesting as hay fever, rhino-conjunctivitis and asthma (Bagarozzi and Travis, 1998; Coutinot *et al*., 2008; Bosio *et al*., 2014; Montagnani *et al*., 2017).

O. communa is a multivoltine beetle in which the larvae and adults can completely skeletonize the leaves of common ragweed and cause severe damage to the inflorescences, with negative effects on pollen production, or they may even kill the host plants before flowering (Müller-Schärer *et al*., 2014; Zhou *et al*., 2014; Bonini *et al*., 2016; Cardarelli *et al*., 2018). Fertile adult females overwinter in soil debris, the eggs are laid in clusters on the leaves of the host plant, and the mature larvae spin cocoons on leaves and stems (Meng *et al*., 2007; Bosio *et al*., 2014).

In 1996, *O. communa* was accidentally introduced into Japan (Yamazaki *et al*., 2000) and Taiwan (Wang and Chiang, 1998). In 2000 and 2001, it was also detected in South Korea (Sohn *et al*., 2002) and then in China (Meng and Li, 2005).

The first records of *O. communa* in Europe occurred in 2013 in northern Italy, in several localities of the Lombardy region (Boriani *et al*., 2013), and subsequently in the Piedmont and Emilia Romagna regions (Bosio *et al*., 2014; Nicoli Aldini, 2014), as well as being detected in Canton Ticino in southern Switzerland (Müller-Schärer *et al*., 2014). Most recently, *O. communa* had its first recorded sightings in localities of Veneto (Zanella, 2017), Slovenia (Seljak *et al*., 2017; EPPO, 2018) and in a limited area of central Croatia (Zadravec *et al*., 2019).

It is not clear how *O. communa* arrived in Europe. However, because high levels of attack were observed on *A. artemisiifolia* plants in the area of Milan Malpensa International Airport (Lombardy), its accidental introduction by air traffic has been suggested (Müller-Schärer *et al*., 2014).

O. communa is currently considered as one of the most promising candidates for biological control of common ragweed in several countries of the world, including the European context (Palmer and Goeden, 1991; Meng and Li, 2005; Kiss, 2007; Zhou *et al.*, 2014; Shaw *et al.*, 2016; 2018). Several native European insects that have colonized *A. artemisiifolia* are polyphagous and appear unsuitable for an augmentative release approach because they cause little damage to this invasive neophyte (Igrc *et al.*, 1995; Kiss *et al.*, 2008; Gerber *et al.*, 2011). Other phytophagous insects from the native North American range of *A. artemisiifolia* have been considered as possible candidates for *A. artemisiifolia* control in Europe (Gerber *et al.*, 2011; Müller-Schärer *et al.*, 2014; Kim and Lee, 2019). Among them, *Acontia candefacta* (Hubner) (Lepidoptera Noctuidae) has spread successfully from southern Russia to various eastern and south-eastern European countries (Stojanović *et al.*, 2017), but has been ineffective at controlling common ragweed (Gerber *et al.*, 2011). Moreover, the nearctic *Zygogramma suturalis* (F.) (Coleoptera Chrysomelidae) was released in Croatia, but its populations remained low and the efficacy against common ragweed seemed very limited (Igrc *et al.*, 1995; Reznik *et al.*, 2008).

After our first accidental finding of *O. communa* in July 2017 in Friuli Venezia Giulia, which coincided with the first records reported from Slovenia and Veneto (Seljak *et al.*, 2017; Zanella, 2017; EPPO, 2018), this study was conducted in the same year to verify the occurrence of this beetle, associated with *A. artemisiifolia*, throughout the Friuli Venezia Giulia region and also took into account several sites from the neighbouring Veneto, western Slovenia and north-western Croatia. Moreover, on the basis of the data collected, the occurrence of *O. communa* on other host plants and a possible time and pathway of the beetle's entry into Friuli Venezia Giulia is proposed.

Materials and methods

From July to October 2017, an extensive survey was carried out to detect the presence of *O. communa* in Friuli Venezia Giulia, with a number of inspections also in eastern Veneto (north-eastern Italy), western Slovenia and north-western Croatia.

Visual samplings were conducted in 79 sites colonized by *A. artemisiifolia*: in ruderal and human-disturbed areas such as along roadsides, cultivated and abandoned fields, and in some cases on gravel in river beds and dry grassland alongside rivers. The sampling session at each site ranged from 15 to 30 minutes during which potential host plants were checked. Their number varied from site to site from 10 plants to more than 100 plants. All sites were visited once, except four sites that were visited twice or three times.

Data were recorded for *O. communa* abundance, developmental stages and damage to *A. artemisiifolia* and, occasionally, other herbaceous host plants. When specimens at any stage or eggs of the beetle were detected on the host plants, the damage observed was subdivided into six levels: 0, no trace of feeding damage but adults or eggs of the beetle present; 1, light feeding damage on a few leaves; 2, feeding damage on some leaves; 3, many leaves with feeding damage; 4, defoliation, plants partially dried; 5, complete defoliation and reproductive structures injured.

The sites were mapped using Google Earth (©2007 Google TM), which provided the coordinates and the altitude for each site, and the traits of the investigated habitats were included. All these data, with the different stages of the beetle observed on plants, are presented in the supplemental table S1.

Several *O. communa* specimens were collected and transferred to the laboratory. Larvae and pupae were reared to the adult stage. The studied material was deposited in the entomological collection of the Department of Agricultural, Food, Environmental and Animal Sciences - Entomology, University of Udine (Italy).

Results and discussion

First record

O. communa was first detected in the Friuli Venezia Giulia region on 13 July 2017 on some *A. artemisiifolia* plants growing at a site along the right bank of the Tagliamento river (Spilimbergo, Pordenone district) (site 14, supplemental table S1). Some host plants were discovered with leaves severely damaged by the beetle, and numerous larvae and cocoons, and a number of adults were observed and collected.

Distribution and occurrence in different habitats

O. communa was detected in 70 out of the 77 sites surveyed with *A. artemisiifolia* plants present in the study areas of Friuli Venezia Giulia (54 out of 56 sites), eastern Veneto (5 out of 5 sites) western Slovenia (7 out of 9 sites) and north-western Croatia (4 out of 7 sites) (figure 1 and supplemental table S1). In addition, at two Friulian sites without *A. artemisiifolia* present, *O. communa* was observed on other two Asteraceae, namely *Helianthus annuus* L. and *Erigeron canadensis* (L.) Cronquist (sites 39 and 60, respectively, supplemental table S1).

In the Friuli Venezia Giulia region the beetle was detected on *A. artemisiifolia* plants growing in urban or ruderal areas or along roadsides (63%), in cultivated areas or abandoned fields (24%) and in less human-disturbed areas located on gravels on the margins of the Tagliamento river or on "magredi" land (broad alluvial areas of gravel and sand) (13%) (figure 1 and supplemental table S1). In the Friulian hills and mountains (sites with altitude over 250 m a.s.l.), 10 out of 12 sites with *A. artemisiifolia* plants growing along the verges of main roads were infested with *O. communa* (figure 1 and supplemental table S1). In particular, the beetle was found along the Canal del Ferro valley, but did not occur along the higher part of the Valcanale valley (sites 19-22 *vs.* sites 23 and 24) (supplemental table S1).

In the nearby Veneto region, *A. artemisiifolia* plants infested with the beetle were located in some urban areas and along farm roads (supplemental table S1).

Concerning the Friulian and Veneto plains, the distribution of *O. communa* was continuous for over 4,000 km^2 with the beetle being present in all sampled habitats that were colonized by common ragweed.

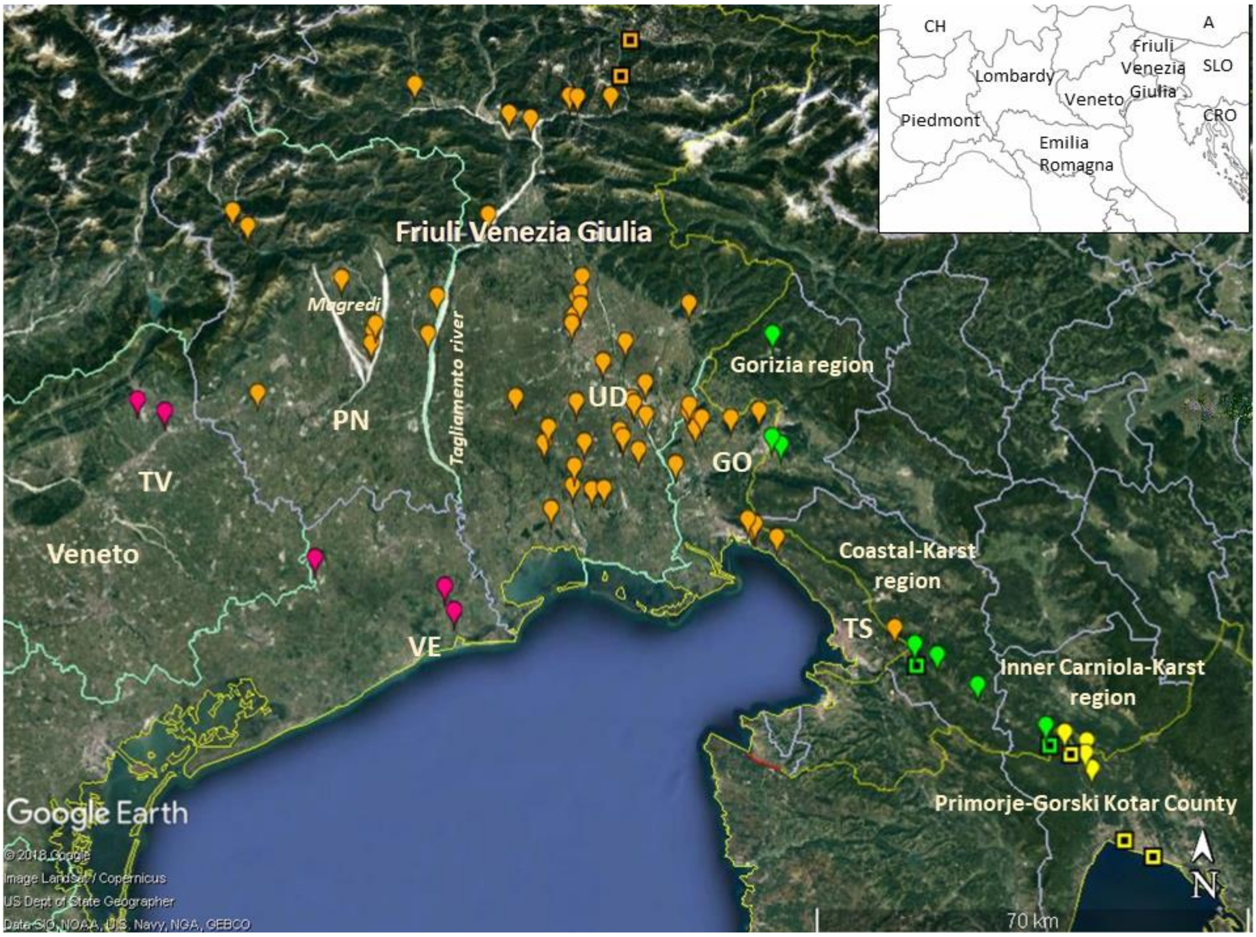

Figure 1. Survey of *O. communa* on *A. artemisiifolia* carried out in 2017 in north-eastern Italy, western Slovenia and north-western Croatia. Sites with the occurrence (drops) and absence (squares) of *O. communa* on *A. artemisiifolia*, and in a few cases on *X. orientale italicum*, *E. canadensis* and *H. annuus* (as reported in supplemental table S1). In Italy, sites in the Friuli Venezia Giulia region are marked in orange: PN Pordenone, UD Udine, GO Gorizia, TS Trieste; sites in the Veneto region are marked in violet: TV Treviso, VE Venezia. In Slovenia, sites are marked in green: Gorizia region, Coastal Karst region, Inner Carniola-Karst region. In Croatia, sites are marked in yellow: Primorje-Gorski Kotar County.

On the territory of western Slovenia contiguous to the Italian national border, the beetle was always found on *A. artemisiifolia* plants growing along suburban roads, such as rest areas or construction yards (figure 1 and supplemental table S1). In the same period, the first records of *A. artemisiifolia* damaged by the beetle were reported in western Slovenia (Seljak *et al*., 2017).

In north-western Croatia, *O. communa* was detected on common ragweed along roadsides in urban and suburban areas (figure 1 and supplemental table S1).

Damage and population levels

Considering the 70 sites with *O. communa* presence on *A. artemisiifolia*, in 20% of cases high levels of defoliation were observed (figure 2). In particular, plants were detected partially dried due to feeding traces on leaves and stem tissue (9% of sites, damage level 4) or plants were even completely dried with the reproductive structures also injured (11% of sites, damage level 5). In 57% of cases, feeding traces on some leaves (28% of sites, damage level 2) or most leaves (29% of sites, damage level 3) were recorded. Lastly, in 23% of cases light feeding traces occurred on a few leaves (17% of sites, damage level 1) or even no trace of feeding was evidenced on the plants (6% of sites, damage level 0).

High damage levels to *A. artemisiifolia* were often associated with high *O. communa* population densities (supplemental table S1). In these cases, over 20-30 adults and larvae feeding on each plant were detected and a large number of cocoons, empty or containing pupae, attached to leaves and stems, and eggs had been laid in clusters on both leaf surfaces. Low population densities of *O. communa* were observed on plants with less severely damaged leaves. In these cases, only some larvae and adults, and few egg clusters and pupae per plant were counted. At sites where low numbers of adults or egg clusters were observed, feeding activity was not evident.

Overall, the highest levels of feeding traces (damage levels 3-5) due to the beetle were observed in a half of the Italian sites from the Friulian and Veneto plains as well as one site from the Gorizia region valley in western Slovenia next to the Italian border (table 1 and supplemental table S1). In contrast, in almost all the study

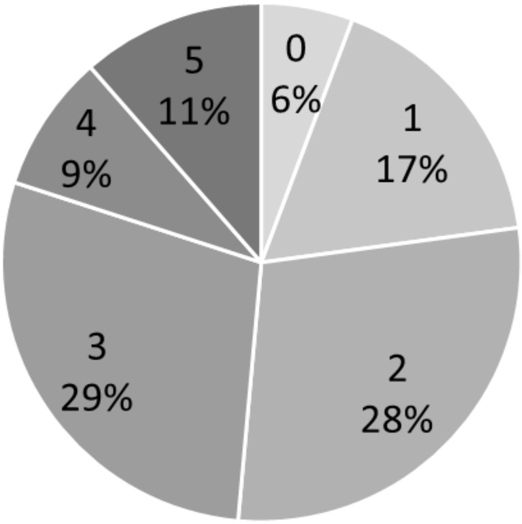

Figure 2. Percentages of the total 70 sites in north-eastern Italy, western Slovenia and north-western Croatia with *O. communa* different damage levels on *A. artemisiifolia* observed in 2017. Damage levels considered: 0, no trace of feeding damage but adults or eggs of the beetle present; 1, light feeding damage on a few leaves; 2, feeding damage on some leaves; 3, many leaves with feeding damage; 4, defoliation, plants partially dried; 5, complete defoliation and reproductive structures injured.

sites towards the south-east, i.e. from those located in the Trieste district in Italy to those easternmost in Croatia, the lowest levels of damage were found (levels 0-1) although the samplings were done at the end of the summer (table 1 and supplemental table S1).

Friulan sites visited in late August-October demonstrated greater severities of *A. artemisiifolia* attack by *O. communa* than those visited in July-early August. Other studies have also reported that the maximum amount of damage due to ragweed leaf beetle occurs at the end of the *A. artemisiifolia* growing season (Miyatake and Ohno, 2010; Fukano *et al*., 2013; Cardarelli *et al*., 2018).

Overlapping of generations

Adults, eggs, larvae and cocoons of *O. communa* were detected on *A. artemisiifolia* plants from July to September, indicating a general overlapping of summer generations. Larvae and adults of the beetle were observed feeding on leaves and inflorescences, mature larvae had spun cocoons on leaves and stems, and eggs were laid in clusters on the leaves. In late October, only adults were found, except in the case of one site where some larvae were also detected (site 21, supplemental table S1). It is already known from the literature that the beetle overwinters as an adult female (Palmer and Goeden, 1991; Tanaka *et al*., 2015; Kim and Lee, 2019).

Population increase during the season

The capacity of the beetle populations to increase during summer and autumn has been observed at a number of sites on the Veneto and Friulian plains that were visited more than once, with *A. artemisiifolia* plants growing in dense stands (sites 4, 27, 29 and 51, supplemental table S1). At one of these sites (site 29), neither *O. communa* specimens nor damage to plants due to the beetle were detected in mid-July, whereas in mid-August many mating adults and eggs laid on leaves were observed, and in mid-October the majority of plants were completely defoliated with several beetle adults present on them. At the other three sites, the beetle was already detected in mid-summer on partially damaged plants, but in mid-October plants had completely died with several *O. communa* adults still being

Table 1. Number of visited sites in 2017 in north-eastern Italy, western Slovenia and north-western Croatia, with different levels of damage caused by *O. communa* to *A. artemisiifolia*. Damage levels considered: 0, no trace of feeding damage but adults or eggs of the beetle present; 1, light feeding damage on a few leaves; 2, feeding damage on some leaves; 3, many leaves with feeding damage; 4, defoliation, plants partially dried; 5, complete defoliation and reproductive structures injured. *FVG: Friuli Venezia Giulia.

Geographic area	Number of sites with *O. communa* and damage to *A. artemisiifolia* (levels 0-5)						Number of sites without *O. communa*
	0	1	2	3	4	5	
North-eastern Italy	-	8	18	19	6	8	2
Venezia district (Veneto)	-	-	*1*	*1*	-	*1*	-
Treviso district (Veneto)	-	-	*1*	-	-	*1*	-
Pordenone district (FVG)*	-	*2*	*3*	*3*	-	*1*	-
Udine district (FVG)	-	*3*	*11*	*10*	*6*	*3*	*2*
Gorizia district (FVG)	-	-	*2*	*5*	-	*2*	-
Trieste district (FVG)	-	*3*	-	-	-	-	-
Western Slovenia	2	2	2	1	-	-	2
Gorizia region			*2*	*1*			
Inner-Karniola Karst region	*1*						*1*
Costal Karst region	*1*	*2*					*1*
North-western Croatia	2	2	-	-	-	-	3
Primorje-Gorski Kotar County	*2*	*2*					*3*
Total number of sites	4	12	20	20	6	8	7

present on them. Because the population density of the beetle increased at these sites throughout the season, the damage level on the plants was also the highest at the end of summer/beginning of autumn.

Host range

At two sites, adults, larvae, pupae and eggs of *O. communa* were found on *Xanthium orientale italicum* (Moretti) Greuter (Asteraceae), with the plants showing damage on young apical leaves (sites 5 and 15, supplemental table S1). Individuals of this plant species grew in mixed stands with *A. artemisiifolia* plants infested with the beetle. *X. orientale italicum* has already been reported as a host of *O. communa* (Yamazaki *et al*., 2000; Bosio *et al*., 2014).

At one site, many adults of *O. communa* were detected feeding on leaves of the Canadian horseweed, *E. canadensis*, during flowering (site 60, supplemental table S1). This plant was also reported as host for *O. communa* by Bosio *et al.* (2014).

In addition, some *O. communa* adults were observed on leaves of one plant of ornamental sunflower, *H. annuus*, at one site (site 39, supplemental table S1). However, the damage caused by the beetle to this economically important crop has been reported as negligible (Dernovici *et al*., 2006; Cao *et al*., 2011; Zhou *et al*., 2011; Müller-Schärer *et al*., 2014; Lommen *et al*., 2017).

In cultivated areas, the beetle was only associated with *A. artemisiifolia* in maize, potato or soybean fields. In vineyard inter-rows with herbaceous vegetation and in abandoned fields (sites 52 and 54, supplemental table S1), common ragweed plants grew alongside other Asteraceae, such as *Artemisia verlotiorum* Lamotte and *Erigeron annuus* (L.) Pers., and weeds belonging to other families, such as *Amaranthus retroflexus* L. (Amaranthaceae), *Chenopodium album* L. (Chenopodiaceae), *Portulaca oleracea* L. (Portulacaceae) and *Sorghum halepense* (L.) Pers. (Poaceae). Neither the beetle nor traces of feeding damage on leaves were observed on any of these plants. In a disturbed dry grassland on the right bank of the Tagliamento river (site 13, supplemental table S1), other than the scattered stands of *A. artemisiifolia* that were infested by the beetle, the following plants were also detected: *E. annuus*, and *X. orientale italicum* in the surroundings, as well as *Amorpha fruticosa* L. (Fabaceae), *Bothriochloa ischaemum* (L.) Keng (Poaceae), *Bromus erectus* Huds. (Poaceae), *Oenothera biennis* L. (Onagraceae), *Sanguisorba minor* Scop. (Rosaceae) and *Scabiosa triandra* L. (Dipsacaceae). All of these plants lacked any trace of feeding damage or the presence of any specimens of *O. communa*.

Time of introduction

It is likely that *O. communa* arrived in Friuli Venezia Giulia not long before 2017 because among the flora investigations conducted along the middle course of the Tagliamento river, where the first finding occurred in July 2017 (site 14, supplemental table S1), no damaged *A. artemisiifolia* individuals were detected in the previous years (summer 2011-2016). Furthermore, a specific survey carried out in 2014 at several localities in Friuli Venezia Giulia by the Regional Agency for Rural Development (ERSA) on the occurrence of the ragweed leaf beetle produced no sightings (Carpanelli and Valecic, 2016). Moreover, in agreement with the results of the present study, a fairly recent occurrence of *O. communa* in eastern Veneto (Zanella, 2017) and western Slovenia (Seljak *et al*., 2017) has been presumed.

However, the possible presence of *O. communa* in Friuli Venezia Giulia had been hypothesized since 2015. In fact, a lower level of common ragweed pollen in the air was detected in 2015 compared to the average level of previous years (source data from the regional pollen monitoring network: ARPA FVG, 2015). A reduction in airborne ragweed pollen concentration was previously reported for Lombardy (Milan area) in northern Italy and Canton Ticino in southern Switzerland after the beetle was detected in these areas (Müller-Schärer *et al*., 2014; Bonini *et al*., 2015; 2016).

Pathway and rapidity of colonization

Taking into account the very high population levels of *O. communa* detected so far in the regions of Lombardy and Piedmont (Bosio *et al*., 2014; Müller-Schärer *et al*., 2014) and the generally lower population density of the beetle detected in the present study, its colonization in north-eastern Italy seems to have taken place from west to east. In the Friuli Venezia Giulia region, where the sampling effort was denser, the population levels of the beetle seemed the highest in the mid plains. Thus, a contiguous distribution of the beetle westward to the Veneto region could suggest its invasion from this latter region and spread towards the east. However, an accidental introduction of the beetle at a site on the Friulian mid-plain and its spread towards the east would also be possible. In any case, entry of *O. communa* into Friuli Venezia Giulia from the east is the least plausible due to i) the very low population levels of the beetle recorded at sites located from the Trieste district to Croatia (table 1), and ii) the discontinuous distribution of these populations, especially during the September-October period when higher densities of the beetle and higher damage levels on plants would be expected. In fact, in the study areas from Slovenia and Croatia, it is possible that the *O. communa* population had no time to increase in number during the July-August period, unlike the sites located on the Friulian plains toward Veneto that were well colonized by the beetle.

In 2018, the beetle was detected in central Croatia, at several localities of the Sava river basin near the border with Bosnia and Herzegovina (Zadravec *et al*., 2019). The damage levels to *A. artemisiifolia* plants were never as high as in Italy and Switzerland. In further samplings carried out towards both the east and north-west of this area the species was not observed (Zadravec *et al*., 2019), suggesting a rather recent colonization of this part of Croatia.

Within about four years of the first records in Italy (Boriani *et al*., 2013), the beetle has reached the western areas of Slovenia and the north-western areas of Croatia, which are over 400 km away from the presumed site of its introduction into Europe at the Milan Malpensa International Airport (Müller-Schärer *et al*., 2014).

Therefore, the species has demonstrated a high dispersal potential. It has the capacity to expand more than 70 km per year by flight (Kim and Lee, 2019), but even greater speed of expansion can be assumed via human transportation. These data are in agreement with observations conducted on the rapid spread of *O. communa* at the beginning of its colonization of Japan (Moriya and Shiyake, 2001).

Further observations in the higher part of the Valcanale valley in the Friuli Venezia Giulia region, where the species was not found, will give us additional information about the colonization pattern of this potential biocontrol agent of *A. artemisiifolia*. In fact, in the early phases of colonization in Lombardy (northern Italy), *O. communa* had been observed at a site in the lower part of the Valtellina valley, but it was absent from sites in the High Valtellina valley (Augustinus *et al*., 2015).

Concluding remarks

A high efficacy demonstrated by *O. communa* against the invasive neophyte *A. artemisiifolia* and a very poor effect against other non-target plants could encourage national authorities of a number of European countries (e.g., Italy, France, Switzerland and Hungary; see Kiss, 2007; Müller-Schärer *et al*., 2014; Bonini *et al*., 2016; Shaw *et al*., 2016; Cardarelli *et al*., 2018) to promote deliberate release of the beetle in new areas affected by common ragweed, applying classical biological control. In fact, in a recent study (Mouttet *et al*., 2018), it was estimated that establishment of *O. communa* in the Rhône-Alpes region in south-eastern France could lead to a 50% reduction in the numbers of days during which ragweed pollen concentrations cause sensitive people to express allergic symptoms. Regarding the containment of *A. artemisiifolia* by *O. communa*, our results are in agreement with those from other studies (see for Italy: Müller-Schärer *et al*., 2014; Bonini *et al*., 2016; Cardarelli *et al*., 2018; and for Asia: Guo *et al*., 2011; Zhou *et al*., 2014). Indeed, according to a recent distribution model of the biocontrol agents predicted across the range of *A. artemisiifolia* in Europe (Sun *et al*., 2017), *O. communa* seems to be the most suitable natural antagonist of this weed in Italy.

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