



Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle, *Ophraella communa*, to native European plant species

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ABSTRACT

In 2013, the North American oligophagous leaf beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), was found in Europe for the first time. Recent studies in Northern Italy and Southern Switzerland record extensive defoliation by *O. communa* on its preferred host, common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae, Tribe Heliantheae), and reductions in its flowering and seeding. In some regions in Northern Italy, this has reportedly led to a > 80% depletion in airborne *A. artemisiifolia* pollen concentrations. The potential for non-target damage by *O. communa* to closely related native European plant species was previously unknown. During extensive field surveys covering 18 populations of nine potential non-target species, we found adult *O. communa* on a single plant individual. In a common garden field experiment in Northern Italy in an area with high *O. communa* densities, leaf damage was highest on two other Asteraceae species, *Dittrichia graveolens* (L.) Greuter and *Pentanema helveticum* (Weber) D.Gut.Larr. (both in the Tribe Inuleae). While adult feeding was observed on most of the test plant species, only *D. graveolens*, which has recently extended its range and is now an invasive species in Western Europe, sustained all life stages of *O. communa* in the common garden and in laboratory experiments. We found no evidence of substantial non-target effects by *O. communa* that could potentially threaten populations of European native plant species that are taxonomically closely related to ragweed.

1. Introduction

The rate of biological invasions has substantially increased over the past 200 years and the present rate of new introductions is expected to continue (Seebens et al., 2017). Invasive alien plant species can cause devastating impacts on ecosystems and ecosystem services (Vilà et al., 2011). With new introductions of invasive weed species, accidental introductions of their natural enemies are also expected to increase: such introductions are already a relatively common phenomenon, with some positive outcomes (Fand et al., 2013; Shaw et al., 2018). For example, the accidentally introduced weevil *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Eirrhinidae) has controlled water fern, *Azolla filiculoides* Lam., populations in Great Britain (Bacon et al., 2018), and the cochineal insect *Dactylopius opuntiae* (Cockerell) (Hemiptera: Coccoidea: Dactylopiidae) has suppressed the invasive cactus, *Opuntia ficus-indica* (L.) Mill., in Spain (Shaw et al., 2016).

While accidental introductions of natural enemies of invasive weeds

may result in successful control of the target weed, they bear the risk of non-target effects on taxonomically closely related crops, ornamental plants, or native species. A crucial first step in a classical biological control programme is the assessment of the candidate's host specificity (Müller-Schärer and Schaffner, 2008). In pre-release studies, native and economically important plant species are selected for host range testing based on the centrifugal phylogenetic hypothesis (Wapshere, 1974), which posits that the likelihood that a non-target species will be attacked by a biological control agent decreases with increasing phylogenetic distance between the non-target and the target species. After selecting suitable plant species, host range tests are conducted to assess whether the plants are within the fundamental or realized host range of the potential biological control agent. The fundamental host range is the range of plants on which the biological control agent can complete its life cycle. The realized host range is the range of hosts that are attacked under natural conditions and tends to be considerably narrower than the fundamental host range (Fowler et al., 2012; Schaffner et al.,

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2018). In classical biological control of weeds, potential biological control agents whose fundamental host range includes native or economically important species are often not approved for field release. This conservative approach is one explanation of why significant non-target attack by deliberately introduced classical biological control agents of weeds is rare (Hinz et al., 2019).

The ragweed leaf beetle, *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), is an oligophagous insect native to North America. It preferentially feeds on common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae), which is also native to North America and invasive in different parts of the world, including Australia, Asia and Europe (Montagnani et al., 2017). In Europe, it is a noxious agricultural weed with a wide distribution mainly in central and Eastern Europe (Bullock et al., 2012; Gentili et al., 2017; Kazinczi et al., 2009; Skjøth et al., 2019), resulting in significant economic losses in agricultural systems (Kazinczi et al., 2008; Kómvives et al., 2006). Moreover, this monoecious and wind-pollinated plant is of concern due to its highly allergenic pollen, which causes serious health problems and costs in the invaded range (Essl et al., 2015; Müller-Schärer et al., 2018; Schaffner et al., 2020).

Ophraella communa was declared as not safe for deliberate release in Australia after it was shown to be able to complete its life cycle on sunflower under laboratory conditions (Palmer and Goeden, 1991). However, it was detected in Japan and Taiwan in 1996 (Wang and Chiang, 1998; Takizawa, 1999), probably due to accidental introductions, and subsequently in South Korea (Sohn et al., 2002) and China (Meng et al., 2007). In China, the beetle was first reported in 2001. A wide range of subsequent laboratory and field experiments, as well as field surveys, did not find significant non-target effects by *O. communa* on sunflower (Zhou et al., 2011). Since then, the beetle has been mass-reared and mass-released for the biological control of *A. artemisiifolia* in various locations (Guo et al., 2011; Zhou et al., 2017) and has become widely distributed in China (Sun et al., 2017).

More recently, *O. communa* was accidentally introduced into Europe, where it was first found in Northern Italy and Southern Switzerland in 2013 (Müller-Schärer et al., 2014). The beetle spread across Northern Italy (Augustinus et al., 2015; Lommen et al., 2017) and reached Croatia in 2018 (Zadravec et al., 2019). It has been found in high densities in Northern Italy, defoliating the host plant and reducing flower and seed production, where aerial *A. artemisiifolia* pollen concentrations in some regions have dropped by 80%, a change that could not be explained by meteorological data or land-use changes (Bonini et al., 2015a, 2015b). Projections of the potential range and abundance of *O. communa* suggest that it could reach population densities comparable to those in Italy in other parts of Europe, particularly on the Balkan Peninsula (Supplementary Fig. 1, Augustinus et al., 2020). The potential economic benefits of *A. artemisiifolia* control by *O. communa* in Europe are likely to be considerable (Mouttet et al., 2018; Schaffner et al., 2020).

Preliminary studies indicate that *O. communa* does not pose a substantial risk to sunflower or to ornamentals (Müller-Schärer et al., 2017). However, *O. communa* has not yet been submitted to careful host range screening with native European plant species. Thus, there is a need to evaluate its biosafety in Europe to accurately balance the benefits reported so far with the potential risks that could be caused by this accidentally introduced biological control agent. In this paper we report on a two-year field survey, a common-garden experiment and laboratory studies to assess the likelihood of a non-target attack on native European plant species, with a focus on rare and endangered plant species closely related to *A. artemisiifolia*.

2. Material and methods

2.1. Study organisms

Ambrosia artemisiifolia is an annual Asteraceae, which has invaded

ranges in every continent except Antarctica (Essl et al., 2015; Montagnani et al., 2017). This predominantly outcrossed plant produces racemes with flowerheads containing flowers that produce highly allergenic pollen, causing allergic rhinitis and severe asthma (Thibaudon et al., 2010), which result in high medical costs and reduction in quality of life among the allergic population. Estimates from one region (Rhônes-Alpes) in southern France amounted to € 5–7 million per year (Mouttet et al., 2018) and estimates for health care reductions in Europe are projected to exceed € 1.1 billion per year once the beetle has exploited its full range (Schaffner et al., 2020).

Ambrosia artemisiifolia plants are highly prolific seed producers, and seeds remain viable in the ground for more than 40 years (Toole and Brown, 1946). In Northern Italy, the first seedlings emerge in early April, but the germination period is protracted (Kazinczi et al., 2008) and germination rates are variable (Fogliatto et al., 2019). Plants release pollen in August and September (Bonini et al., 2015a) and produce seeds from mid- to late-September (Lommen et al., 2018). *Ophraella communa* is a multivoltine, oligophagous herbivore which can achieve up to seven generations per year in its introduced range in southern China (Zhou et al., 2014) and up to four generations in Europe (Mouttet et al., 2018). The leaf beetle overwinters in the adult stage and, in Europe, the first eggs are found in spring as soon as *A. artemisiifolia* seedlings emerge (Bosio et al., 2014). The females lay eggs in batches of 10–60 eggs, and the three larval stages and the highly mobile adults feed on the green parts of their host plant (Müller-Schärer et al., 2014).

To gain an overview of host species known to date, we searched via Google Scholar for “*Ophraella communa*” AND ‘host’, resulting in 633 hits. We followed the list of publications according to their apparent relevance to our study. Papers, including host species already investigated by studies already on the list, were not included if they did not use a novel method or technique (laboratory or field, choice or no choice experiments). We only included plant species for which both larval and adult *O. communa* feeding was reported, since the highly mobile larvae and adults can be found on plants far beyond their host range, and scoring these incidental observations would overpredict both the ecological and fundamental host range of the beetle.

We focused on native plant species present in Northern Italy (i.e., the Lombardy Region) and in Southern Switzerland that belong to the tribes *Coreopsideae* and *Inuleae* and from which populations were found < 6 km away from *A. artemisiifolia* and *O. communa* populations. These tribes are the most closely related taxa to the tribe *Heliantheae* (sunflowers): the latter has no native species in the study region. For the laboratory experiments, we included two species (*Pentanema britannicum* L. and *Pentanema helveticum* (Weber) D.Gut.Larr.) that were not found in the study area, but could be at risk if the beetle will cross the Alps. We also considered *Centaurea nigrescens* Willd., a more distantly related plant species from the tribe *Cardueae*, as adult *O. communa* feeding was observed in the field on this plant species in summer 2015 (B. Augustinus, pers. obs.). In total, we included ten native plant species in our study. Of these, according to the Red List of Switzerland and based on the IUCN categories and criteria at the regional level (Bornand, 2016) (Table 1), one species is categorized as near threatened (NT), two species as vulnerable (VU) and four species as endangered (EN). Some of these are also included in the list of species protected in the Lombardy Region (Regione Lombardia, 2010). In addition, we selected one species native to Southern Europe and Northern Africa, *Dittrichia graveolens* (L.) Greuter, and the North American species *Bidens frondosa* L., which is naturalized in the study region, to increase the number of representatives of the *Coreopsideae* tribe (Table 1). To illustrate the relationship between the test plant species to *A. artemisiifolia*, we constructed a phylogenetic tree using iTOL (interactive tree of life) v4 (Letunic and Bork, 2019) (Supplementary Fig. 2).

Table 1
Plant species examined in this study.

Tribe	Name	EPPO code	State of threat	Field survey	Common garden field	Laboratory	Flowering time in month
Heliantheae	<i>Ambrosia artemisiifolia</i> L.	Ambar	NP	X	X		8–10
Coreopsideae	<i>Bidens cernua</i> L. *	Bidce	VU	X			7–9
Coreopsideae	<i>Bidens frondosa</i> L. ^a	Bidfr	NP	X			8–10
Inuleae	<i>Carpesium cernuum</i> L.	Carce	EN	X			7–8
Inuleae	<i>Dittrichia graveolens</i> (L.) Greuter ^b	Ditgr	NP		X	X	8–11
Inuleae	<i>Pentanema britannicum</i> (L.) D.Gutt.Larr. (<i>Inula britannica</i>)	Penbr	EN		X	X	7–9
Inuleae	<i>Pentanema conyzae</i> (DC.) D.Gut.Larr. (<i>Inula conyzae</i>)	Penco	LC	X	X	X	7–10
Inuleae	<i>Pentanema helveticum</i> (Weber) D.Gut.Larr. (<i>Inula helvetica</i>)	Penhe	VU		X	X	7–9
Inuleae	<i>Pentanema hirtum</i> (L.) D.Gut.Larr. (<i>Inula hirta</i>)	Penhi	EN	X	X	X	6–7
Inuleae	<i>Pentanema salicinum</i> (L.) D.Gut.Larr. (<i>Inula salicina</i>)	Pensa	NT	X	X	X	6–9
Inuleae	<i>Pentanema spiraeifolium</i> (L.) D.Gut.Larr. (<i>Inula spiraeifolia</i>)	Pensp	EN	X			7–10
Inuleae	<i>Xerolekia speciosissima</i> (L.) Anderb * / **	Xersp	NE	X			7–8
Cardueae	<i>Centaurea nigrescens</i> Willd. ^b	Cenni	LC	X	X	X	6–8

Plant species studied for potential non-target effect of *Ophraella communa* feeding, ordered in phylogenetic relationship to *Ambrosia artemisiifolia* (cf. Supplementary Fig. 2). State of threat is according to the “Red List of Vascular Plants” of the Swiss Federal Office for the Environment (NP = neophyte, LC = least concern, NT = near threatened, VU = vulnerable, EN = endangered, NE = not evaluated) (Bornand, 2016). * = species under legal protection (Regione Lombardia, 2010); ** = endemic species in Northern Italy. ^a: plant species selected to increase the number of representatives of the *Coreopsideae* tribe. ^b: selected because feeding was observed in the field.

2.2. Field survey

Because the accidentally introduced *O. communa* has established in a region where several native plant species related to the target weed occur naturally, we conducted a field survey to assess the levels of non-target effects by this biological control agent under field conditions. We referred to ‘info flora’ (<https://www.infoflora.ch/en/>), and received advice from local botanists, to locate populations of the selected non-target species in Southern Switzerland. We selected plant populations that were close to field sites (< 6 km; Supplementary Fig. 3 and Supplementary Table 1) with observations of *A. artemisiifolia* and *O. communa* made between 2013 and 2015. In 2016 and 2017, we visited five sites in Southern Switzerland and found a total of ten populations of three native endangered species, two populations of two native species of least concern (Bornand, 2016; Info flora) and one population of *B. frondosa* (Table 1). We also surveyed eight populations with three additional plant species (a species endemic to Italy, and two species protected by law) in the Lombardy region in Italy (Table 1, Supplementary Tables 1 and 2). Additionally, we surveyed five populations of *A. artemisiifolia* that were near surveyed plant populations to confirm whether *O. communa* was present.

We monitored all selected plant populations between the end of June and the beginning of August, i.e., during peak abundance of *O. communa*. For plant populations of less than 50 individuals, we checked all plants for the presence of *O. communa* eggs, larvae and adults. For plant populations with more than 50 individuals, we divided the site into four equal sectors and sampled 50 plants per site, with at least 10 plants sampled in each of the four sectors. Where possible, we sampled individual plants at a minimum distance of 1 m from each other. If the population density of the plants was high enough, we achieved this by walking through the sectors in straight transects, choosing plants along the transect. We searched every leaf of the selected plants for *O. communa* presence. Leaf damage without any sign of *O. communa* was recorded as ‘no damage by *O. communa*’, since the plants surveyed typically also had native herbivores feeding on them.

2.3. Common garden field experiment

Plants for the common garden experiment and the laboratory experiments (see below) were grown from seeds (for the origin of the seed material, see Supplementary Table 3). The seeds were germinated on blotting paper in Petri dishes at day/night temperatures of 26/18 °C, a

photoperiod of L:D 16:8 hr and relative humidity between 50 and 70%. Seedlings were first transplanted into seed trays and then, when the plants were 15–33 cm tall, to 10 cm diameter pots filled with a mix of 1/3 sand and 2/3 standard garden soil (Selmaterra, Eric Schweizer AG, Thun, Switzerland). Plants used in the experiments were all in the vegetative stage.

On 10 July 2017, seven non-flowering individuals from each of seven native plant species (Table 1) were transplanted from the greenhouse to an experimental field near Magnago, Lombardy, Italy (45.581°N, 8.793°E), which had a moderate density of *A. artemisiifolia* in the same field, high densities of *A. artemisiifolia* in adjacent fields (~150 m away), and high numbers of *O. communa*. Before transplanting, we mowed the experimental plot to ensure that the test plant species were not outcompeted by the resident vegetation, and we planted the experimental plants in a Latin-square design (seven rows with seven individuals per plant species), with 50 cm distances between the plants. Plants were watered twice a week to prevent desiccation, since some of these species normally grow in moister environments. We left three naturally occurring *A. artemisiifolia* plants in close proximity (~3 m) to the experimental plot as an indicator for *O. communa* presence at the site. All other ragweed plants within a radius of 10 m around the experimental plot were repeatedly removed. At three-weekly intervals, we measured plant sizes, monitored the plants for the presence of *O. communa* and counted the number of egg batches, eggs per egg batch, larvae at the 1st, 2nd or 3rd instar stage, the number of pupae and adults, and estimated the percent leaf damage by *O. communa*.

2.4. Laboratory experiments

We conducted three replicates of two different laboratory experiments in 2017: (i) on non-target plant species exposed to adults of *O. communa*; and (ii) on non-target species exposed to newly-hatched larvae. We set up cages with six plants (one individual of *D. graveolens*, *P. helveticum*, *P. britannicum* and *P. salicinum*, and two *C. nigrescens* plants from different populations), and released either one adult per plant (3♂, 3♀ per cage), or four larvae on each of the plants. The plants were kept in six plastic cat litter boxes (35 × 45 × 15 cm), each 10 cm away from the others, in a randomized design. Each box was placed in a netted cage (60 × 60 × 60 cm) in the quarantine facility of the University of Fribourg.

The adults and larvae used were reared in the quarantine facility

from adults, eggs and pupae sourced in Northern Italy during 2013. To minimize inbreeding, the laboratory stocks were repeatedly supplemented by field populations taken from the same localities as the original laboratory population. The quarantine facility was kept at a day/night temperature of 25/16 °C, a photoperiod of L:D 14:10 hr and a relative humidity of 50 ± 5%. To ensure that freshly emerged adults (< seven days old) were used, pupae were collected one week before the experiments were started and kept in a separate container on an *A. artemisiifolia* plant. Freshly hatched larvae were taken from egg batches that were transferred on damp filter paper in Petri dishes one week before the experiments. Nine and 21 days after the start of the experiments we counted the number of egg batches, larvae, adults and recorded feeding damage. Eggs that were counted at day 9 were not counted a second time since eggs take < 9 days to hatch. Larvae were likely double-counted, since only two larvae pupated during the experiment.

2.5. Statistical analyses

The data from the common garden experiment and the laboratory experiments were analysed by assessing the effects of plant species on the sum of the number of egg batches, larvae and adults observed during the experiment, as well as on percent leaf damage at the end of the experiment. For the experiments using adults of *O. communa*, we compared the sum of the number of egg batches and adults as well as the percent leaf damage by plant species at the end of the experiment. For the experiments using larvae, we assessed the effects of plant species on the sum of the number of larvae observed on the two sampling occasions, and on percent leaf damage at the end of the experiment. All comparisons were made using a Kruskal-Wallis test because of the non-normal distributions of the data, and groups were identified post-hoc using a Fisher's least significant difference test. All analyses were conducted in R version 3.6.1, using the packages *readxl* (Wickham and Bryan, 2016), *tidyr* (Wickham and Henry, 2017), and *agricolae* (de Mendiburu, 2019). Figures were produced in *ggplot2* (Wickham, 2009) and *ggpubr* (Kassambara, 2019).

3. Results

3.1. Reported host range of *O. Communa*

Previous no-choice host specificity tests with *O. communa* have revealed that it can complete its life-cycle on several plant species in the tribe Heliantheae (Bosio et al., 2014; Cao et al., 2011; Cardarelli et al., 2018; Futuyama, 1990; Hu and Meng, 2007; Lee et al., 2007; Lommen et al., 2017; Palmer and Goeden, 1991) (Supplementary Table 4). However, only a few test plant species from other tribes within the Asteraceae were included in these no-choice tests. On *Dahlia pinnata* Cav., the only species in the tribe Coreopsidae tested, six eggs were laid by *O. communa*, but neither adults nor larvae fed on the test plant (Palmer and Goeden, 1991). In the field, *O. communa* has primarily been recorded on species within the subtribe Ambrosiinae, including several *Ambrosia* and *Xanthium* species, *Parthenium hysterophorus* L., *Iva axillaris* Pursh., but also from *Ratibida pinnata* (Vent.) Barnhart (subtribe Rudbeckiinae) and *Helianthus ciliaris* DC. (subtribe Helianthinae) (Dernovici et al., 2006; Futuyama and McCafferty, 1990; Goeden and Ricker, 1985; McFadyen and McClay, 1981; Palmer and Goeden, 1991; Watanabe and Hirai, 2004). In earlier studies, sustained feeding by *O. communa* was only observed on plants in the tribes Ambrosiinae and Rudbeckiinae (Supplementary Table 4).

3.2. Field survey

During the 43 visits conducted in 2016, we did not find *O. communa* on any of the non-target species sampled (Supplementary Table 1). In 2017, we found three adults of *O. communa* feeding on a single plant of

B. cernua during one visit in Trezzo sull'Adda, Lombardy, Italy (< 1% leaf damage). The infested *B. cernua* plant was next to an *A. artemisiifolia* plant that had been completely defoliated and that was crowded with larvae and adults of *O. communa* (see Supplementary Plate 1). No attack of non-target species was observed during any of the other 42 visits made in 2017 (Supplementary Table 2), although we found *O. communa* feeding during all 13 visits to the five nearby *A. artemisiifolia* sites. During our sampling period, *A. artemisiifolia* was heavily damaged by abundant *O. communa* (Supplementary Table 1).

3.3. Common garden field experiment

On *A. artemisiifolia* plants, 3–150 m away from the common garden experiment, we found all stages of *O. communa* from the beginning of the experiment onwards. By mid-August, *O. communa* had almost completely (> 95%) defoliated the *A. artemisiifolia* plants surrounding the experimental site, and by early September 2017, all *A. artemisiifolia* plants were completely defoliated.

Adults of *O. communa* were found on test plant species included in the common garden experiment from mid-August onwards, i.e., 3 weeks after the experiment was set up (Supplementary Fig. 4). The number of adults ($\chi^2 = 23.37$, $p < 0.001$, $df = 6$) and of larvae ($\chi^2 = 26.91$, $p < 0.001$, $df = 6$) differed significantly between the plant species, but not the number of egg batches ($\chi^2 = 9.95$, $p = 0.126$, $df = 6$). Egg batches (three egg batches with 9–20 eggs each) and larvae were only found on *D. graveolens*. The highest number of adults (32) per plant was found on *C. nigrescens* in late September 2017.

Overall leaf damage at the end of the experiment differed significantly among the plant species (Supplementary Fig. 4; $\chi^2 = 33.60$, $p < 0.001$, $df = 6$). It was highest on *P. helveticum* (55–95%), followed by *D. graveolens* (10–80%) and *C. nigrescens* (10–35%). Damage levels on the other test plant species were low and varied between 0 and 10% (Supplementary Fig. 4d).

3.4. Laboratory experiments

The non-target plant species used in the laboratory experiments (Supplementary Fig. 5) sustained significantly different degrees of leaf damage from the *O. communa* adults ($\chi^2 = 16.23$, $p = 0.006$, $df = 5$) and differed marginally in the numbers of egg batches per plant species ($\chi^2 = 10.24$, $p = 0.069$, $df = 5$), but not in the number of adults observed per plant ($\chi^2 = 7.51$, $p = 0.185$, $df = 5$). *Dittrichia graveolens* sustained the highest amount of damage (ca. 20%). We found eggs on all the plants except on *C. nigrescens* that came from a population originating near Pavia. Yet, egg hatching was only observed on *D. graveolens*; on all other plants, the eggs desiccated before the larvae could hatch.

Populations of *A. artemisiifolia* plants were exposed to females of *O. communa* from the same rearing colony, under identical experimental settings, one week before the experiments presented here; in these experiments, a mean (± SE) of 0.8 ± 0.04 egg batches (or 22.8 ± 1.4 eggs) were laid per female and plant after 21 days (Litto et al., unpublished results). In the present experiment, we found a mean of only 0.3 ± 0.07 egg batches (or 2.9 ± 0.8 eggs) laid per female per plant; on *D. graveolens*, the mean was 0.7 ± 0.19 egg batches (or 5.2 ± 1.7 eggs) per female and plant after the same period of time.

When non-target plants were exposed to newly hatched larvae, the non-target plant species did not differ significantly in the total number of larvae recovered ($\chi^2 = 2.43$, $p = 0.787$, $df = 5$), nor in the percentage of leaf damage sustained ($\chi^2 = 8.52$, $p = 0.130$, $df = 5$) (Supplementary Fig. 6). Two larvae had pupated prior to the last sampling (after 21 days) and one larva had developed into an adult. In a control experiment with a similar set-up as the experiment using adult *O. communa* (see legend of Supplementary Fig. 7 for details), when *O. communa* adults were offered *A. artemisiifolia*, *P. conyzae*, *P. helveticum*, *P. hirtum* and *P. spiraeifolium*, we found many more eggs, larvae and

adults on *A. artemisiifolia* than on any of the other plants, while leaf damage was more evenly distributed amongst the different plants (Supplementary Fig. 7).

4. Discussion

Hinz et al. (2019) defined degrees of non-target feeding/attack by biological control agents as follows. (i) 'Sustained' attack is where the biological control agents are able to fully develop and maintain populations on the non-target plant species, regardless of the presence or absence of the target weed. This is most likely to negatively influence plants at the population level (Blossey et al., 2018; Louda et al., 2005; Zimmermann et al., 2000). (ii) In contrast, 'collateral' or 'spill-over' non-target attack, usually occurs at high biological control agent densities resulting in the depletion of the target weed populations, which leads to opportunistic attacks on non-target plant species. In the latter cases the biological control agents are not able to maintain permanent populations on the non-target hosts and are thus unlikely to become a threat to non-target plants at the population level (Blossey et al., 2018). Hinz et al. (2019) further refined the latter terms: 'spill-over' non-target attack is on confamilial non-target species, which support full or partial development of the biological control agents; whereas 'collateral' non-target attack is on taxonomically-unrelated non-target species on which the biological control agents cannot develop.

During our 2-year field surveys, we found *O. communis* feeding damage in only one of 85 instances on non-target species, while we observed feeding damage during all 13 surveys on nearby *A. artemisiifolia* populations. On one occasion, we observed three *O. communis* adults feeding on the leaves of a single *B. cernua* plant (< 1% defoliation). The plant was directly adjacent to *A. artemisiifolia* plants that were heavily colonized by all developmental stages of *O. communis* (see Supplementary Plate 1). We consider this case to be a spill-over attack on *B. cernua*, but no-choice tests are required to assess to what extent *O. communis* can actually develop on this non-target species.

To ensure that all field surveyed populations of the non-target species were within the short-term dispersal range of *O. communis*, we chose survey locations close to *A. artemisiifolia* populations with *O. communis* present, often in high densities. Moreover, previous studies indicate that *O. communis* is a highly mobile insect species. For instance, we observed *O. communis* colonizing transplanted *A. artemisiifolia* plants that were at least 3 km away from and at an elevation 1,000 m higher than the nearest naturally occurring locations of *A. artemisiifolia* populations (Augustinus, unpublished results). Reports on the dispersal of *O. communis* after its introduction in Japan indicate that the beetle can spread at a rate of about 100 km per year (Moriya and Shiyake, 2001). Factors other than host plant suitability, including the micro-habitat of the surveyed populations, may explain why some of the non-target species were not colonized by *O. communis*. Nevertheless, the almost entire absence of non-target attack by *O. communis* on the surveyed populations of representatives of the tribes Coreopsidae and Inuleae agrees with the results from previously published field records (Futuyma and McCafferty, 1990; Palmer and Goeden, 1991; Hu and Meng, 2007) and indicates a low risk of sustained non-target attack on the non-target species included in the field survey.

The common-garden experiment was conducted in a location with large *O. communis* densities, and we observed feeding on non-target species, particularly on *D. graveolens*, *C. nigrescens* and *P. helveticum*, after the adjacent *A. artemisiifolia* plants had been completely defoliated. Except for *D. graveolens*, feeding was by adults only, indicating that most of the observed damage can be considered as a spill-over non-target attack. The laboratory experiments provide additional evidence that *O. communis* can complete its life cycle on *D. graveolens* but as the latter species has extended its range and become an invasive element in the flora of western Europe, non-target attack by *O. communis* is not a concern. Nevertheless, we propose to monitor the level of the non-target effect by *O. communis* on *D. graveolens* to assess whether sustained

non-target attack by *O. communis* may also occur on a plant species outside of the tribe Heliantheae.

Our findings provide no evidence of sustained non-target attack on vulnerable and near-threatened native European plant species of the tribes Inuleae and Coreopsidae by *O. communis*. In cases of the coexistence of *A. artemisiifolia* with the rare *Pentanema* species, high densities of *O. communis* may incur considerable spill-over damage, as with adult feeding damage on *P. helveticum* in the common garden experiment. Spill-over attacks can cause conspicuous damage to individual plants, but there is no evidence that this type of non-target attack can lead to negative consequences at the population level for non-target species (Blossey et al., 2018; Hinz et al., 2019).

Centaurea nigrescens was also attacked by adult *O. communis*, but we did not find evidence that *O. communis* can develop on this non-target species which is confamilial with *A. artemisiifolia*, but in a different subfamily (Carduoideae). Adult feeding in this case is categorized as collateral damage, rather than as spill-over damage, but no-choice larval development and oviposition tests are needed to confirm this. If the host-range of *O. communis* is indeed restricted to the tribe Heliantheae, then the number of plant species that are potentially at risk in Europe is limited. The only native European species within the tribe Heliantheae is *Ambrosia maritima* L., which grows in a few places in Mediterranean Europe (Gerber et al., 2011; Orsenigo et al., 2017), and might be conspecific with *A. artemisiifolia* (Martin et al., 2018). The genus *Pentanema* (former *Inula*) (Gutiérrez-Larruscain et al., 2019), which includes several threatened or vulnerable native European species, was previously placed in the tribe Heliantheae, but is now placed in a separate tribe, Inuleae (<http://tolweb.org/Heliantheae/22924>).

In the common garden and the laboratory experiments reported in this study, most feeding damage on non-target species was caused by adults of *O. communis*. This is in line with earlier assessments in a field experiment in Northern Italy where *O. communis* individuals feeding on non-target plant species were predominantly adults, indicating a wider host range for adults compared to larvae (Cardarelli et al., 2018). Adult chrysomelids have often been shown to have a wider host range than the conspecific larval stages (e.g., Huang et al., 2011; Pemberton and Witkus, 2010; Wang et al., 2008). The highest number of *O. communis* adults was found on *C. nigrescens*, the test plant species phylogenetically most distantly related to *A. artemisiifolia* (Supplementary Fig. 2). In contrast, oviposition preferences roughly followed the phylogeny of the plant species included in the bioassays. As expected from other studies (Fowler and Witter, 1982; Cullen, 1990), the laboratory cage experiments on *O. communis* increased the range of host plants chosen for egg laying: we observed oviposition on all plant species in the laboratory experiment, but eggs hatched only on *D. graveolens*.

In summary, the results obtained from our field survey, the common garden and the laboratory experiments provide evidence that *O. communis* is unable to cause significant non-target damage on native European plant species. We found no evidence that rare and endangered plant species belonging to the closely related tribes Inuleae and Coreopsidae are likely to experience sustained attack by *O. communis*. Nevertheless, we suggest to continue monitoring populations of selected native plants, including *D. graveolens* and *P. helveticum*, for potential non-target attack by the recently introduced *O. communis* in Europe.

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CRedit authorship contribution statement

Benno Andreas Augustinus: Formal analysis, Investigation, Writing - original draft. **Rodolfo Gentili:** Investigation, Resources, Writing - review & editing. **David Horvath:** Investigation. **Ruhollah Naderi:** Conceptualization, Methodology. **Yan Sun:** Formal analysis, Writing - review & editing. **Anne-Marthe Truce Eleonoor Tournet:** Investigation. **Urs Schaffner:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Heinz Müller-Schärer:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2020.104356>.

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