Occurrence monitoring and non-target survey of *Ophraella communa* in Ticino and Northern-Italy

Final Scientific Report

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Proposed work

As commissioned by FOEN, we:

i) monitored the further spread of *O. communa* in Switzerland in 2016 and 2017, and

ii) conducted field surveys to assess non-target effects by *O. communa* on native plant species under field conditions.

iii) In addition to the tasks proposed to FOEN, we also report on a common-garden experiment with native plant species that was set up in the frame of our ongoing research activities on integrated management of common ragweed and that assessed the likelihoods of non-target effects by *O. communa* when the plants grow in an environment that harbours high beetle densities.

iv) Besides and as requested by FOEN, we will (1) give recommendations on the use of *Ophraella communa* as a biological control agent for ragweed in Europe, balancing its impact on ragweed with its risk for non-target plants, (2) give an overview of the results from COST-SMARTER (end: Nov. 2016), and (3) give an overview of our presently on-going studies on the *Ambrosia- Ophraella* study system.

Introduction

The North American plant common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae), has become an invasive alien plant in several continents, including Europe. Next to being a noxious weed with a persistent soil seed bank in agriculture, the plant emits highly allergenic pollen, which results in severe negative effects on human well-being and subsequently high economic costs.

One of the main aims of the COST Action "SMARTER" (Sustainable Management of *Ambrosia artemisiifolia* in Europe; FA1203) is the implementation of long-term management options of common ragweed in Europe, including biological control (Müller-Schärer and Lommen, 2014). As a part of this 4-year Action, we set out to assess the host specificity of biological control candidates, to use plant population modelling to predict the efficacy of candidate biological control agents in controlling common ragweed densities, and to provide a scientific basis for the future implementation of biological control of common ragweed in Europe.

Ophraella communa LeSage is a North-American leaf beetle that was first detected in Europe in 2013 in the Po plains, Italy, and in southern Ticino, Switzerland. Initially, the highest population densities were found close to Malpensa International Airport; Milano, Italy, hinting to an accidental introduction of the beetle in Europe. In Japan and China, the beetle was also introduced accidentally and is now considered to be a very successful biocontrol agent of common ragweed (Müller-Schärer et al., 2014).

Originally, *O. communa* was excluded from the list of promising biocontrol agents of common ragweed for Europe because of its rather oligophagous nature: besides common ragweed, the beetle can conclude its life cycle on several other host plants, including sunflower, *Helianthus annuus* (Gerber et al., 2011) However, although the insect can reproduce on sunflower, several experiments in Italy, Switzerland and China suggest that the beetle is of little concern for this crop plant. Still, the impact on native European plant species remains to be investigated. In particular, rare and endangered plant species that are phylogenetically closely related to common ragweed should be of concern. To address this knowledge gap, we proposed to conduct a range of field surveys in the Ticino and in the Lombardy to assess non-target effects of *O. communa* on native endangered plant species that are closely related to *A. artemisiifolia*. Additionally, we proposed to continue monitoring the spread of this insect in Europe.

Methods

A) Distribution of *Ophraella communa*

We used the SMARTER network to monitor occurrences of the beetle in Northern Italy, Austria, Slovenia, Croatia, France (especially Rhône-Alpes) and in Switzerland, both south (Ticino) and north of the Alps. North of the Alps, common ragweed populations were surveyed at ragweed sites close to Thun and Geneva. Emphasis was put on surveying common ragweed populations at the border and outside of the area colonized in 2015.

Sampling of locations with common ragweed populations was restricted to short time periods (~15 minutes), during which leaf damage and beetle presence was searched for. In some cases, additional sweep-netting with a 40cm-diameter net was conducted, which increased the likelihood of finding beetles when population densities are very low.

Sites at the boundary of the known distribution range of *O. communa* were sampled between mid August and mid September, because beetle densities reach their peak in late summer.

B) Field surveys for non-target effects by *Ophraella communa*

We used a test plant list created in the frame of COST-SMARTER using the centrifugal phylogenetic method to determine native species that are closely related to common ragweed and that are rare and/or endangered in Switzerland. We focused on the tribes *Inuleae* and *Coreopsideae* and selected nine species for field monitoring (Table 1). Prior to the field surveys, we requested occurrence data of these species from 'info flora' (infoflora.ch) and created maps with these data as well as with data on records of *A. artemisiifolia* and *O. communa* in previous years. We selected several populations that were close (< 10 km) from field sites where common ragweed and/or *O. communa* had been observed between 2013 and 2015 (Table 2, Annex 1).

Table 1: Plant species monitored during the NES field surveys, arranged along a gradient of increasing phylogenetic distance from *A. artemisiifolia,* and the number of sites visited in 2016 and in 2017.

Species			# Sites visited		Status according to Swiss red list of vascular plants
Tribus	**Full species name**	**Abbreviation**	**2016**	**2017**	
Heliantheae	*Ambrosia artemisiifolia* L.	Ambart	0	5	Invasive
Heliantheae	*Helianthus annuus* L.	Helann	1	1	Crop
Coreopsideae	*Bidens frondosa* L.	Bidfro	1	1	Low concern
Coreopsideae	*Bidens cernua* L.	Bidcen	1	1	Vulnerable
Inuleae	*Inula hirta* L.	Inuhir	2	2	Endangered
Inuleae	*Inula conyzae* (Griess.)	Inucon	4	3	Low concern
Inuleae	*Inula spiraeifolia* L.	Inuspi	3	3	Endangered
Inuleae	*Inula salicina* L.	Inusal	1	1	Near threatened
Inuleae	*Carpesium cernuum* L.	Carcer	2	2	Endangered
Inuleae	*Xerolekia speciosissima* (L.) Anderb.	Xerspe	1	2	Endemic to Italy
Cardueae	*Centaurea nigrescens* Willd.	Cennig	2	2	Low concern

We visited 11 sites in the Ticino (Table 2, Figure 1, Annex 1) and found 16 populations of 3 native endangered species, two native species of low concern (Bornand 2016) and two exotic species (*Bidens frondosa* to increase sites for *Coreopsidae* and sunflower). To supplement the species list, we surveyed a total of 5 more populations of 3 additional plant species (1 near threatened species, 1 vulnerable species according to Bornand [2016] and a species endemic to Italy) in the Lombardy region (Italy; Table 2; Figure 1). The plant species monitored are listed in Table 1. We monitored the populations between the end of June and the beginning of August. Because some of these species only start flowering late in the season, and some species were very difficult to identify at the vegetative stage, not all sites were visited three times.

Table 2: Number of times sites in the monitoring study were visited.

Species	Reference number (cf. Fig.1)	#visits 2016	#visits 2017	Distance (km) to closest ragweed population	Country (CH=Switzerland ,IT=Italy)
Ambart	1	0	3	0	IT
Ambart	2	0	3	0	CH
Ambart	3	0	2	0	CH
Ambart	4	0	2	0	IT
Ambart	5	0	3	0	CH
Helann	6	3	3	3	IT
Helann	7	0	3	0	CH
Bidfro	8	3	3	3	CH
Bidcer	9	1	1	0	IT
Inuhir	10	3	3	3	IT
Inuhir	11	3	3	1	CH
Inucon	12	3	3	3	CH
Inucon	13	3	2	3	IT
Inucon	14	3	0	6	IT
Inucon	15	3	3	5	IT
Inucon	16	2	2	1	IT
Inuspi	17	3	3	3	IT
Inuspi	18	3	3	5	CH
Inuspi	19	2	3	1	CH
Inusal	20	3	1	1	IT
Carcer	21	3	2	6	CH
Carcer	23	1	2	4	IT
Xerspe	24	3	3	1	IT
Xerspe	25	0	1	6	IT
Cennig	26	1	2	1	IT
Cennig	27	0	2	1	IT

Whenever plant populations consisted of more than 50 individuals, we used a standardized sampling protocol, for which we divided the site with the plants into 4 equal sectors. The aim was to sample 50 plants per plant species and site, with at least 10 plants sampled in each of the four sectors. If the populations were smaller than 50 individuals, we sampled all plants. Whenever plants were disturbed by recent mowing, we decided not to sample the population.

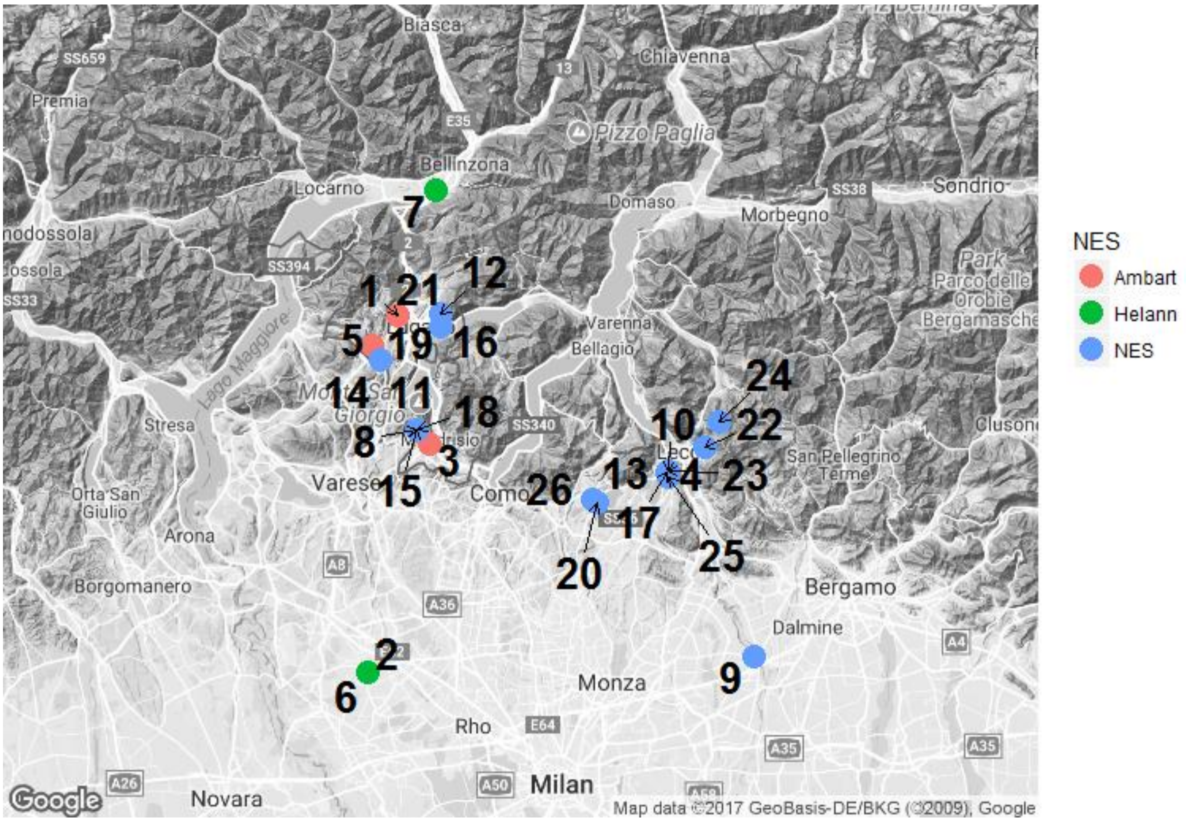

Figure 1: Sampling sites in the Ticino and in the Lombardy. The numbers correspond with the site numbers in Table 2. Red dots indicate sites with *A. artemisiifolia, g*reen dots indicate sites with *H. annuus,* and blue dots indicate sites with native endangered species (NES).

C) Common-garden experiment

In order to assess the risks of non-target attacks of some of the surveyed native plant species when they would grow in sites with high *O. communa* densities, we set up a field experiment in Magnago, Lombardy, and monitored attack of the transplanted native species by *O. communa*. This approach also allowed us to test *Inuleae* species that were not found in the Ticino or the Lombardy during the surveys.

Seeds of seven different native plant species (Table 3) were sown in batches starting April until beginning of June at the University of Fribourg on moist blotting paper in petri dishes in a germination room of the University of Fribourg at 18-26°C, 18:6h light cycle and relative humidity at 50-70%. After germination, the seedlings were transferred to pots filled with a mixture of 1/3 sand and 2/3 standard garden soil and grown in the quarantine greenhouse of the University of Fribourg. The plants were brought to the field on the 3rd of July, 2017. The experiment was set up in a former park made available by the municipality of Magnago (N45.580, E8.793) to perform research on *A. artemisiifolia.*

Table 3: Species used in the transplant experiment, with origin of seed material.

Species	Tribe	Obtained from	Comments
Dittrichia graveolens (L.) Greuter	Inuleae	Biotechnical faculty, Ljubljana, Slovenia	Not found during the surveys; attack by *O. communa* reported from Northern Italy (Petr Toth, 2015)
Inula britannica L.	Inuleae	Botanical garden Berlin-Dahlem, Germany	Not found during the surveys
Inula conyzae (Griess.)	Inuleae	Botanical garden Fribourg	
Inula helvetica Weber	Inuleae	Botanical garden Geneva, Switzerland	Not found during the surveys
Inula hirta L.	Inuleae	Botanical garden Fribourg	
Inula salicina L.	Inuleae	Rareplants.eu	
Centaurea nigrescens Willd.	Cardueae	Centro flora Autoctona, Galbiate, Italy	attack by *O. communa* in the field observed in 2016

Prior to transplanting, the experimental plot was mown to assure that the test plant species were not outcompeted by the original vegetation. The test plant species were set up using a latin-square design (seven rows with seven individuals per plant species). At three-weekly intervals, we monitored the plants for plant size, presence of *O. communa* (number of egg batches, number of eggs per egg batch, number of larvae at 1st or 2nd instar, number of larvae at 3rd instar, number of pupae, number of adults) and % leaf damage by *O. communa*. A few ragweed plants was left in close vicinity (< 3m) of the experimental plot and served as an indicator for *Ophraella* presence and phenology at the site. All other ragweed plants within the experimental plot and around it (in a radius of 10m were repeatedly removed.

Results

A) Distribution of *Ophraella communa*

The distribution of *O. communa* in Europe by the end of August 2017 is shown in Figure 2. In 2017, the beetle has been recorded for the first time in Slovenia and Croatia, but did apparently not cross the Alps to France or Northern Switzerland, as we did not receive any records despite our awareness campaign throughout the large SMARTER network.

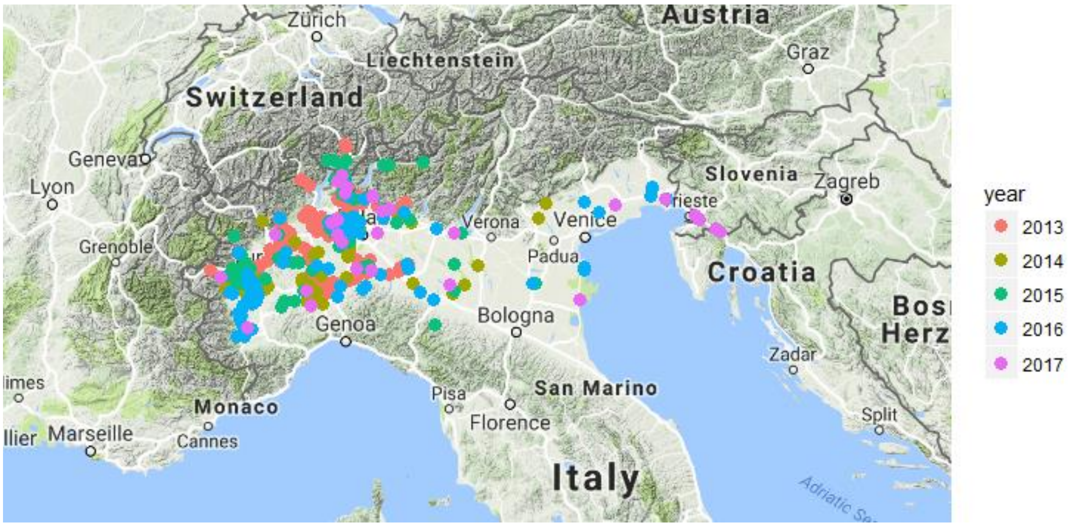

Figure 2: Distribution of *O. communa* in Europe by end of August 2017. Different colours indicate the different years of observations.

B) Field survey for non-target effects by *Ophraella communa*

During the 48 visits of populations made in 2017, we found *O. communa* feeding on none except one of the selected native plant species. When visiting the field site with *B. cernua* in Trezzo sull' Adda, Lombardy, we observed 3 *O. communa* adults on leaves that showed feeding damage. The three beetles were sitting on plants directly adjacent to *A. artemisiifolia* plants that were highly infested with *O. communa* in all life stages (see picture 1 and picture 2). However, incidence and severity of attack were lower than on *A. artemisiifolia* in the field (see Table 4). Compared to the surveys made in 2016, *O. communa* densities on *A. artemisiifolia* at the field sites were considerably lower in 2017 (see Table 4).

Picture 1: Completely defoliated *A. artemisiifolia* plant by *O. communa* larvae, pupae and adults directly adjacent to a *B. cernua* plant.

Picture 2: *Ophraella communa* on *Bidens cernua.*

Table 4: Incidence (percent *A. artemisiifolia* plants on which *O. communa* was detected) and severity (percent leaf area removed by *O. communa*) of attack observed on plant species surveyed in 2017.

Species (see Table 1 for full name)	Site reference to map (Figure1)	Site Name	Incidence of attack end of June 2017	Severity of attack end of June 2017	Incidence of attack mid-July 2017	Severity of attack mid July 2017	Incidence of attack beginning August 2017	Severity of attack beginning August 2017
Ambart	1	Bioggio	NA	NA	6	1.7	10	2.8
Ambart	2	Busto	14	0.6	8	2.2	68	4.12
Ambart	3	ExitMendrisio	2	3.5	100	3.3	64	7. 3
Ambart	4	MBarro600	0	0	0	0	0	0
Ambart	5	Pura	NA	NA	36	6.5	100	9. 4
Helann	6	Busto	0	0	0	0	0	0
Bidfro	8	ArzoQuarry	0	0	0	0	0	0
Bidcer	9	TrezzoAdda	NA	NA	NA	NA	3.0	1
Inuhir	10	MBarroPath	0	0	0	0	0	0
Inuhir	11	MteCaslano	0	0	0	0	0	0
Inucon	13	Eremo	NA	NA	0	0	0	0
Inucon	14	Caslano	NA	NA	0	0	0	0
Inucon	15	ArzoQuarry	0	0	0	0	0	0
Inucon	16	Gandria	0	0	0	0	0	0
Inuspi	17	MBarroRock	0	0	0	0	0	0
Inuspi	18	ArzoQuarry	0	0	0	0	0	0
Inuspi	19	Gandria	0	0	0	0	0	0
Inusal	20	LagoPusiano	NA	NA	0	0	NA	NA
Carcer	21	Curreggia	NA	NA	0	0	0	0
Carcer	23	ErnaParking	NA	NA	0	0	0	0
Xerspe	24	Morterone	NA	NA	0	0	NA	NA
Xerspe	25	MBarroPath	0	0	0	0	0	0
Cennig	26	Pusiano	0	0	0	0	NA	NA
Cennig	27	Fogliaro	0	0	0	0	NA	NA

C) Common-garden experiment

During the first two samplings of the common-garden experiment (mid-July and beginning of August) we did not find any *O. communa* on the transplanted species. During the third inspection (end of August), however, we found *O. communa* on some plants in the plot. We found *O. communa* adults on 3 out of 7 *C. nigrescens* plants, with an average of 3 beetles per plant, inflicting a maximum of 1% leaf damage. On 2 out of 7 *I. helvetica* plants, we found *O. communa* adults (2 and 12 individuals), inflicting up to 30% leaf damage. We did also find eggs and adults on *D. graveolens,* with low leaf damage (<2%). This prompted us to keep conducting the experiment for 6 more weeks until the experiment will be stopped in late September.

Discussion

As predicted in our last report, *O. communa* has now reached Slovenia and Croatia, but it did not spread at the same rate as during the last two years. We presume that the extraordinary cold April in 2017 might have hindered the population build-up and therefore slowed down the spread. However, we expect the beetle will continue to spread over the next years towards east (Austria) and south-east (Balkans). We also expect that the beetle will colonize in the near future the Rhône valley in southern France, which is heavily invaded by common ragweed.

Host range testing in 2017 has been expanded with four species that had not been tested before in a transplant experiment: Two of them were Inuleae species that we had not found in the study area: *I. helvetica* (classified vulnerable) and *I. britannica* (classified endangered). *Dittrichia* (*Inula*) *graveolens* has not been classified for Switzerland yet, but we included this plant because feeding had been observed on it in earlier years. In 2016, we also observed occasional feeding of *O. communa* adults on *C. nigrescens* in the absence of *A. artemisiifolia*, hence we included this Cardueae species in the transplant experiment. Additional to that, we were able to monitor two sites with established *C. nigrescens* plants.

During this summer, we observed *O. communa* on four non-target plant species. On three species, we only observed one adult beetle, which indicates that beetle do not complete their life cycle on these plants, a critical aspects of the non-target risk assessment in biological control. On two of these plant species (*C. nigrescens, I. britannica)*, damage was very limited (<<5%), so we do not consider these species at risk by *O. communa* feeding. On one plant, *I. helvetica*, *O. communa* caused considerable damage (~30% defoliation). While this can have detrimental effects on the plant, it should be noted that the phenology of this plant in the field experiment was very late; in natural populations, *I. helvetica*'s flowering period is from July to August. This means that even if the beetle starts attacking the plants at the end of August, this might have only a low impact on the plant's fitness since it already has produced seeds before the beetle attacks the plants to an extent that it has a detrimental effect. We therefore do not expect *I. helvetica* to experience significant damage by adult *O. communa* under natural field conditions.

However, the 4th plant on which we observed *O. communa*, *Dittrichia (Inula) graveolens*, was attacked by adult beetles, which also laid eggs on the plants. In a quarantine laboratory experiment carried out at the University of Fribourg, we found that the beetle can complete its life cycle on *D. graveolens.* We continue monitoring the transplant experiment till end of September to assess the damage and the final developmental stage of the beetle. While it is well possible that *O. communa* can and will develop on *D. graveolens* in the field, it should be mentioned that the plant has gained the status of a 'noxious weed' in the USA, specifically in California, where it overlaps with the natural distribution of *O. communa.* The plant is generally considered to be a Mediterranean species, where it can reach high densities. This means that the plant grows in a climatic niche largely outside the potential distribution of *O. communa* in Europe.

In summary, the results obtained in this study suggest that *O. communa* is unlikely to cause significant non-target effects on native European plant species. In particular, we found no evidence that rare and endangered plant species belonging to the closely related tribes

Inuleae and Coriopsideae are likely to be attacked by *O. communa*. Except for the common species *D. graveolens*, we only found a few adults on native plant species, without evidence of a permanent establishment.

However, we recommend to further monitoring the impact of *O. communa* on *D. graveolens* in the field. It is possible that the beetle causes damage on *D. graveolens* in Northern Italy and Switzerland, but *D. graveolens* is a widespread species and most of the native range of *D. graveolens* is outside the geographic range that is predicted to be colonized by *O. communa* in Europe. More insights will be gained when the various host specificity experiments with *O. communa* will be analysed and published that were carried our over the past 4 years in the Quarantine facility of the University of Fribourg. These involved, besides native endangered species also crop, ornamental and other Ambrosia species, as well as various common ragweed populations from all over the world.

SMARTER achievements

The COST Action FA1203 - Sustainable management of *Ambrosia artemisiifolia* in Europe (SMARTER; www.ragweed.eu) was approved in 2012 and ended in December 2016. The Action brought together experts involved in the control of ragweed, key-experts in biological control and legislation from non-COST member countries, health care professionals, aerobiologists, economists, atmospheric and agricultural modellers and numerous stakeholders. The main objectives of EU-COST SMARTER were to (i) initiate and coordinate trans-national and trans-sectoral cooperation in integrated management of ragweed, with special emphasis on elaborating biological control and vegetation management schemes, (ii) assess local and large-scale effects of the proposed integrated management measures and develop tools for the evaluation of the success of implemented strategies for present and predicted future climate change scenarios, (iii) train young scientists in the field of understanding, monitoring and managing plant invasions, and finally (iv) assist national and European authorities in establishing and implementing regulations for the prevention and management of common ragweed and other IAS, and for the import and release of biological control organisms.

One of the primary goals of SMARTER was to develop biological control options for the sustainable management of common ragweed in Europe. Specifically, SMARTER responded to the accidental introduction of the ragweed leaf beetle *Ophraella communa* in Europe (Müller-Schärer et al. 2014), which is used as a biological control agent of ragweed in China, by building an interdisciplinary team of weed scientists, biological control experts, aero-biologists, medical doctors and economists that aimed to assess both the potential risks and benefits of this accidentally introduced biological control agent. We conducted extensive host specificity studies, both under controlled (in the quarantine facility at the University of Fribourg) and open field conditions (in Switzerland, Italy and China). Biosafety studies included egg and larval transfer tests in the quarantine and in the field, host choice experiments in the field, where *Ophraella* occurs now naturally, using a series of different experimental designs both in the presence and absence of *Ambrosia*. In total, we conducted some 80 experiments, testing all 6 *Ambrosia* species invasive in Europe (*A. artemisiifolia*, *A. trifida*, *A. psilostachya*, *A. tenuifolia*, *A. confertiflora* and *A. maritima*) and the closely related invasive *Parthenium hysterophorus*, 4 ornamental species (*Zinnia* spp.), 4 sunflower varieties (reflecting varieties used for oil production, as ornamentals and for green manure) and 10 native endangered species (NES) from European tribes closely related to the tribe Heliantheae, to which *Ambrosia* belongs. Field tests we carried out during 2015 and 2016 at 4 sites in Switzerland and Italy, exposing the test plants at each occasion during 3 time periods (cohorts in early May, mid-July and early September, each lasting 9 weeks) to mimic different levels of *Ophraella* densities and ratios of co-occurrence with the target species, including the late season conditions when availabilities of *A. artemisiifolia* are lowest and beetle densities highest. In addition, we performed extensive non-target field surveys on a total of 25 plant species in 55 localities (crops, other exotic species and NES) across Southern Switzerland and Northern Italy to monitor potential *O. communa* occurrence and damage of non-target species under natural conditions.

Our biosafety studies conducted so far show a strong preference of *O. communa* for *Ambrosia* species, with *A. trifida* the least preferred. In no-choice tests in the quarantine, a few *O. communa* were able to fully develop on sunflower seedlings and impose considerable damage (above 80% leaf area removed). In our field experiments with sunflower, no eggs were laid at any site during the first and last cohort, and only a few ones during the second cohort (less than 3% of all eggs laid). A few larvae developed to pupae on sunflower during the second cohort, imposing only non-significant damage. In contrast, presence and damage by the adults on sunflower increased over the season at all sites, inflicting damage with yield reduction in cohort 3 (established in early September) especially at the site in Rovio, Ticino. During our extensive non-target surveys in 2014 and 2015, we found considerable damage by *O. communa* on *A. trifida*, *Xanthium strumarium* and occasionally significant damage on Jerusalem artichoke (*Helianthus tuberosus*), but only very limited damage by adults on sunflower leaves, with no impact on yield.

The results of our bioassays and non-target surveys classify the limited feeding of *Ophraella* on sunflower as a “spill-over” effect, meaning that the beetle would not be able to maintain persistent populations on sunflower. This confirms findings from earlier experiments made in China and from extensive field observation in the native range in North America, where *O. communa* has never been reported to occur on sunflower. However, adult feeding may well occur on sunflower later in the season (our third cohort). Importantly to note, however, is that sunflower for oil production is already harvested at the end of August, thus escaping the situation with high beetle densities in the absence of the target weed later in the season. More at risk will be sunflower grown as ornamentals and used as green manure that are still growing from September to December (until the first frost), but these two uses of sunflower varieties are not in practice south of the Alps (in Ticino and Northern Italy), where the beetle presently occurs. We thus estimate this effect on sunflower yield as non-significant, but propose to quantify this in future studies.

Within the frame of SMARTER, we also conducted studies to assess the potential benefits of the establishment of *O. communa* in Europe. Pollen monitoring studies in the Milan area revealed that since the establishment of *O. communa* ragweed pollen concentrations have dropped by approximately 80%. Bonini et al. (2015, 2016) showed that the low amounts of airborne *Ambrosia* pollen observed in the Milan area in 2013 and 2014 could not be explained by meteorology in those years. Moreover, insect enclosure experiments in northern Italy revealed that at the densities observed in 2014 and 2015 *O. communa* inflicted a high mortality and reduction in reproductive output of *Ambrosia* at the population level (Lommen et al., 2017), further supporting the notion that the decrease in aerial *Ambrosia* pollen concentration in the Milan region is related to the presence of large numbers of *O. communa*.

In late 2014, the French Agency for Food, Environmental and Occupational Health & Safety (Anses) created a working group with external experts to conduct a Pest Risk Analysis of an establishment of *O. communa* in France, with particular emphasis on assessing the risks for crop species. SMARTER provided published and unpublished information to the working group to make sure that the analysis is capitalizing on the research conducted within the frame of the COST action. Based on the analysis of published and unpublished data and information gathered from expert interviews, the working group concluded that the establishment of *O. communa* in France would cause a minimal risk of non-target effects on sunflower and would not need any specific management measures to limit the negative impact of *O. communa*, while drawing attention to the precautionary measures that need to be adopted if the insect were to be used as a biological control agent against common ragweed. Specifically, the range of plants attacked in the field could change in a context of inundative release, which could lead to *O. communa* attacking sunflower seedlings under certain conditions (i.e. in early spring) due to strong demographic pressure, given the insect's oligophagous nature.

Because of the potentially significant positive impact of *O. communa* on health costs, the French Ministries of Health, Agriculture and the Environment mandated an expert appraisal to assess the efficacy of *O. communa* as a BCA against common ragweed in France (ANSES 2017). This is a remarkable development, since French authorities have so far not considered classical biological control as a reasonable management option against invasive weeds in continental France, only in overseas territories. In the final document, it was suggested that the benefits of an establishment of *O. communa* to France could be significant, but that further host-specificity studies with native plant species are warranted (ANSES 2017).

Besides *O. communa*, biocontrol work in SMARTER also included research on other candidate biological control agents of common ragweed, mainly those that have already established in Europe. The moth *Epiblema strenuana*, which is considered to keep common ragweed in check in Australia, has accidentally established in Israel, and the moth *Ponometia candefacta*, which was deliberately released in Russia, was found to have spread to Serbia and Bulgaria. Moreover, the mite *Aceria artemisiifoliae* n.sp., which was recently described from Serbia (B. Vidović, University of Belgrade, Serbia, pers. communication) and which largely prevents seed set of common ragweed, is probably of North American origin. All these herbivores are currently under consideration for use as biological control agents against common ragweed and/or other invasive ragweed species (e.g. *Ambrosia psilostachya* DC) in Europe. Investigations on the suitability of these specialist herbivores for the biological control of common ragweed in Europe will continue beyond the COST action.

As we initially proposed *O. slobidkini* as a suitable biological control agent for ragweed in Europe but rejected its oligophagous congener *O. communa* (Gerber et al. 2011), we also conducted several studies with *O. slobodkini* (collected in Florida, USA) in our quarantine facility at the University of Fribourg (Lommen et al. 2017). The results confirmed that

O. slobodkini does not survive on, and is hence unlikely to cause severe damage to sunflower, while *O. communa* can survive but develops more slowly on sunflower than on ragweed. In parallel, our species distribution models predict no suitable area for the establishment of *O. slobodkini* in Europe, while *O. communa* is likely to expand its current range to include a maximum of 18% of the European ragweed distribution. Based on this early assessment, the prioritization and further assessment of *O. slobodkini* seem unwarranted whereas the results urgently advocated further risk-benefit analysis of *O. communa* (cf. above).

Finally, SMARTER was most successful in the communication of their results, both for science (publications in high impact journals; more to come), the stakeholders (cf. e.g. our stakeholder meeting in 2016 in Rho, Italy with contributions under the reference section Proceedings of the International Rho Meeting (28th October 2016) on "*Ragweed management and the potential benefit and risk of Ophraella communa in Northern Italy: researchers meet their stakeholders*"), as well as for the wider audience (cf. e.g. a collection on Newspaper, radio and television reports on http://www.unifr.ch/ecology/groupmueller/press). This overall success is reflected in the fact that SMARTER was chosen to be one of the 10 success stories among all COST Actions that ended in 2016! (cf. additional attachment to this report).

On-going and follow-up studies

To better understand the likelihood of future evolutionary changes in host specificity, we initiated specific studies to assess the potential of *O. communa* to develop a sunflower strain using selection experiments, bioassays and genomic tools in the framework of the recently granted SNF project to Heinz Müller-Schärer. In addition, we will illuminate the invasion and spread history of *O. communa* both in Asia and Europe in order to identify the sources of the introduced populations and the spreading genotypes. By phenotyping *O. communa* populations from their native range in North America, from China and Northern Italy, we will also estimate its potential to rapidly adapt to new abiotic (temperature) environment to predict its speed and spatial spread into suitable habitats across Europe, and (cf. lay summary in Annex 2). Furthermore, we assess the potential selection of *O. communa* and warming (as a part of climate change) on common ragweed populations to see if ragweed populations might evolve that are more tolerant or resistant to *O. communa* and thus lowering the long-term biocontrol effect of *O. communa* (Novartis grant to Yan Sun and Heinz Müller-Schärer).

We recently estimated the potential economic benefits of an establishment of *O. communa* in the heavily invaded Rhône-Alpes region in south-eastern France, where detailed data on the health costs caused by common ragweed are available. Extrapolating from the change in airborne ragweed pollen concentrations observed in the Milan area, we estimated that an establishment of *O. communa* in the Rhône-Alpes region will reduce the number of days with a ragweed pollen risk of ≥3 (threshold of pollen concentration at which sensitive people express symptoms) by 50% and the medical costs due to common ragweed by 5-7 M € annually. It should be noted that these are conservative cost estimates, as they only include costs related to the consumption of medical care and medical goods by persons affiliated to the general health insurance scheme. Nevertheless, our findings suggest that investments of public funds are justified to conduct a complete risk analysis of *O. communa* for Europe.

We are currently upscaling the estimates of the potential benefits of a further spread and establishment of *O. communa* to the European level. This study integrates spatial analyses of the distribution of common ragweed, the potential number of generations per year for *O. communa*, the current airborne ragweed pollen concentrations, the density of the human population and the sensitization rates among the population across Europe. The outcome will be an estimate of the reduction in health costs in the human population in Europe once *O. communa* has colonized all climatic regions that support at least three complete generations of this beetle.

Furthermore, we applied species distribution models to assess the suitable habitats in Europe of the various potential biological control agents to rank their potential effectiveness, to identify which combination of candidates is expected to cover the most area, and for which abiotic conditions to select in order to develop climatically adapted strains for particular regions, where ragweed is currently unlikely to be controlled (Sun et al. 2017). Similarly, we explored biological control opportunities of ragweed in East Asia with climate change. Our results show that *O. communa* has a larger overlap with the geographic range of *A. artemisiifolia* than *E. strenuana*, both under current and future climatic scenarios. Importantly, climate change is expected to reduce the total geographic overlap of *A. artemisiifolia* by the two agents combined, with a higher reduction by *O. communa* than by *E. strenuana* (Sun et al. 2017b).

Recommendations

Ophraella communa as a biological control agent of common ragweed in Europe

The COST-SMARTER project significantly increased the basic knowledge on *A. artemisiifolia,* a most prominent invasive alien species in Europe, and evaluates innovative management solutions needed for its control. Based on field and laboratory studies and associated cost-benefit analyses, we provisionally propose that *O. communa* is an effective candidate biocontrol agent of common ragweed to be used in European countries that are greatly invaded by this species and that are characterized by a climate that is favorable for rapid population build-up of *O. communa*. Sunflower, which supports complete development of *O. communa* under laboratory conditions, appears to be at low risk under field conditions. This is particularly the case for sunflower for oil production as these are already harvested at the end of August, thus escaping high beetle densities later in the season. This conclusion, which is based on a number of field studies conducted in the frame of SMARTER, is further supported by the fact that *O. communa* does not cause damage on sunflower in China, where the beetle has been mass-reared and mass-released for more than ten years, and that *O. communa* has never been reported to inflict any damage to sunflower in its native North American range. More at risk (particularly by adult feeding damage) will be sunflower grown as ornamentals and used as green manure that are still growing from September to December. Moreover, the data from the field surveys presented above suggest that most native European plant species, including the rare and endangered species of the closely related family Inuleae, do not support development of *O. communa* and/or escape from attack in the field. Further tests on host specificity are still on-going.

Along the lines of the conclusions drawn in the report by ANSES (2017), we suggested that the benefits of a wider establishment of *O. communa* in Europe could be significant, but that further host-specificity studies with native plant species are warranted. These additional risk assessment studies should be done using the European-wide test plant list that was compiled in the frame of SMARTER, in order to coordinate risk assessment studies in biological control projects against common ragweed across Europe and to adjust results to the new regulations, which require a European-wide risk assessment.

SMARTER - a template for a more efficient and sustainable management of invasive plants and weeds

Ragweed is just one example from a large number of invasive plants that require integration across different disciplines. Other species may require cooperation among other sectors and research disciplines, e.g. environment with engineering (*Reynoutria* spp.) or agriculture with environment and trade (*Eichhornia crassipes*). Increasingly, typical invasive alien plants are also being reported from crop field (e.g. *Ageratum conyzoides, Abutilon theophrasti*), and native weeds are conquering disturbed and some non-crop land (e.g. *Cirsium arvense*, *Echinochloa crus-galli, Chenopodium album*). Invasive species do not stop at habitat or political boundaries, calling for a trans-national and trans-sectoral coordination of management activities. The SMARTER approach is based on a close integration of and cooperation among weed science, invasion science as well as other research disciplines and sectors affected by ragweed invasion and management across Europe. We propose that it can serve as a template for establishing trans-national, trans-sectoral and interdisciplinary consortia towards a comprehensive impact analysis, efficient management and subsequent success evaluation of numerous other weeds and invasive alien species that impact multiple sectors, habitat types and regions.

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Annex 1: Sites visited in field surveys

Table 5: List of sites visited during the field surveys in 2016 and 2017 with exact GPS coordinates

Species	Site code in figure 1	Site Name	Latitude	Longitude	Country
Ambrosia artemisiifolia	1	Bioggio	46.015005	8.915643	CH
Ambrosia artemisiifolia	2	Busto Arsizio	45.594905	8.864614	IT
Ambrosia artemisiifolia	3	Exit Mendrisio	45.86285	8.969094	CH
Ambrosia artemisiifolia	4	Monte Barro 600m	45.828567	9.372178	IT
Ambrosia artemisiifolia	5	Pura	45.980156	8.872142	CH
Helianthus annuus	6	Busto Arsizio	45.594905	8.864614	IT
Helianthus annuus	7	St. Antonino	46.16127	8.97816	CH
Bidens frondosa	8	Quarry, Arzo	45.88085	8.948	CH
Bidens Cernua	9	Trezzo sul'Adda	45.61313	9.516826	IT
Inula hirta	10	Monte Barro path	45.83184	9.372301	IT
Inula hirta	11	Monte Caslano	45.962117	8.884983	CH
Inula conyzeae	12	Curréggia	46.016283	8.988383	CH
Inula conyzeae	13	Monte Barro road	45.831	9.3701	IT
Inula conyzeae	14	Monte Caslano	45.962117	8.884983	CH
Inula conyzeae	15	Quarry, Arzo	45.88085	8.948	CH
Inula conyzeae	16	Sentiero di Gandria	46.00197	8.9877	CH
Inula spiraeifolia	17	Monte Barro road	45.831	9.3701	IT
Inula spiraeifolia	18	Quarry, Arzo	45.88085	8.948	CH
Inula spiraeifolia	19	Sentiero di Gandria	46.00197	8.9877	CH
Inula salicina	20	Lago di Pusiano	45.79406	9.25149	IT
Carpesium cernuum	21	Curréggia	46.016283	8.988383	CH
Carpesium cernuum	22	Piani d'Erna parking	45.8598	9.4337	IT
Xerolekia speciosissima	23	Monte Barro path	45.83184	9.372301	IT
Xerolekia speciosissima	24	Morterone road	45.889214	9.4598	IT
Centaurea nigrescens	25	Fogliaro	45.825412	9.373465	IT
Centaurea nigrescens	26	Pusiano	45.799252	9.244697	IT

Annex 2: SNSF Project Heinz Müller-Schärer: Lay Summary

Einwanderungsgeschichte und Evolutionsfähigkeit in einer neuen Umwelt: ein innovativer Ansatz zur Risiko-Nutzen Abschätzung eines potentiellen biologischen Kontrollorganismus

Demographic history and recent selection in novel environments: an innovative approach to assess benefits and risks of a potential biological control agent

Lead
Ein gross angelegtes europäisches Forschungsprojekt untersucht die "Nachhaltige Bekämpfung von *Ambrosia artemisiifolia* in Europa" (EU-COST Aktion FA1203). Ambrosia ist aufgrund ihres äusserst allergenen Pollens und ihrer Wichtigkeit als Ackerunkraut die wohl prominenteste invasive Pflanzenart in Europa. Ein Forschungsschwerpunkt ist zur Zeit die Abklärung der Auswirkungen des seit 2013 in der Südschweiz und in Norditalien nachgewiesenen, ursprünglich aus Nordamerika stammenden Ambrosia-Blattkäfers *Ophraella communa* in Hinblick auf dessen Eignung also biologischer Kontrollorganismus. Das Projekt baut auf diesen Erkenntnissen auf und ergänzt diese durch den Einbezug von genetischen und genomischen Untersuchungen zur Evolutionsfähigkeit der Anpassung an veränderte Temperaturbedingungen und an Nicht-Zielpflanzen (z.B. Sonnenblumen), als Grundlage einer wissenschaftlich verbesserten Risiko-Nutzen Abschätzung dieses potentiell äusserst erfolgreichen Antagonisten.

Inhalt und Ziele des Forschungsprojekts
Die Einfuhr von wirtsspezifischen Antagonisten aus dem Herkunftsgebiet invasiver Pflanzen zu deren biologischen Bekämpfung im Einfuhrgebiet kann grossartige Erfolge aufweisen, sie birgt jedoch auch das Risiko, selber invasive Arten hervorzubringen. Die bis heute erfolgten Untersuchungen zur Risiko-Nutzen Abschätzung von *Ophraella* deuten auf ein enormes Potential dieser Käferart zur Bekämpfung und Verhinderung der Blütenbildung von *Ambrosia* hin, können jedoch ein Risiko für Sonnenblumen nicht vollumfänglich ausschliessen. Hier schliesst unser Forschungsprojekt nahtlos an mit dem Ziel 1) die Einwanderungsgeschichte und Ausbreitung von *Ophraella* in Asien und Europa aufzudecken, und 2) das Potential abzuschätzen, dass diese Käferart sich schnell an andere Temperaturbedingungen und Pflanzenarten anpassen kann, um die weitere Ausbreitung sowie die Gefahr eines zunehmenden Befalls der Sonnenblume besser eruieren zu können. Wir verwenden die neusten genetischen und genomischen Analyseverfahren (RAD-seq, pool seq) und führen Phänotypisierungs-Experimente in unserer Quarantäne sowie ein Selektionsexperiment im Feld in Nord-Italien auf Sonnenblumen durch, um mit Hilfe von verschiedenen Verfahren der Bioinfomatik und Statistik die Genetik der Anpassung zu untersuchen.

Wissenschaftlicher und gesellschaftlicher Kontext des Forschungsprojekts
Das Projekt trägt dazu bei, grundlegende Prozesse der lokalen, evolutiven Anpassung von Populationen besser zu verstehen, was insbesondere für den Umgang mit invasiven Arten und im Artenschutz im Zusammenhang mit globalem Klimawandel und steigender Mobilität von zunehmender Wichtigkeit ist. Die Studien werden aber auch Grundlagen liefern für nationale und international Institutionen und Behörden in Bezug auf die Regulierung und der

biologischen Bekämpfung von invasiven Arten, sowie praktische Massnahmen zur Bekämpfung der wohl wichtigsten Allergiepflanze liefern.

Key words
biological invasions, biological control, ragweed, Ambrosia, genomics, selection, evolution, neobiota

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