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**On potential risks and benefits of an accidentally
introduced weed biological control agent in Europe:
the case of the ragweed leaf beetle, *Ophraella communa***

Thesis

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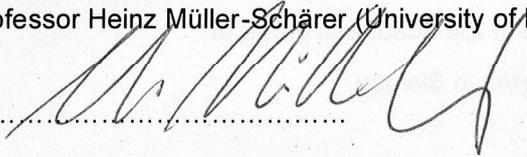
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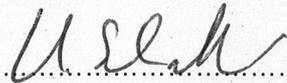
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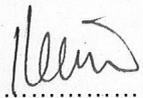
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University of Fribourg, Switzerland

2020

*All models are wrong,
some are useful*
George E. P. Box

TABLE OF CONTENTS

Summary	1
Zusammenfassung	4
General Introduction	7
Chapter 1	16
Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle on native European plant species. BA Augustinus, R Gentili, D Horvath, R Naderi, Y Sun, ATE Tournet, U Schaffner, H Müller-Schärer, Biological Control (2020), 150:104356	
Chapter 2	43
In-season leaf damage by a biocontrol agent explains reproductive output of an invasive plant species. BA Augustinus & STE Lommen, S Fogliatto, F Vidotto, T Smith, D Horvath, M Bonini, RF Gentili, S Citterio, H Müller-Schärer, U Schaffner, NeoBiota (2020), 55:117-46	
Chapter 3	76
Predicting impact of a biocontrol agent: Integrating distribution modelling with climate-dependent vital rates. BA Augustinus, Y Sun, C Beuchat, U Schaffner, H Müller-Schärer, Ecological Applications (2020), 30(1):e02003	
General Discussion	111
Appendix 1	124
Predicting insect herbivore population increase in a biological control field experiment with a demographic model driven by surface temperatures. BA Augustinus, M Blum, S Citterio, RF Gentili, D Helman, D Nestel, U Schaffner, H Müller-Schärer, IM Lensky, in prep.	
Appendix 2	142
Diffusione di <i>Ambrosia artemisiifolia</i> L. e <i>Ophraella communa</i> LeSage in Valtellina (Alpi Centrali, Lombardia), BA Augustinus, MF Guarino, F Colombo, S Citterio, U Schaffner, H Müller-Schärer, RF Gentili, Natura Bresciana (2015), 39, 235-238	
Appendix 3	147
Biological weed control to relieve millions of allergy sufferers in Europe. U Schaffner, S Steinbach, Y Sun, C Skjøth, LA de Weger, STE Lommen, BA Augustinus, M Bonini, G Karrer, B Šikoparija, M thibaudon, H Müller-Schärer, Nature communications, in press.	
Appendix 4	166
Estimating economic benefits of biological control of <i>Ambrosia artemisiifolia</i> by <i>Ophraella communa</i> in southeastern France. R Mouttet, BA Augustinus, M Bonini, B Chauvel, N Desneux, E Gachet, T LeBourgeois, H Müller-Schärer, M Thibaudon, U Schaffner, Basic and Applied Ecology (2018), 33, 14-24	

Appendix 5	178
Direct effects of insecticides on common ragweed – implications for natural enemy exclusion trials. STE Lommen, S Fogliatto, F Vidotto, S Citterio, BA Augustinus, H Müller-Schärer, Journal of Pesticide Science (2018) D17-048	
Appendix 6	189
Development and impact of <i>Ophraella communa</i> in Europe. STE Lommen, BA Augustinus, U Schaffner, H Müller-Schärer and the COST-SMARTER Task Force <i>Ophraella</i> , the COST-SMARTER Task Force Population Dynamics, Notiziario della Società Botanica Italiana (2017), 1(1): 1-10	
Appendix 7	192
Taxonomic issues related to biological control prospects for the ragweed borer, <i>Epiblema strenuana</i> (Lepidoptera: Tortricidae). TM Gilligan, DJ Wright, RL Brown, BA Augustinus, U Schaffner, Zootaxa (2020), 4729 (3), 347-358	
Acknowledgements	204
Ehrenwörtliche Erklärung	206
Curriculum vitae	207

SUMMARY

As a result of an increasing rate of introductions of species in novel ranges, management of invasive alien plant species will be a continuous challenge. One curative method to control invasive alien plants is classical biological control. In conventional classical biological control programmes, extensive host range testing to assess potential risks is one of the earliest steps, with the evaluation of the agent's impact (potential benefit) after introduction in the novel range. While novel introductions undoubtedly result in the introduction of species that have unwanted impacts, the chance of introductions of their natural enemies increases as well, with accidental biological control as a result. For accidentally introduced biological control agents, both risks and benefits can (and should) be evaluated once the agent has established in the novel range.

One example for such an accidentally introduced biological control agent is *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), a natural enemy of *Ambrosia artemisiifolia* L. The North-American *A. artemisiifolia*, common ragweed, is a flagship invasive plant in Europe, since it is not only a noxious weed in agriculture and extensively managed disturbed soils, but also produces vast amount of highly allergenic airborne pollen that reduces the quality of life and results in high medical costs in the sensitized population. Since 2013, *O. communa*, a North-American leaf beetle is found in Northern Italy, probably after accidental introduction. Both adults and larvae feed on *A. artemisiifolia*, which can lead to complete defoliation and death of the plants. Since the observation of high number of *O. communa* in Northern-Italy, aerial *Ambrosia* pollen concentrations are reduced by 80%, and the beetle is spreading over Northern-Italy and has reached Croatia in 2017. In this thesis, we present a post-release evaluation of the host specificity of an accidentally introduced biological control agent, an analysis of its impact on its target weed, and propose a method to use both temperature and humidity to predict potential population build-up of an accidentally introduced biological control agents in its potential range.

In the first study of this thesis, we combined a two-year field survey with a common garden field and two laboratory experiments to assess the level of non-target feeding of *O. communa* on European plants closely related to *A. artemisiifolia*. Sustained feeding of *O. communa* has only been described on plants within the Heliantheae tribe, which has no native representatives in Europe. We investigated the level of feeding on European plants of the taxonomically closely related tribes Inuleae and Coreopsideae, and did not find any substantial feeding of *O. communa*. The field and laboratory experiments showed considerable feeding

damage on *Pentanema helveticum* and *Centaurea nigrescens* by adult *O. communis*, and that *Dittrichia graveolens* could sustain the full life cycle of the beetle. However, *P. helveticum* and *C. nigrescens* were only attacked after surrounding *A. artemisiifolia* plants were completely defoliated, and no larval feeding was observed in the field. Therefore, the level of attack on *P. helveticum* (Weber) D.Gut.Larr. (former *Inula helvetica*) and *C. nigrescens* Willd. is considered as collateral damage, which is not likely to have a considerable effect on populations of these plant species. *Dittrichia graveolens* L. is a Mediterranean plant species and considered a neophyte in Northern and Western Europe. Thus, our results indicate that it is unlikely that *O. communis* will cause significant non-target effects on native European plant species in the tribes Inuleae and Coreopsideae.

In the second study, we investigated how *O. communis* abundance and damage on individual *A. artemisiifolia* during summer affects the plant's reproductive output. In a field experiment, we followed individual plants in four sites, estimated plant size, number of *O. communis* individuals and leaf damage in three-weekly intervals. Reproductive output of the plants was estimated at the end of the season. Leaf damage, together with plant volume, explained the chance of male flower production from 6 weeks before male flower set. However, difference in site explained far more of the variation within the plants. In contrast, likelihood of seed formation (female reproduction) could be explained with leaf damage and plant volume much earlier (up to 10 weeks), with explanatory power similar to that of the site effect. Models including leaf damage always explained more variation than models including abundance *O. communis* parameters, suggesting that leaf damage is a better estimator for impact than beetle abundance.

In the third study, we extended a published species distribution model with temperature- and relative humidity driven vital rates of *O. communis* that we had determined experimentally. The relationship between temperature and developmental time was deduced from a two-year field experiment. Additionally, we included the effect of relative humidity on egg hatching success, since laboratory experiments showed that egg hatching success strongly decreases from > 80% to < 20% when relative humidity drops from 55% to 45% during the warmest time of the day. The combination of the models showed that the current range of *O. communis* in Northern Italy is among the geographic ranges with the highest potential population build up, together with western Georgia and the Russian Krasnodar region. Comparing the relative importance of temperature and relative humidity for potential population build up showed that the importance of these climatic factors greatly differs spatially

across the range that is climatically suitable for *O. communa* and *A. artemisiifolia*. This indicates that both factors should be considered in models predicting potential build-up of *O. communa* in Europe.

We discuss the findings of the three studies above in the context of an expected increase of accidental introductions of biological control agents. We describe the potential pitfalls and opportunities of studying insect demography in order to make predictions on potential impact of biological control agents of weeds. Furthermore, we describe how the presented work has been used in risk-benefit analyses of *O. communa*, and how these analyses can be conducted for the investigation of potential risks and benefits of future accidental introductions.

ZUSAMMENFASSUNG

Aufgrund der zunehmenden Einschleppungsrate von Arten in neue Regionen wird das Management derjenigen gebietsfremder Arten, die invasiv werden, eine ständige Herausforderung bleiben. Eine vielversprechende Methode zur Bekämpfung invasiver gebietsfremder Pflanzen ist die klassische biologische Schädlingsbekämpfung. Bei herkömmlichen Programmen zur klassischen biologischen Kontrolle bilden umfangreiche Tests über das Wirtsspektrum des Nützlings zur Bewertung potenzieller Risiken ein zentrales Element der Voruntersuchungen, während die Bewertung des potenziellen Nutzens des Nützlings oft erst nach dessen Einfuhr erfolgt.

Während zufällige Einschleppungen von Arten in neue Regionen zweifellos zur Einfuhr von unerwünschten Arten führt, steigt durch diesen Prozess auch die Wahrscheinlichkeit, dass Nützlinge eingeführt werden, welche zur Bekämpfung invasiver Arten genutzt werden könnten. Das Problem besteht aber darin, dass bei versehentlich eingeführten Nützlingen zu Beginn kaum Daten über die Risiken oder den möglichen Nutzen vorliegen, insbesondere nicht für die Region, in die der Nützlich zufällig eingeschleppt wurde.

Ein Beispiel für einen versehentlich eingeführten Nützlich ist *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), ein natürlicher Gegenspieler des Nickenden Traubenkrauts, *Ambrosia artemisiifolia* L. Diese nordamerikanische Pflanze ist ein prominentes invasive Unkraut in Europa, das nicht nur Probleme in der Landwirtschaft verursacht, sondern auch durch seine hoch allergenen Pollen die Lebensqualität der sensibilisierten Bevölkerung reduziert und zu hohen medizinischen Kosten führt. Im Jahr 2013 wurde der nordamerikanische Blattkäfer *O. communa* erstmals in Nord-Italien und in der Südschweiz nachgewiesen. Sowohl Larven als Adulte fressen *A. artemisiifolia*, was zur vollständigen Entlaubung und zum Tod der Pflanzen führen kann. Seit den ersten Beobachtungen hoher Dichten von *O. communa* in Norditalien sind *Ambrosia*-Pollenkonzentrationen in der Gegend um Milano in der Luft um 80% gesunken. In der Zwischenzeit hat sich der Käfer über Norditalien nach Slowenien und Kroatien ausgebreitet.

In dieser Dissertation präsentieren wir Analysen des Wirtsspektrums von *O. communa* und des Einflusses auf das Zielunkraut in Norditalien und in der Südschweiz und schlagen eine Methode vor, die mit Hilfe von Temperatur und Luftfeuchtigkeit die Populationsdynamik des Nützlings in seiner potentiellen Verbreitung abschätzen kann.

In der ersten Studie dieser Dissertation haben wir eine zweijährige Feldstudie mit einem Feldexperiment und zwei Laborexperimenten kombiniert, um die Wahrscheinlichkeit

eines Befalls europäischer, mit *A. artemisiifolia* verwandter Pflanzen durch *O. communa* zu analysieren. Kontinuierlicher Frass von *O. communa* wurde nur an Pflanzen des Tribus Heliantheae beobachtet, der in Europa keine einheimischen Vertreter beinhaltet. Europäische Vertreter der taxonomisch eng verwandten Triben Inuleae und Coreposideae zeigten meisten keine oder nur schwache Frassschäden; erheblicher Frass durch adulte Käfer wurde einzig auf *Pentanema helveticum* (Weber) D.Gut.Larr. (ehemals *Inula helvetica*) und *Centaurea nigrescens* Willd. gefunden, und auf *Dittrichia graveolens* L. kann *O. communa* den gesamten Lebenszyklus durchlaufen. *Pentanema helveticum* und *C. nigrescens* wurden im Feld jedoch erst befallen, nachdem die in der Umgebung vorkommenden *A. artemisiifolia* komplett entblättert waren. Der Befall von *P. helveticum* und *C. nigrescens* kann deshalb als Kollateralschaden („collateral damage“) bezeichnet werden, was wesentliche Auswirkungen auf die Populationen dieser Pflanzenarten unwahrscheinlich macht. *Dittrichia graveolens* ist eine mediterrane Pflanzenart und gilt in Nord- und Westeuropa als Neophyt. Daher weisen unsere Ergebnisse darauf hin, dass *O. communa* bei einheimischen Vertretern der Triben Inuleae und Coreposideae wohl nur geringe Schäden verursachen wird.

In der zweiten Studie untersuchten wir, wie sich Befallsdichte und Frassschaden von *O. communa* auf die Fitness von *A. artemisiifolia* Pflanzen auswirkt. In einem Feldversuch bestimmten wir an individuell markierten Pflanzen an vier verschiedenen Standorten in Norditalien Pflanzengröße, Anzahl der *O. communa* Individuen und Frassschaden in regelmäßigen Abständen vom Frühsommer bis Herbst. Am Ende der Saison wurde von jeder Pflanze die Pollen- und Samenproduktion geschätzt. Frassschaden erklärte zusammen mit Pflanzenvolumen die Wahrscheinlichkeit, dass eine Pflanze männliche Blüten bildet, etwa 6 Wochen vor Blühbeginn. Der Standortunterschied erklärte jedoch weitaus mehr Variation zwischen Pflanzen als der Frassschaden an den einzelnen Pflanzen. Die Wahrscheinlichkeit der weiblichen Fortpflanzung (Samenbildung) wurde schon 10 Wochen vor der Samenbildung durch Frassschaden (Prozent Blätter gefressen) miterklärt, wobei die Erklärungskraft von Frassschaden ähnlich gross war wie diejenige des Standorts. Modelle mit Blattschaden erklärten immer mehr Variation als Modelle mit Abundanzen von *O. communa*, was darauf hindeutet, dass Blattschaden ein besserer Schätzer für die Auswirkung des Käfers ist als Abundanz.

In der dritten Studie erweiterten wir ein bestehendes Artenverbreitungsmodell von *O. communa* mit Angaben zum experimentell bestimmten Einfluss von Temperatur oder relativer Luftfeuchtigkeit auf Überleben oder Entwicklung dieses Blattkäfers. Die temperaturabhängige

Entwicklungszeit wurde aus einem zweijährigem Feldversuch abgeleitet. Zusätzlich haben wir den Einfluss von Luftfeuchtigkeit auf Schlupfrate berücksichtigt, da Laborexperimente zeigten, dass Schlupfrate von Eiern von >80% auf <20% abnimmt, wenn die relative Luftfeuchtigkeit während der wärmsten Tageszeit von 55% auf 45% sinkt. Die Kombination der beiden Modelle zeigt, dass das derzeitige Verbreitungsgebiet von *O. communa* in Nord-Italien zu den Gebieten in Europa mit dem potentiell höchsten Populationswachstum gehört, zusammen mit Westgeorgien und der russischen Krasnodar-Region. Ein Vergleich der relativen Bedeutung von Temperatur und relativer Luftfeuchtigkeit für die Populationsdynamik zeigt, dass der Einfluss dieser zwei Faktoren je nach geographischer Region variiert. Dies weist darauf hin, dass Modelle, die die Populationsdynamik von *O. communa* in Europa vorhersagen sollen, beide Faktoren berücksichtigen müssen.

Wir diskutieren die Ergebnisse der drei oben genannten Studien im Kontext einer zu erwartenden Zunahme des versehentlich eingeschleppten Nützlings in Europa. Wir erwähnen dabei den möglichen Nutzen und potentielle Fallstricke, die sich ergeben, wenn man damit Voraussagen zur Populationsdynamik von Insekten wie z.B. *O. communa* machen möchte. Darüber hinaus beschreiben wir, wie die oben erwähnten Befunde in Risiko-Nutzen Analysen von *O. communa* verwendet wurden und wie diese bei einer Analyse von zukünftigen versehentlich eingeschleppter Nützlinge eingesetzt werden können.

GENERAL INTRODUCTION

Invasive alien plant species can have devastating effects on the invaded ecosystems (Hejda et al. 2009, Vilà et al. 2011) and threaten human livelihoods globally (Early et al. 2016). One promising management method of invasive alien plants is classical biological control, i.e. the use of specialist natural enemies from the native range of the plant to reduce its densities below a given threshold (Müller-Schärer and Schaffner 2008, Winston et al. 2014). While classical biological control of weeds has been economically and ecologically very successful (Moran et al. 2005, Winston et al. 2014), it still is met with skepticism by some stakeholders, invasion scientists or conservation biologists (Seastedt 2015). Critics of classical biological control are mainly concerned about biosafety and likelihood of success (Havens et al. 2019).

Especially biosafety has been a topic of discussion, since there is a chance of non-target attack, and the introduction of a classical biological control agent is irreversible in most cases (Follett and Duan 2012). To address the issue of biosafety, pre-release host-specificity tests are essential for biological control programmes (Müller-Schärer and Schaffner 2008). Host specificity of potential biological control agents is examined on plants that are selected based on the centrifugal phylogenetic hypothesis (Wapshere 1974), which states that the likelihood of herbivore attack decreases with increasing phylogenetic distance of a test plant species from the primary host.

Host range tests are used to estimate the fundamental and realized host range of the agent, with the fundamental host range being the range of plants on which the biological control agent can complete its life cycle, and the realized host range the range of hosts that are attacked under natural conditions. The realized host range tends to be considerably narrower than the fundamental host range (Fowler et al. 2012, Schaffner et al. 2018), but a fundamental host range including native or economically important species is often used as a reason to reject potential biological control agents for release. This host range testing has resulted in high biosafety for biological control of weeds (Winston et al. 2014). There have been reports of unexpected significant adverse effects for less than 1% of the deliberately released biological control agents (Suckling and Sforza 2014). Since biosafety testing protocols have improved with experience, there is a decrease in non-target attack in weed biological control projects over time to less than 5.3% of the agents released for the period after 2008 (Hinz et al. 2019), with significant adverse effects in non-target plant populations known for less than 1% of the released biological control agents of weeds (Suckling and Sforza 2014). Still, to address questions on the biosafety of classical biological control of weeds, both

sceptics and advocates of this method are calling for more post-release evaluations of non-target attacks (Paynter et al. 2018, Schaffner et al. 2018, Havens et al. 2019, Hinz et al. 2019, van Wilgen et al. 2020).

Although weed biological control has been practiced for more than 150 years, predictability of the success of biological control agents remains a challenge (Heimpel and Cock 2018, Paynter et al. 2018). Schwarzländer et al. (2018) reported that 36.8% of the released biological control agents of weeds did not establish after introduction, and in 46.5% of the establishments, damage on the target weed was considered less than 'medium'. This highlights that both the establishment rates and the impact are not guaranteed in biological control programs. In the selection process for potential biological control agents, the climatic niche is generally estimated using species distribution models (Zalucki and Van Klinken 2006, Robertson et al. 2008). While the use of these stochastically-based models helps to inform on the suitable range, they lack in explanatory power for potential population densities that could be reached in their new range (Thuiller et al. 2014, Csergő et al. 2017). Herbivore impact on their host plant populations is often highly dependent on the herbivore's population density (Myers and Sarfraz 2017, McEvoy 2018), and successful biological control programs are usually characterized by population outbreaks with biological control agent densities that are several orders higher than known from their native range (Gassmann 1996, Müller-Schärer and Schaffner 2008). Forecasting insect herbivore densities to predict potential impact is common in agricultural applications, where the timing of management interventions is coordinated using pest predictive modelling (Blum et al. 2015, Magarey et al. 2015). The field of biological control of weeds is in principle very similar, with the difference that high herbivore impact is desirable. However, estimating potential population densities based on climate or weather data is an underutilized tool to make predictions on weed biological control success.

While biological control safety has improved over time and tools for making biological control success more predictable are available and used, accidental introductions of potential biological control agents circumvent the selection procedure. The rate of introductions of alien species worldwide has been increasing in the past 200 years, with no sign of slowing down (Seebens et al. 2017). With this, the chance of accidental introductions of potential biological control agents (the introduction of organisms used as biological control agents in other geographical ranges) increases as well (Shaw et al. 2016, Shaw et al. 2018). Classical biological control programs have protocols, legislation, and experience in place. In contrary, there are no blueprints on how to react to accidental introductions of biological control agents,

which should be addressed in light of the expected increases of accidental introductions of alien species. Meanwhile, once a potential biological control agent has been accidentally introduced, this can be seen as an opportunity to assess potential risks and benefits more accurately than in the case of (intentional) classical biological control in pre-release studies.

This PhD project was carried out within the framework of the EU-COST Action FA1203 SMARTER (“Sustainable management of *Ambrosia artemisiifolia* in Europe”, which was an international and interdisciplinary network of weed scientists, ecologists, health care professionals, aerobiologists, economists, and atmospheric and agricultural modellers. A central aim of SMARTER was to create a template for management of invasive alien plant species in Europe (Müller-Schärer et al. 2018). A part of this template is also how to react to accidental introduced biological control agents. In the following studies, we lay out how both positive and negative potential impacts of an accidentally introduced biological control agent can be assessed and projected on a temporal and spatial scale.

I aimed to address the lack of post-release evaluations on host specificity of accidentally introduced biological control agents (cf. **Chapter 1**), to improve the understanding of the impact of a potential biological control agent on its target weed (cf. **Chapter 2**), and the lack in prediction of potential biological control impact (cf. **Chapter 3**) by studying *Ophraella communa* LeSage on *Ambrosia artemisiifolia* (L.) in Europe. This system is particularly rewarding, since it is an example of an accidental introduction of a potential biological control agent, or so-called fortuitous biological control (DeBach 1971, Fand et al. 2013). This gives us the opportunity to weigh the risks and benefits of a biological control agent that has not passed a risk assessment before introduction.

Ambrosia artemisiifolia L., common ragweed, originates from North America and has invaded ranges in Asia, Australia, Africa, South America and Europe, where it is one of the most prominent plant invaders (Essl 2015, Müller-Schärer et al. 2018). This annual Asteraceous plant is considered a noxious weed in Europe since the early 1920s (Csontos et al. 2010). It is a costly agricultural weed in spring-sown crops (Komives et al. 2006), and has attracted particular attention because of its production of highly allergenic air-borne pollen (Müller-Schärer et al. 2018). In Europe, *A. artemisiifolia* grows on disturbed soil, especially in agricultural fields and along linear infrastructure like roads, rivers and train tracks. This renders control with mechanical and chemical methods financially unsustainable over large areas, which makes *A. artemisiifolia* a suitable target for classical biological control (Gerber et al. 2011, Essl 2015).

The North-American *O. communis* is a prospective biological control agent of *A. artemisiifolia*. The multivoltine beetle overwinters as adult, and females start laying eggs in spring, where the first eggs are laid as soon as the first seedlings of host plants emerge (Bosio et al. 2014). The three larval instars and the adults feed on the green parts of the plants, which can result in total defoliation and death of the host plants. *Ophraella communis* was found in Japan in 1996 (Ohno 1997), probably after accidental introduction, and spread via Korea and Taiwan to mainland China, where it arrived in 2001 (Meng and Li 2005). It is presently used successfully as a biological control agent of *A. artemisiifolia* in combination with the noctuid moth *Epiblema strenuana* Walker (Zhou et al. 2017). A potential issue with *O. communis* is that it is oligophagous, with a fundamental host range including various Heliantheae, including sunflower (Palmer and Goeden 1991). While this was the reason for not releasing *O. communis* in Australia and for not considering it as an antagonist for ragweed control in Europe (Gerber et al. 2011, Müller-Schärer and Schaffner 2016), no negative effects of *O. communis* on commercial sunflower or native Asteraceae have been found in intensive host range studies in China (Meng and Li 2005, Zhou et al. 2011). Since 2013, *O. communis* is found in Northern Italy and Southern Switzerland (Müller-Schärer et al. 2014), probably after accidental introduction in connection with activities linked to the airport in Milan Malpensa, which raises the question whether the oligophagous beetle will pose a risk to native European species.

In Northern Italy, aerial *A. artemisiifolia* pollen levels have decreased by 80% after the arrival of *O. communis*, which could not be explained by meteorological or land use changes (Bonini et al. 2015). Since 2013, *O. communis* has spread further through Italy and the Balkan peninsula (Augustinus et al. 2015, Lommen et al. 2017, Zadavec et al. 2019), and is expected to spread further through Europe. To predict the potential impact of *O. communis* on *A. artemisiifolia* in Europe, further studies of climate-dependent demography of the beetle and its impact on *A. artemisiifolia* are essential.

In the first study of the thesis (**Chapter 1**), “Assessing the risks of non-target feeding by *Ophraella communis* (Chrysomelidae) on native and endangered European plant species”, we focus on potential non-target feeding. Specifically, we report on a two-year field survey, a common garden and two laboratory experiments exploring the fundamental and ecological host range of *O. communis* in Europe, with a special focus on native and endangered plants that are closely related to *A. artemisiifolia*. We investigated the non-target feeding of larvae and adults in laboratory and common garden choice experiments, and conducted a two-year

field survey on populations of native and endangered Asteraceae in Northern Italy and Southern Switzerland to evaluate the level of non-target feeding.

In the second study (**Chapter 2**), “In-season leaf damage by a biological control agent explains reproductive output of an invasive plant species”, we focused on impact on the level of the individual plant, and whether damage or abundance measurements during the season can be used to explain plant reproductive output at the end of the season. Male reproductive output, i.e. pollen formation, is of interest since ragweed pollen are highly allergenic, while female reproductive output, i.e. seeds, affects the soil seedbank and thus the long-term plant population density. In a field experiment on four sites in Northern Italy, we followed individual plants from mid-June until late-September. Per site, we selected plants of different sizes, and created a gradient in insect herbivore pressure by applying insecticides to a part of the experimental plot. We then measured plant size, *O. communa* abundance and leaf damage, and measured male and female reproductive organ production in three-weekly intervals. The explanatory power of *O. communa* abundance and leaf damage was then compared to explore which parameters, together with plant volume, explain reproductive output best.

In the third study of the thesis (**Chapter 3**), “Predicting impact of a biological control agent: integrating distribution modelling with climate-dependent vital rates”, we extended a published species distribution model with temperature- and relative humidity driven vital rates of *O. communa*. Temperature-dependent developmental time of *O. communa* in Europe was deduced in a field experiment in Northern Italy, which was conducted in caged experiments along an altitudinal gradient. The effect of relative humidity on egg hatching success was inferred from laboratory experiments, where we mimicked fluctuating temperatures and relative humidity like the beetle would be exposed to in the field. We then combined the data with a stochastically based species distribution model (Sun et al. 2016) to project potential population growth of *O. communa* in Europe, and performed elasticity analyses to estimate where temperature or relative humidity have a higher relative importance for potential population increase.

In the General Discussion, I synthesize the findings of the three chapters and discuss them in the perspective of the expected increase of accidental introductions of potential biological control agents. I explore how demographic models can be of use to make predictions of biological control agent’s impact on their target, and how this can be of short- and long-term use for practitioners. Furthermore, I make recommendations for how to approach the investigation of potential risks and benefits of an accidentally introduced

biological control agent. In the Annex, I list published and unpublished manuscripts I contributed to during this project.

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CHAPTER 1

Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle on 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 nontarget 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.

Keywords:

biological control, accidental introduction, *Ambrosia artemisiifolia*, biological control of weeds, biosafety, host range, *Ophraella communa*, post-release evaluation

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 rufinusus* 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 ficusindica* (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., 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 nontarget attack by deliberately introduced classical biological control agents of weeds is rare (Hinz et al., 2019).

). 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 of the explanations 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; Kőmives 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 for non-target effect of *Ophraella communa* feeding experiments, ordered in phylogenetic relationship to *Ambrosia artemisiifolia* (cf. supplementary Fig. S2). State of threat is according to the “Rote Liste Gefäßpflanzen” 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.

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

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 nontarget 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 nontarget 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 threeweekly 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 analysis

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. communis*, 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. communis*

Previous no-choice host specificity tests with *O. communis* 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; Futuyma, 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. communis*, 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; Futuyma 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 (\pm 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' nontarget 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. communa* 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. communa* 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. communa* (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. communa* 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. communa*, we chose survey locations close to *A. artemisiifolia* populations with *O. communa* present, often in high densities. Moreover, previous studies indicate that *O. communa* is a highly mobile insect species. For instance, we observed *O. communa* 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. communa* 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. communa*. Nevertheless, the almost entire absence of non-target attack by *O. communa* 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. communa* 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 nontarget attack. The laboratory experiments provide additional evidence that *O. communa* 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. communa* is not a concern. Nevertheless, we propose to monitor the level of the nontarget effect by *O. communa* on *D. graveolens* to assess whether sustained non-target attack by *O. communa* 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. communa*. In cases of the coexistence of *A. artemisiifolia* with the rare *Pentanema* species, high densities of *O. communa* 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. communa*, but we did not find evidence that *O. communa* 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. communa* 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 Coreopsideae 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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Appendix A. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2020.104356>

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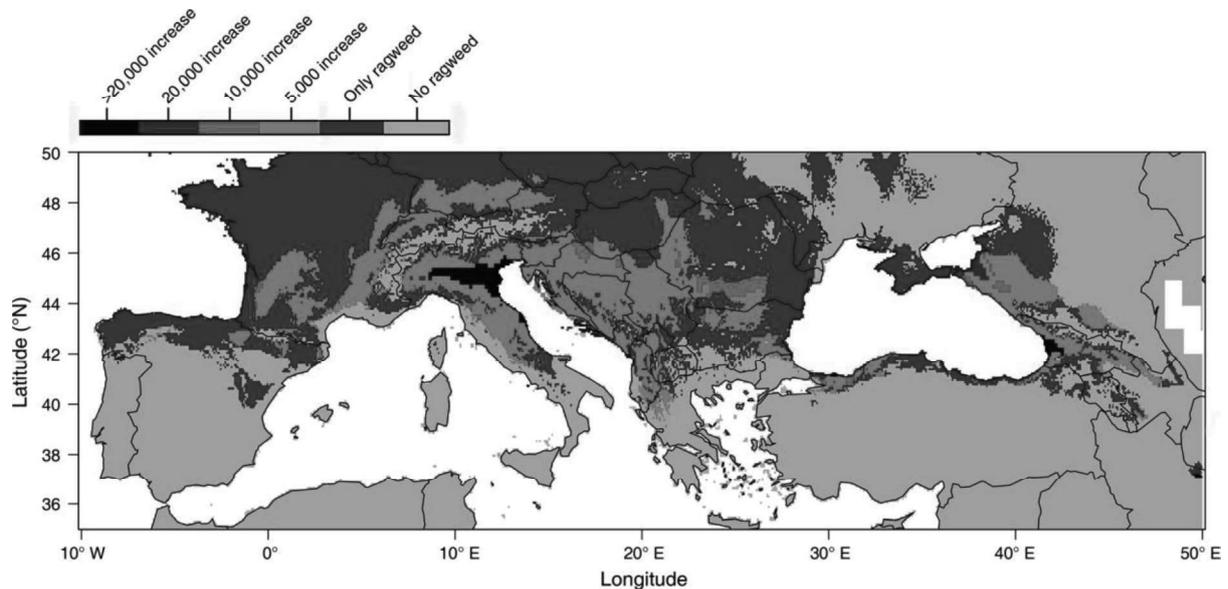
Supplementary data

Supplementary Table S1: Incidence of *Ophraella communa* attack and severity of attack (in average percent leaf damage) per site with plants sampled in 2017. NA refers to not sampled

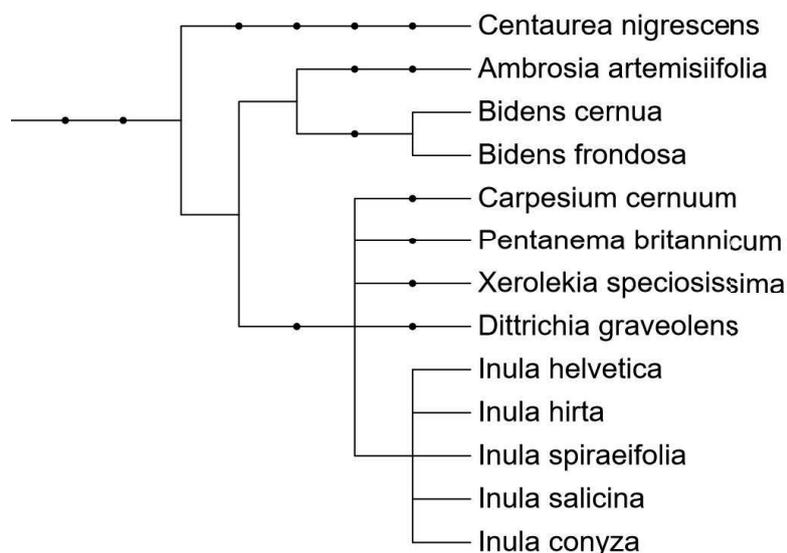
Species (see Table 1 for full name)	Site reference to map (Fig. S3)	Site Name	Incidence of attack	Severity of attack	Incidence of attack	Severity of attack	Incidence of attack	Severity of attack	Distance (km) to closest ragweed population
			end of June 2017		mid-July 2017		beginning August 2017		
Ambar	1	Bioggio	NA	NA	6	1.7	10	2.8	0
Ambar	2	Busto	14	0.6	8	2.2	68	4.12	0
Ambar	3	ExitMendrisio	2	3.5	100	3.3	64	7.3	0
Ambar	4	MBarro600	0	0	0	0	0	0	0
Ambar	5	Pura	NA	NA	36	6.5	100	9.4	0
Bidfr	8	ArzoQuarry	0	0	0	0	0	0	3
Bidce	9	TrezzoAdda	NA	NA	NA	NA	3.0	1	0
Inuhi	10	MBarroPath	0	0	0	0	0	0	3
Inuhi	11	MteCaslano	0	0	0	0	0	0	3
Inuco	13	Eremo	NA	NA	0	0	0	0	3
Inuco	14	Caslano	NA	NA	0	0	0	0	6
Inuco	15	ArzoQuarry	0	0	0	0	0	0	5
Inuco	16	Gandria	0	0	0	0	0	0	1
Inusp	17	MBarroRock	0	0	0	0	0	0	3
Inusp	18	ArzoQuarry	0	0	0	0	0	0	5
Inusp	19	Gandria	0	0	0	0	0	0	1
Inusa	20	LagoPusiano	NA	NA	0	0	NA	NA	1
Carce	21	Curreggia	NA	NA	0	0	0	0	6
Carce	23	ErnaParking	NA	NA	0	0	0	0	4
Xersp	24	Morterone	NA	NA	0	0	NA	NA	1
Xersp	25	MBarroPath	0	0	0	0	0	0	6
Cenni	26	Pusiano	0	0	0	0	NA	NA	1
Cenni	27	Fogliaro	0	0	0	0	NA	NA	1

Supplementary Table S2 Origin of seeds used for the field, the common garden and laboratory experiments

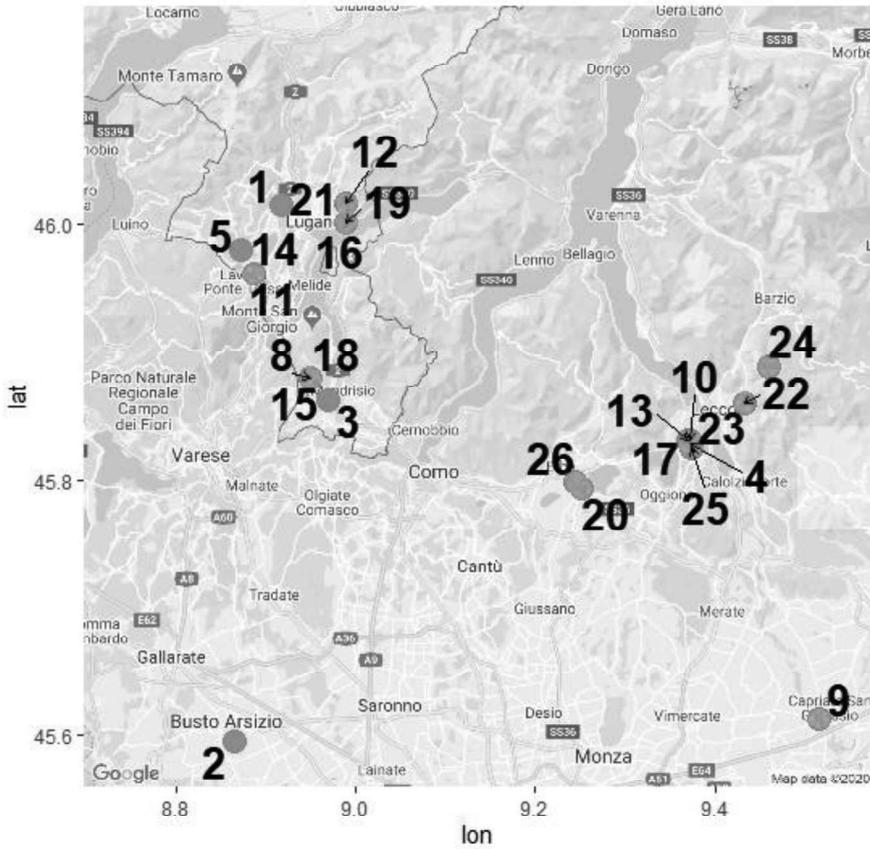
Plant species	Obtained from
<i>Dittrichia graveolens</i> (L.) Greuter	Biotechnical faculty, Ljubljana, Slovenia
<i>Pentanema britannicum</i> L.	Botanical garden Berlin-Dahlem, Germany
<i>Pentanema conyzae</i> (Griess.)	Botanical garden Fribourg, Switzerland
<i>Pentanema helveticum</i> Weber	Botanical garden Geneva, Switzerland
<i>Pentanema hirtum</i> L.	Botanical garden Fribourg, Switzerland
<i>Pentanema salicinum</i> L.	Rareplants.eu
<i>Centaurea nigrescens</i> Willd.	Centro flora Autoctona, Galbiate, Italy (CenniP, for population collected in Pavia) Collected by B.A. Augustinus on Monte Barro in 2016 (Cenni)



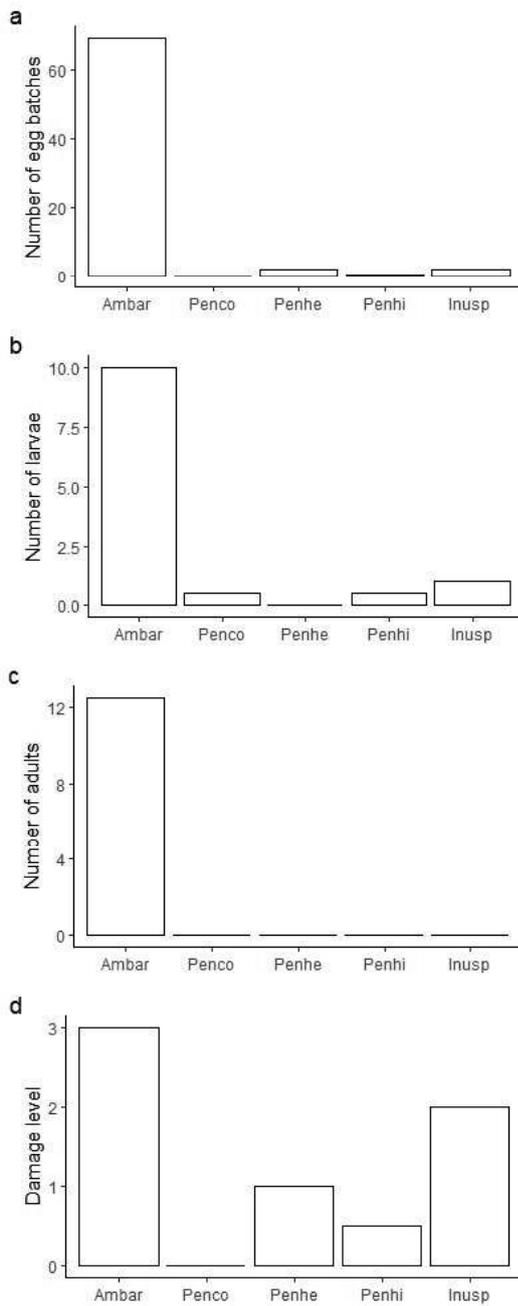
Supplementary Fig. S1 Population density of *Ophraella communa* based on model predictions of habitat suitability, temperature, and relative humidity (Augustinus and Sun et al., 2020). Gray color indicates the area not suitable for both *Ambrosia artemisiifolia* and *O. communa*; green color indicates the area suitable for *A. artemisiifolia* but not for *O. communa*; reddish colors indicate the area suitable for both *A. artemisiifolia* and *O. communa*: the darker the reddish color, the higher the density of *O. communa*.



Supplementary Fig. S2: Phylogenetic tree of the plant species tested, based on iTOL v.4 (Letunic and Bork 2019).

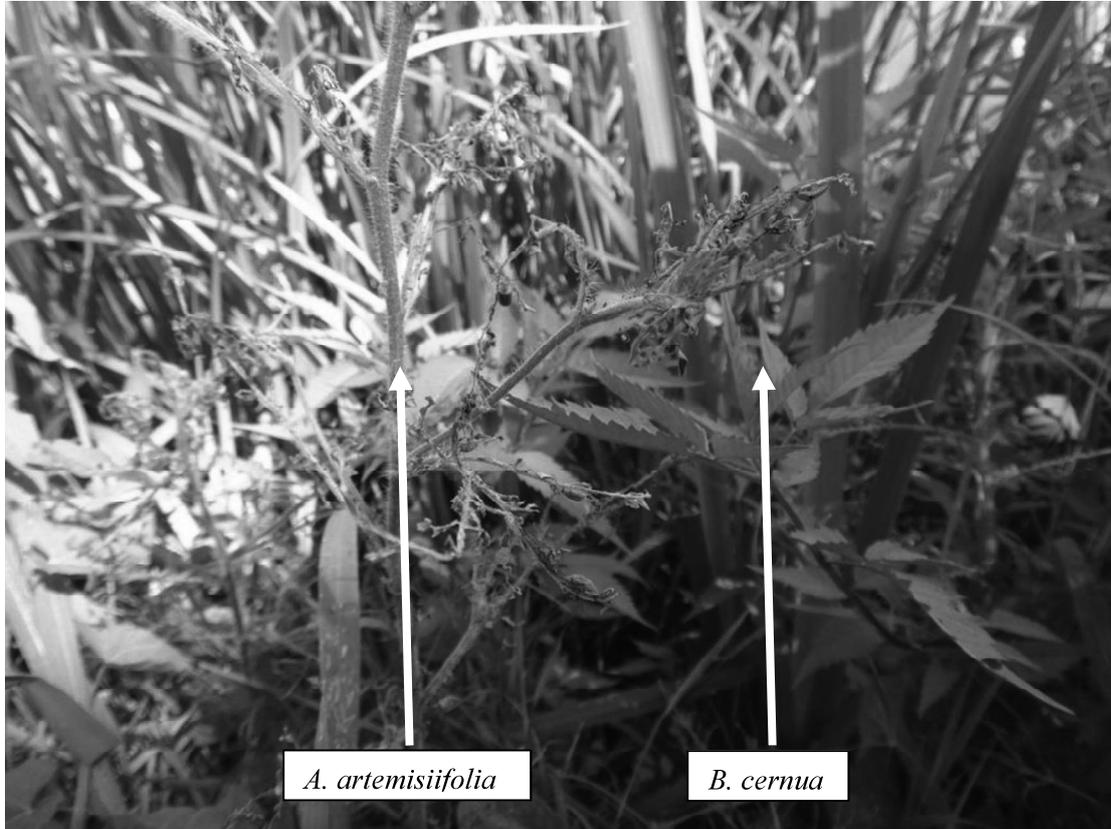


Supplementary figure S3 Sampling sites in the Ticino, Switzerland and in the Lombardy, Italy. The numbers correspond with the site numbers in supplementary Table S1. Red dots indicate sites with *Ambrosia artemisiifolia* and blue dots indicate sites with the other plant species surveyed (cf. Table 1).



Supplementary Fig. S4 Average number of egg batches (a), number of larvae (b), number of adults (c) and damage level (d) per plant at the end of the control experiment with *Ambrosia artemisiifolia* and four *Inuleae* species. In a cage like described for the adult experiment, two plants per species were exposed to two freshly hatched adult *O. communa* (1♂, 1♀) for 38 days.

Supplementary Pic. 1 From field survey: Completely defoliated *Ambrosia artemisiifolia* plant with larvae and adults of *Ophraella communa* and neighboring *Bidens cernua* plants with adult *O. communa* and minor feeding damage (<1%).



CHAPTER 2

In-season leaf damage by a biocontrol agent explains reproductive output of an invasive plant species

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Abstract

One of the biggest challenges in classical biological control of invasive weeds is predicting the likelihood of success. *Ambrosia artemisiifolia*, a North American plant species that has become invasive in Europe, causes economic losses due to health problems resulting from its huge amount of highly allergenic pollen and as a weed to agricultural crops resulting from high seed densities. Here we assessed whether the pollen and seed output of the annual *A. artemisiifolia* (at the end of the season) is related to in-season abundance of, or damage by, the accidentally introduced biological control agent *Ophraella communa*. We monitored the growth and leaf damage of individually labelled *A. artemisiifolia* plants at four locations in Northern Italy and recorded abundance of different *O. communa* life stages at regular intervals. We found that the in-season level of leaf damage by *O. communa* consistently helped explaining seed production in combination with plant volume and site throughout the season. Feeding damage, plant volume and site also explained pollen production by *A. artemisiifolia* six weeks before male flower formation. At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. Leaf damage proved to be a better explanatory variable than *O. communa* abundance. Our results suggest that the monitoring of the in-season leaf damage can help projecting the local impact of *O. communa* on *A. artemisiifolia* at the end of the season and thus inform management regarding the needs for additional measures to control this prominent invader.

Keywords:

Ambrosia artemisiifolia, biological invasions, classical biological control, common ragweed, *Ophraella communa*, herbivory

Introduction

It is now well established that plant species that are introduced into areas outside their native range and become invasive can cause serious impacts on nature and human well-being (Vilà et al. 2011). One option to mitigate the negative impacts of invasive alien species is classical biological control, i.e. the use of specialist natural enemies from the native range to reduce invader densities below an economic and ecological threshold or to slow down their spread (Müller-Schärer and Schaffner, 2008). Classical biological control of invasive alien plant species has been implemented worldwide for more than 120 years (Winston et al. 2014). The method is used either alone or in combination with other weed management practices (Müller-Schärer and Collins 2012, Lake and Minter 2018).

One of the biggest challenges in classical biological control of weeds is predicting the likelihood of success, and thus the necessity for considering additional management practices (Schwarzländer et al. 2018). Once established, biological control agents need to reach high densities to impact the target plant to such an extent that vital rates and ultimately its population growth rate are negatively affected (Jamieson et al. 2012). In cases of successful biological control of invasive alien plant species, population densities of the introduced biological control agents often reach numbers several orders higher than observed within their native range (Müller-Schärer and Schaffner 2008). However, whether biological control agents are able to build up high densities depends, among others, on the weather and climate conditions they encounter in the new range (Weed and Schwarzländer 2014; Mills 2018). Including biological control in integrated weed management presupposes an understanding of the likely abundance of the biological control agent and its impact on the target weed in a given region or year.

Predicting the impact of herbivore abundance on plants has a long history in crop pest forecasting (Magarey and Isard 2017), where it is used to support decision making regarding the scheduling of pest management interventions. For example, Lemic et al. (2016) found a strong positive correlation between the number of noctuid moths caught in pheromone traps and damage to sugar beet crops, which can be used to inform the timing of insecticide application to keep damage under an economical threshold level. This principle is also used, among many others, in the management of codling moth in apple orchards (Rather et al. 2018), of western corn rootworm in maize (Kos et al. 2014) and of navel orangeworm in almond plantations (Rosenheim et al. 2017). Thus, economic threshold levels are used in pest predictive models to decide when to apply pesticides. Along the same lines, a threshold level

could be used in biological control programs to inform weed managers whether or not additional control measures are required to achieve pre-defined management objectives. While the use of threshold levels in integrated weed management seems intuitive, there are only a few examples in classical biological control of weeds where in-season parameters related to herbivore abundance or damage have been identified that would allow informed management decisions. For example, Ding et al. (2006) estimated the number of larvae of the leaf beetle *Galerucella birmanica* necessary to control growth and reproduction of water chestnut, an invasive weed in several continents. Similarly, Häfliger et al. (2006) showed a negative linear relationship between attack rates of the stem boring noctuid *Archanara geminipuncta* early in the season and the performance of the invasive reed *Phragmites australis* towards the end of the season.

Ambrosia artemisiifolia L. (*Asteraceae*) is one of the most notorious plant invaders in Europe (Essl et al. 2015, Müller-Schärer et al. 2018). Originating from North America, it has been considered a noxious weed in Europe since the early 1920s (Csontos et al. 2010). In some parts of the invaded range, it causes serious yield losses in spring-sown crops such as maize, soy bean, and sunflower (e.g. Kómives et al. 2006). Even more importantly, this annual produces large amounts of highly allergenic pollen, which cause substantial medical costs and reduced quality of life among the allergic population (Smith et al. 2013, Mouttet et al. 2018, Schaffner et al. in press). Current management tools for *A. artemisiifolia* in Europe comprise mechanical and chemical control, which are applied in agricultural environments and along road sides (Milakovic and Karrer 2016, Lommen et al. 2018c). Due to their costs, impracticality, and/or their negative impact on the natural environment, these tools are unsuitable for most other habitat types where common ragweed thrives, such as waste land, riversides, or on small patches of ruderal land. As both the spread and health impacts of common ragweed in Europe are likely to increase with the changing climate (Storkey et al. 2014, Hamaoui-Laguel et al. 2015, Sun et al. 2017), and the number of persons sensitized to *A. artemisiifolia* pollen is rising (Lake et al. 2016), alternative methods such as biological control will be important to help controlling this invasive weed (Gerber et al. 2011). Biological control of *A. artemisiifolia* has already been implemented in Australia and China (Palmer et al. 2010, Zhou et al. 2014). In China, biological control of common ragweed is based on joint mass releases of the deliberately introduced noctuid moth *Epiblema strenuana* Walker and the accidentally introduced leaf beetle *Ophraella communa* LeSage (Zhou et al. 2014).

In Europe, *O. communa* was found for the first time in Northern Italy in 2013, probably also due to an accidental introduction (Bosio et al. 2014, Müller-Schärer et al. 2014). Since then, the beetle has become widely established in Northern Italy and has been spreading all over the Po Plain (Augustinus et al. 2015, Lommen et al. 2017b). In its current range in Europe, the beetle can complete up to four generations per year, which can result in complete defoliation and impaired reproduction of *A. artemisiifolia* plants (Bosio et al. 2014, Müller-Schärer et al. 2014). Since the first sighting of high abundances of *O. communa* in Northern Italy in 2013, yearly airborne ragweed pollen counts in the region have decreased by 80%. As this drop in airborne pollen counts could not be explained by land use change or meteorological factors, it is most likely attributable to feeding damage by *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b). At the field plot level, Lommen et al (2018b) showed a negative effect of the presence of *O. communa* on the density of *A. artemisiifolia* seeds produced. However, the observed effect of *O. communa* on *A. artemisiifolia* plants varied considerably, both at the spatial and temporal scale (Lommen and Augustinus, unpublished data).

In Northern Italy, the yearly peak of *O. communa* population size is only reached at the time when the first flower buds are produced. Identifying earlier, in-season indicators that are related to the level of biological control at the end of the season could help projecting whether in a particular season or location *O. communa* damages *A. artemisiifolia* to such an extent that it prevents plants from reproduction, i.e. from producing pollen (which impacts human health) or seeds (which impacts long-term population dynamics and crop yield).

Here we report on a field experiment to assess whether abundance of or damage by *O. communa* during the season is related to *A. artemisiifolia* reproduction at the end of the season. We followed individually labelled *A. artemisiifolia* plants in four locations in Northern Italy during the summer of 2016 to answer the following questions: (1) what is the in-season variation in a) in-season survival of *A. artemisiifolia*, b) the number of *O. communa* individuals of, and leaf damage caused by *O. communa* on individual *A. artemisiifolia* plants, and (2) what is the effect of in-season *O. communa* abundance or leaf damage on *A. artemisiifolia* reproduction at the end of the season?

Material and Methods

Study species

Ambrosia artemisiifolia is an annual plant that has invaded areas in all continents except Antarctica (Csontos et al. 2010, Essl 2015). The monoecious plant produces racemes with male flower heads that release highly allergenic pollen. The wind-pollinated female flowers are located in clusters in the leaf axils, with each flower producing one single seed. The seeds can remain viable in the soil for up to 40 years (Toole and Brown 1946). In Northern Italy, the first plants emerge in early April but they can emerge anytime until late summer, e.g. after soil disturbance or heavy rains. They form male flowers by mid-August producing pollen in August and September (Bonini et al., 2015a), while female flowers are formed in early September, and produce seeds from mid- to late September (Fogliatto et al. 2019, Lommen et al., 2018b).

Ophraella communa is a multivoltine leaf beetle which overwinters at the adult stage and lays eggs in egg batches in spring. The beetle then goes through three larval stages, which feed on the green parts of the host plant. It then pupates and starts mating shortly after emergence from the lightly woven cocoon. Adults feed on green parts of the plant as well. In Northern Italy, the beetle can complete up to 4 generations per year (Moultet et al. 2018). In southern China, where climatic conditions appear to be particularly suitable, *O. communa* concludes five generations in a year (Meng et al. 2007).

Study sites

We selected three former crop fields and one meadow with natural populations of both *A. artemisiifolia* and *O. communa* in the Po Plain of the Italian Piedmont and Lombardy regions (see Appendix 1). The sites were in different successional stages, thus allowing us to include variation in interspecific competition and ragweed growth rate, which likely affect biocontrol impact. One field site (Magnago) had been converted from woodland to grassland 4 years prior to the experiment. Another site (Unito) was a former crop site that had not been used for agriculture for the past two years and was dominated by grasses. Two other crop sites (Magenta, Busto Arsizio) were in agricultural use until very recently and were still dominated by early-succession weeds. The Busto Arsizio site was tilled and prepared for maize production (as grown adjacent) by a local farmer two weeks before commencing the experiment. The preparations included application of fertilizer, but no application of pre-emergence herbicides. The size of the study plots in each site ranged from 125-400 m². To

ensure that enough plants were available for the experiment, we disturbed the plots in April by mowing and raking to encourage recruitment of *A. artemisiifolia* from the soil seedbank.

Selection of plants

The study plants were selected between 13 and 18 June 2016, when *A. artemisiifolia* was between the 4- and the 12-leaf stage. We maximised the variation in initial size of *A. artemisiifolia* by randomly measuring plants at each site for 10 minutes and separating them into three equally numbered size classes (small, medium, large). We then laid transects of 20 m length through the study plots and selected 20 plants per size class along this transect, with an as homogenous distribution over the site as possible. Minimum distance between selected plants was 50 cm and maximum distance away from the transect 2 m. Plants were individually marked with an aluminium label around the stem and a bamboo stick.

Timing of the study

We decided to start our experiment in mid-June to exclude background seedling mortality from the dataset, since seedling establishment can vary considerably within and among sites (Rothrock et al. 1993). Furthermore, *A. artemisiifolia* has a long germination period (Kazinczi et al. 2008) and possesses high variability in germination rate and onset even among individuals of a population (Fogliatto et al. 2019). Thus, starting our experiment in mid-June allowed us to include plants that had germinated later and thus to cover a larger range in plant size.

Insecticide treatment

In order to increase intra-site variation in abundance of and damage by *O. communa*, two subplots of approximately 5 m long along the transect were selected at random for insecticide application. The two subplots contained in total 12 labelled plants (4 plants per size class) per site. These subplots were sprayed twice a month with insecticides, alternating between contact and systemic insecticides. We used Lambda-Cyhalothrin in a dosage of 20g/ha (Syngenta KarateZeon) as contact insecticide, and a combination of Acetamiprid in a dosage of 100g/ha (Sipkam EPIK), and Deltamethrin in a dosage of 20g/ha (Bayer DecisEVO) as systemic insecticides. Insecticides were applied at a spray volume of 1000L/ha using a backpack sprayer. Previous studies revealed that there is no direct effect of this insecticide

treatment on the measured plant parameters (Lommen et al. 2018a). The rest of the study plots were sprayed with the equivalent amount of water with the same intervals.

Measurements

Plant survival and size, *O. communa* abundance and leaf damage caused by *O. communa* were assessed on individual plants six times ("censuses") at three-week intervals from mid-June until mid-September 2016 (see exact dates in Appendix 2). To assess plant survival, plants were scored as 'alive' as long as parts of their above-ground biomass were green; for example, completely defoliated plants were scored alive when parts of the main stem or lateral shoots were still green. To monitor plant size, we measured height (from the ground to the highest point when the plant was held erect) and width (at the widest span) of the plants and used these values to calculate volume, using the following formula:

$$Volume = height * \pi * \left(\frac{width}{4}\right)^2$$

To assess the abundance of *O. communa* on individual plants, we counted the number of *O. communa* egg batches, larvae >5 mm long (larger L2 and L3 larvae), and the number of adults on each labelled plant at each census, we disregarded egg batches with less than 5 eggs, because laboratory experiments indicated that eggs from small egg batches are mostly unfertilized (Augustinus, unpublished data). As small larvae are difficult to find since they can hide in buds and flowers, we did not count these to minimize observer errors. In addition, we measured leaf damage per plant by estimating the percent leaf area removed by *O. communa* from the total leaf area if the plant was intact (plants without leaves were given a value of 100% area removed). We did not score damage that was clearly not caused by *O. communa* (e.g. with traces of snail mucus). However, we never observed other leaf-chewing insect herbivores on *A. artemisiifolia* than *O. communa*, and rarely found traces of molluscs.

To estimate levels of plant competition early in the season, we assessed percent bare soil in a 50x50cm square around each marked plant in early July. A square frame of 50x50cm was laid around a plant and the fraction of that surface covered by bare soil, when projecting the vegetation onto the ground, was estimated by at least two persons and the average taken. Stones or dead leaf material were scored as bare soil as well. In late August, we measured the summed length of all racemes per plant as a proxy for pollen production (Lommen et al. 2018b). Seed formation was assessed between 19 and 25 September 2016 by counting the

number of seeds and female flowers (each flower gives rise to a single seed) produced per plant before seed rain.

Statistical analysis

To compare the change in leaf damage over time between sites, we conducted a repeated measures ANOVA with damage as response variable, site as fixed variable, and census as random effect. The fit of the residuals was evaluated graphically, and we took the square root of damage to obtain a better fit.

Because of the highly zero-inflated nature of our data, we applied a hurdle approach to analyse the effect of *O. communa* numbers on male (i.e. pollen) and female (i.e. seeds) *A. artemisiifolia* reproduction by first using presence/absence of racemes (pollen-bearing structures) in late August, and of seeds in mid-September to assess the probability of male and female reproduction, respectively. In a second step, we analysed the quantity of male and female reproduction conditional on the probability of reproduction (i.e. only using plants that did produce), using raceme length (as a proxy for the number of pollen produced), and numbers of seeds as response variables, respectively.

In the first part of the hurdle approach, we assessed the effect of *O. communa* on likelihood of raceme or seed formation in separate analyses by formulating generalized linear models for each of the first four (for raceme formation) or five (for seed formation) censuses. As fixed effects we included site, the natural logarithm of plant volume, as well as none or one of the four *O. communa*-related variables (number of eggs per plant, number of pupae per plant, number of adults per plant, and percent leaf damage) in each model, as these were inter-correlated. We produced models with and without an interaction term for the *O. communa*-related variable and plant volume, and with and without percent bare soil. We compared all resulting 18 models for each response variable at each census and selected the model with the lowest conditional Akaike information criterion (AICc) value, which penalizes models with more parameters (Burnham and Anderson 2002). To assess the fit of each model, we added Nagelkerke's pseudo r-squared (Tables 1 and 2). We displayed the effect size of the explanatory variables by plotting the odds ratios for all explanatory variables of the best performing model per census (Figure 4, 5).

In the second step of the hurdle approach, we assessed the effect of *O. communa* on total raceme length or number of seeds of those plants that did produce racemes or seeds, respectively. We formulated a set of linear models for the natural logarithm of raceme length

and number of seeds, assuming a Gaussian distribution of the response variable. We chose to use a Gaussian distribution over a Poisson distribution since it reduced AICc values of the fitted models by more than 5000 for every case. As fixed effects we included the natural logarithm of volume and site and added none or one of the four *O. communis*-related variables. To prevent overparameterization, we did not include bare soil and interactions with plant volume in these models, since the sample size of plants that successfully formed racemes and seeds was too low to include more than three fixed effects.

All analyses were conducted in R version 3.5.1 (2018-07-02) --"Feather Spray" (2018). Data were prepared using the readxl (Wickham and Bryan 2016) and reshape (Wickham 2007) packages, models were formulated in lme4 (Bates et al. 2015), model fits explored in DescTools (Signorell 2017) and MuMIn (Bartoń 2013), comparisons of damage levels between sites were conducted in agricolae (De Mendiburu 2019) and figures produced in ggplot2 (Wickham 2009), sjPlot (Lüdecke 2018) and gridExtra (Auguie 2016) packages.

Results

Ophraella communis abundance and damage

Until late July, we found less than one egg batch, larva or adult of *O. communis* per plant (Fig. 1). The number of egg batches increased in early August but declined again in late August; after that, we found no more egg batches. Larvae and adult counts peaked in late August, with larval counts averaging 5 individuals per plant in late August (Fig. 1).

Plant volume steadily increased until late August, and decreased or stayed stable thereafter (Appendix 4). The few plants with no leaf damage during the course of the experiment were exclusively plants treated with insecticides (Fig. 2). Plants that were not treated with insecticides showed a moderate but continuous increase in percent leaf damage by *O. communis* until early August, reaching an average of approximately 20-50% leaf damage across all sites (Fig. 2). Between early and late August, the percent of leaf tissue damaged rapidly increased to approximately 85%. Plant mortality occurred throughout the experiment, but was most severe between late August and the beginning of September (Fig. 3), before natural mortality due to senescence occurred. Percent leaf damage varied significantly among sites (repeated measures ANOVA, $H=147.33$, $df=3$, $p<0.01$), with Busto Arsizio exhibiting the highest percent leaf damage until early August and Unito the lowest percent leaf damage throughout the whole study period (Fig.2).

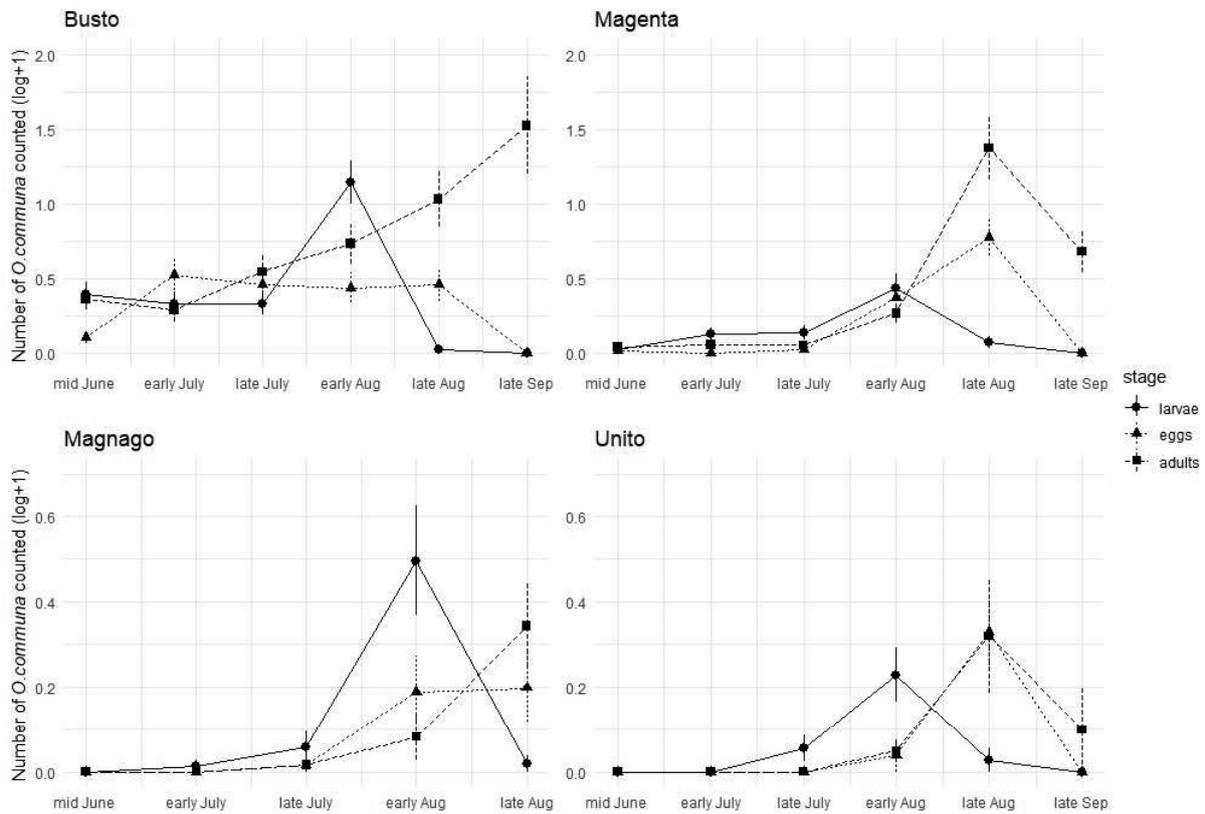


Figure 1: Average number of *O. communa* individuals per plant during the experiment in the four different sites. Different life stages are marked with different lines and symbols. Vertical lines indicate the standard error.

Effect of *O. communa* on *A. artemisiifolia* reproduction

Models with the lowest delta AICc values (compared to the best performing model) for successful raceme formation included *O. communa* abundance parameters measured in early August (number of adults), and models with the lowest delta AICc values for raceme length of the plants that successfully formed racemes included *O. communa* abundance parameters measured in late July (number of egg batches) and early August (number of adults; see Table 1).

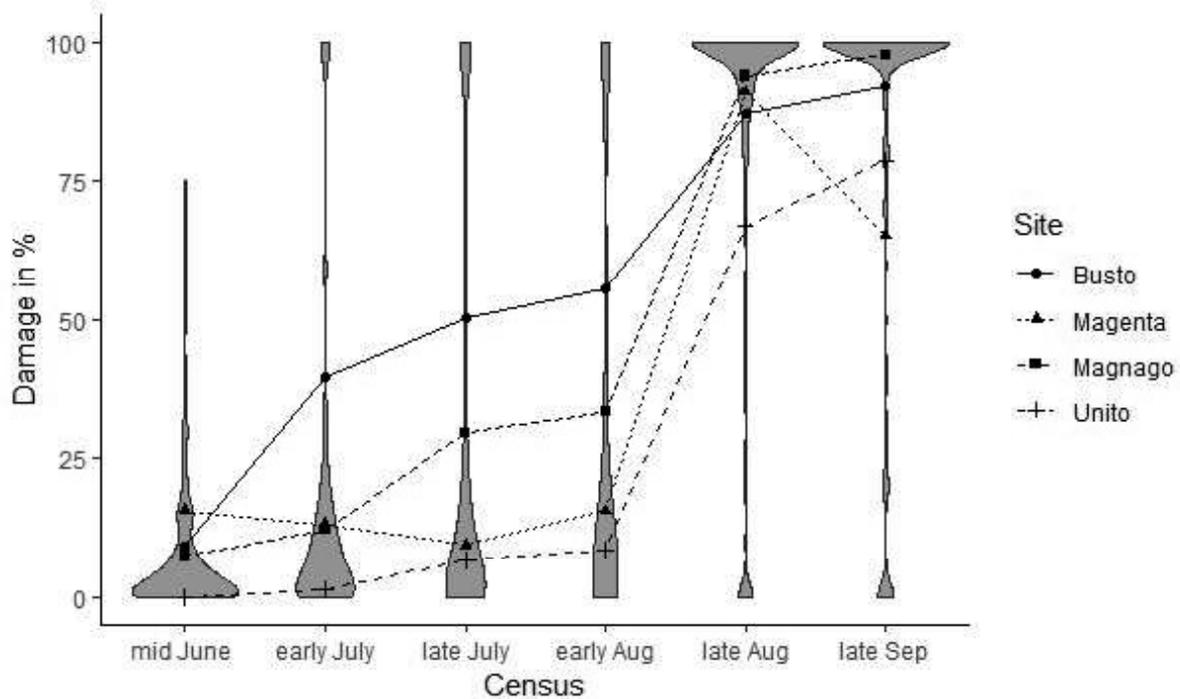


Figure 2: Violin plot of *Ambrosia artemisiifolia* leaf damage by *O. communa* feeding. The lines indicate the mean of the leaf damage scored on living plants in the different sites. The distribution of the damage measurements is shown with the grey shapes. Only damage of plants which were not treated with insecticides are displayed.

In the model with the lowest delta AICc value for successful raceme formation in late July, we found a positive relationship between leaf damage in percent and successful raceme formation (Fig. 4). In the models for raceme length, the number of egg batches per plant in late July was positively related to raceme length, and number of adults per plant was positively related to raceme length (Table 1).

The selected models for successful seed formation included *O. communa* abundance parameters measured in early July (number of adults per plant), early August (number of adults, larvae and egg batches per plant) and late August (number of adults and larvae per plant) Table 2). Successful seed formation could best be explained by models including the number of adults per plant in early July, the number of adults, number of larvae or number of eggs per plant in early August, and the number of adults or larvae per plant in late August. In all cases, the number of *O. communa* had a negative effect on successful seed formation. Five out of six models had a lower AICc when an interaction term with volume and the number of *O. communa* individuals was included (see Table 2, Figure 5). Including percent bare soil

did not increase any model fit. In contrast to the models for successful seed formation, none of the models with the lowest AICc values for number of seeds produced by successfully reproducing plants included an *O. communa* abundance parameter (Table 2).

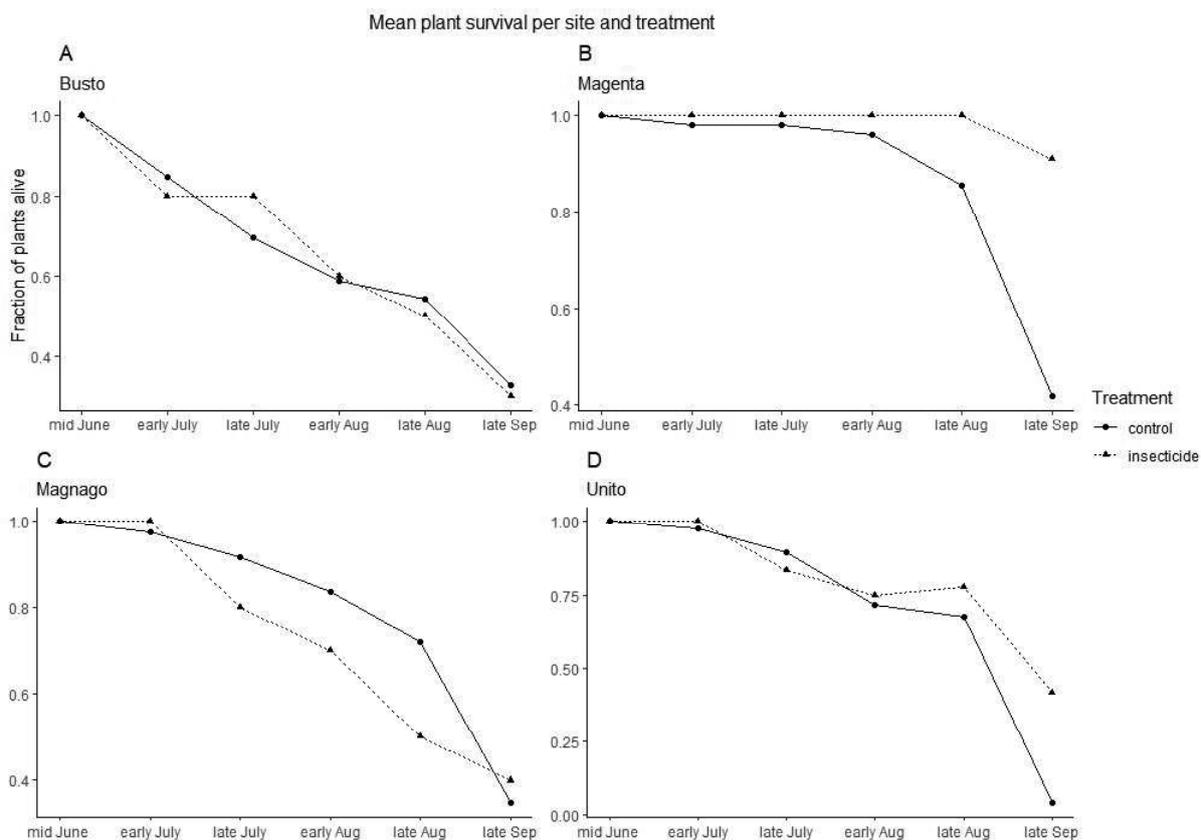


Figure 3: Fraction of plants exposed to *O. communa* and those treated with insecticides alive over time during the experiment.

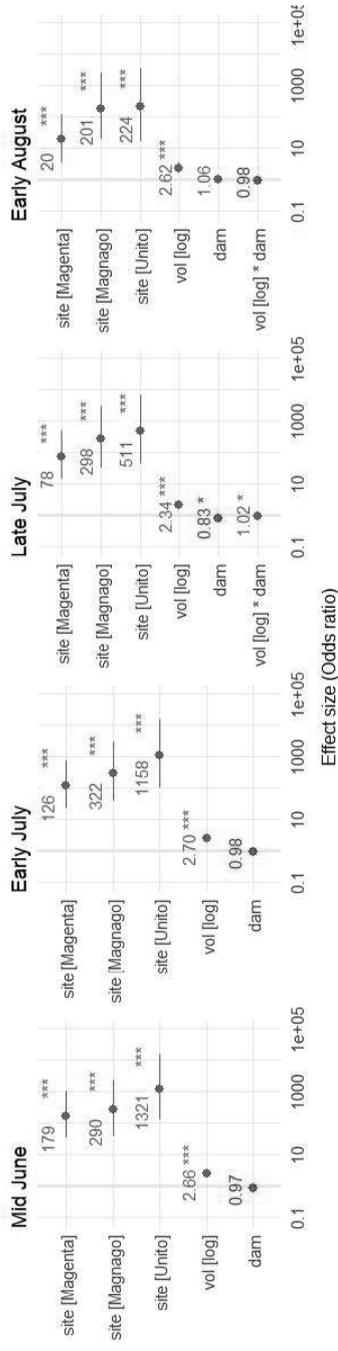


Figure 4: Odds-ratios of effect size of explanatory variables of the models with the lowest AIC per census, explaining successful raceme formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor “site” with the corresponding site name in square brackets show the effect size of site compared to Busto. Plant volume “vol” (in cm³) is log-transformed for the analysis, leaf damage in percent is abbreviated with “dam”. In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as “vol[log]*dam”.

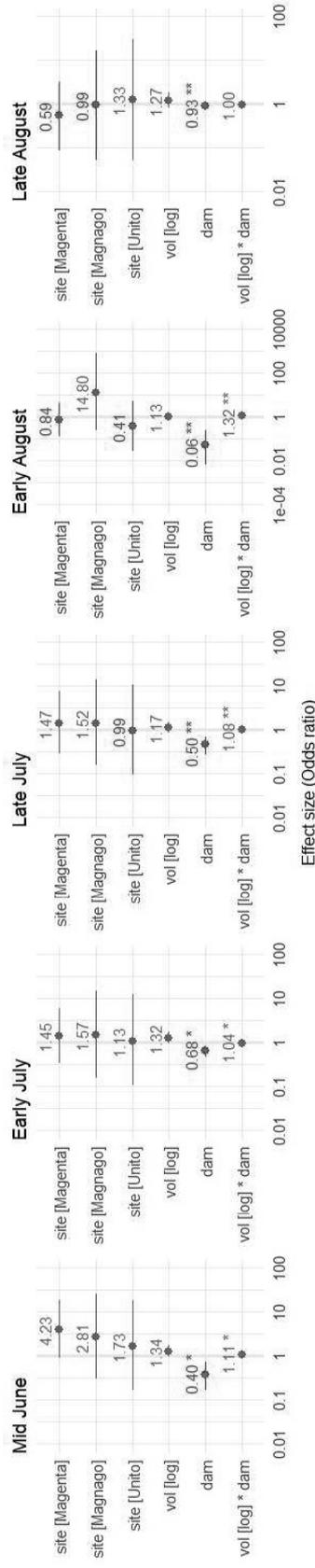


Figure 5: Effect size of explanatory variables of the models with the lowest AIC per census, explaining successful seed formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor “site” with the corresponding site name in square brackets show the effect size of site compared to Busto. Plant volume “vol” (in cm³) is log-transformed for the analysis, leaf damage in percent is abbreviated with “dam”. In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as “vol[log]*dam”.

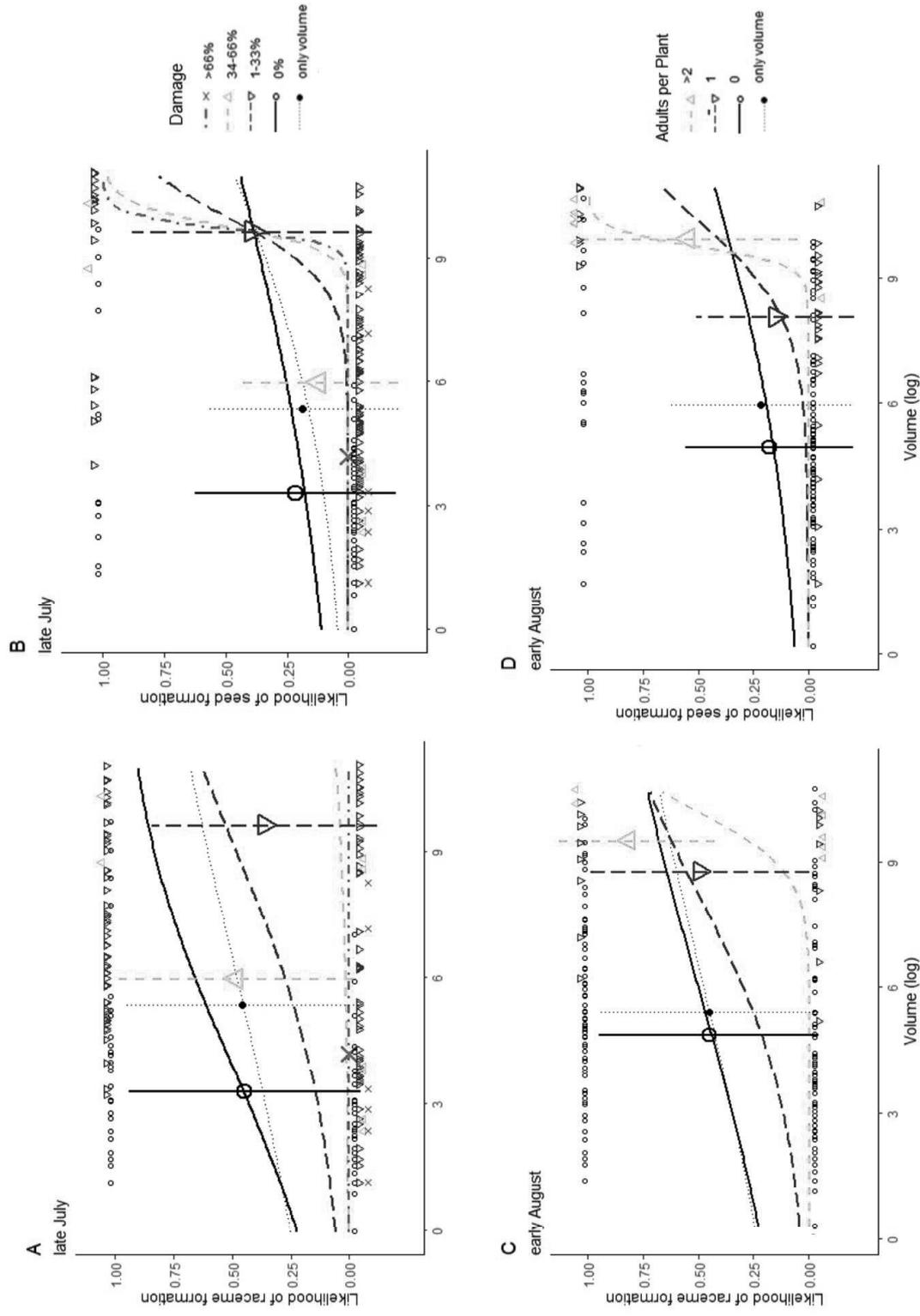
Table 1: Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful raceme formation (left part of table) and total raceme length of raceme-producing plants (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with '*'. Corresponding p-values can be found in Appendix 3.

Factor	Probability of raceme formation dependent on factor					Raceme length dependent on factor ...				
	Mid-June	Early July	Late July	Early August		Mid-June	Early July	Late July	Early August	
No <i>O. communa</i> parameter	59 0.40	58 0.40	46 0.37	6.2 0.40		13 0.724	16 0.737	8.1 0.738	1.5 0.713	
# egg batches	59 0.41 0.74 (0.48,1.15)	60 0.40 0.88 (0.58,1.34)	48 0.37 0.92 (0.52,1.65)	8.2 0.40 1.03 (0.88,1.22)		15 0.72 1.11 (0.70,1.75)	18 0.74 1.15 (0.86,1.55)	3.5 0.74 1.55 (1.12,2.13)	1.5 0.72 1.06 (0.98,1.16)	
# larvae	61 0.40 0.82 (0.25,2.72)	60 0.40 1.01 (0.78,1.32)	47 0.37 0.74 (0.42,1.30)	7.8 0.40 1.13 (0.81,1.57)		14 0.73 1.82 (0.70,4.73)	18 0.74 1.08 (0.91,1.28)	10 0.74 1.20 (0.76,1.91)	2.7 0.72 1.06 (0.95,1.19)	
# adults	60 0.41 0.85 (0.58,1.25)	60 0.40 1.05 (0.65,1.70)	48 0.37 0.95 (0.73,1.23)	4.5* 0.43 24.61 (0.97,624.27)		15 0.73 1.13 (0.72,1.77)	16 0.75 1.25 (0.93,1.67)	10 0.75 1.09 (0.91,1.30)	0 0.73 1.13 (1.00,1.28)	
% leaf damage	59 0.41 0.97 (0.94,1.01)	58 0.41 0.98 (0.96,1.01)	39* 0.43 1.02 (1.00,1.04)	0* 0.46 1.06 (0.96,1.17)		15 0.73 1.01 (0.97,1.04)	18 0.74 0.99 (0.97,1.02)	11 0.74 1.01 (0.98,1.03)	2.6 0.72 0.98 (0.95,1.01)	

Table 2: Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful seed formation (left part of table) and total seeds produced (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with [†]†. Corresponding p-values can be found in Appendix 3.

Factor	Probability of seed formation dependent on...						Number of seeds produced dependent on...					
	Mid-June	Early July	Late July	Early August	Late August	Mid-June	Early July	Late July	Early August	Late August		
No <i>O. communa</i> parameter	69 0.16	70 0.17	63 0.16	63 0.21	51 0.16	9.0 0.85	9.0 0.86	10 0.85	1.2 0.86	9.5 0.85		
# egg batches	66* 0.21 1.79 (0.83,3.87)	66* 0.23 0.00 (0,4.51)	61 0.16 1.30 (0.76,2.23)	28* 0.32 0.03 (0.00,0.44)	51* 0.19 0 (0,inf)	9.5 0.85 1.19 (0.95,1.49)	12 0.86 1.06 (0.85,1.33)	13 0.85 1.16 (0.77,1.73)	1.1 0.85 0.91 (0.82,1.01)	13 0.85 1.04 (0.15,6.99)		
# larvae	60* 0.26 0.00 (0,inf)	71 0.18 1.17 (0.90,1.54)	57 0.17 1.31 (0.79,2.18)	28* 0.32 0.00 (0,0.79)	26* 0.40 0.00 (0,0.27)	12 0.84 1.08 (0.54,2.16)	11 0.86 1.08 (0.92,1.25)	13 0.85 1.05 (0.71,1.55)	4.4 0.83 1.07 (0.68,1.69)	13 0.85 1.00 (0.77,1.30)		
# adults	68* 0.19 0.20 (0,109.11)	64 0.23 2.74 (1.15,6.53)	58 0.17 1.22 (0.90,1.65)	28* 0.31 0 (0,0.58)	31* 0.36 0.14 (0.03,0.74)	12 0.84 0.95 (0.75,1.20)	11 0.86 1.15 (0.91,1.45)	10 0.87 1.12 (0.97,1.30)	3.9 0.84 1.04 (0.93,1.16)	12 0.85 1.02 (0.95,1.09)		
% leaf damage	56* 0.29 0.40 (0.19,0.83)	59* 0.27 0.68 (0.51,0.92)	48* 0.30 0.50 (0.31,0.80)	1.9* 0.51 0.06 (0.01,0.35)	0 0.57 0.93 (0.89,0.98)	12 0.83 1.02 (0.87,1.19)	6.8 0.88 0.98 (0.96,0.99)	12 0.86 0.99 (0.97,1.01)	3.2 0.84 0.96 (0.88,1.04)	0 0.90 0.98 (0.97,0.99)		

Figure 6: Likelihood of successful raceme (A,C) and seed formation (B,D) dependent on plant volume. In A and B the data are presented for four damage classes and in C and D for three *O. communa* infestation classes (0, 1, or ≥ 2 adults per plant). The large symbols give median volume and mean probability of raceme or seed formation, respectively, together with their associated standard errors. "Only volume" shows the average values without consideration of damage or abundance classes.



Effect of percent leaf damage by *O. communa* on *A. artemisiifolia* reproduction

Including percent leaf damage by *O. communa* in models for successful raceme formation generated the models with the lowest AICc values for late July and early August, and including percent leaf damage by *O. communa* measured in late July generated the respective model with the lowest AICc value for raceme length of plants that successfully formed racemes (see Table 1). In those cases where damage reduced the probability of raceme formation, an interaction term with volume increased the fit of the models. The significance of the leaf damage x plant volume interaction term in late July for the probability of raceme formation could be explained when focusing on plants with higher percent leaf damage. Plants with 1-33% leaf damage were on average smaller than plants with 34-66% leaf damage (see Figure 6, A).

With regard to the models for successful seed formation, all models with the lowest AICc values calculated from mid-June to late August included percent leaf damage by *O. communa*. Of the *A. artemisiifolia* plants with more than 10% leaf damage in mid-June (19.7% of all plants), none produced seeds at the end of the season (Table 2). Likewise, only 4% of the plants with more than 10% leaf damage in early August produced seeds at the end of the season. In contrast, 14.5% and 40% of the plants with no leaf damage in mid-June and early August, respectively, produced seeds at the end of the season. Models including percent leaf damage measured in early July and late August also resulted in the respective models with the lowest AICc values for the number of seeds produced by successfully reproducing plants (Table 2). In particular, including percent leaf damage in models for successful seed production generated the lowest AICc values for all censuses from mid-June, when average leaf damage was approximately 5%, to the end of August, when almost all plants were defoliated to 80-100% (Table 2, Fig. 2).

In general, models for the successful formation of racemes and seeds that included percent leaf damage had lower AICc values than those that included *O. communa* abundance parameters (Table 1 and 2). For successful raceme formation, the effect size of sites was much higher than other explanatory factors. In contrary, the effect size of site was comparable to damage, volume or damage*volume interactions for successful seed formation (Figure 4,5). For the plants that successfully formed seeds, we found that models including damage in early July and late August explained the number of seeds produced best, and plants with more damage produced less seeds or racemes (see Table 2). Including percent bare soil did not increase any model fits.

Interaction of *O. communis* abundance or damage with plant volume

In 18 out of 20 cases, the models including an interaction of plant volume and *O. communis* abundance or damage improved the model fit for successful seed formation, and in the two cases where *O. communis* abundance or damage improved the models for successful raceme formation, the model included an interaction of abundance or damage with plant volume. To explore the nature of these interaction terms, we displayed the interactions graphically, splitting the data into groups (by level of damage or abundance) and plotted the probability of successful raceme formation against the log of plant volume (Fig. 4). We chose late July and early August as time points for exploration, since the models with the lowest AICc values for both successful raceme and seed formation contain an interaction term with volume at these points in time, and the relationship between adult abundance and successful raceme formation was positive in late July, contrary to all other cases. Overall, leaf damage decreased and the number of adult beetles increased with plant volume, except for plants with 0% leaf damage, which were generally very small in size. The chance of raceme formation increased with plant size and decreased with percent leaf damage and the number of adults per plant.

Explained variation

The models with the lowest AICc values within one census also had the highest pseudo R-squared values. *Ophraella communis* presence and/or damage explained the likelihood of seed formation better than the likelihood of raceme formation. Including leaf damage increased the pseudo-R² value of models for successful seed formation much more (max. 40%) than for raceme formation (max. 6%). In contrast, including *O. communis* abundance parameters hardly improved the pseudo-R² value of models of seed numbers (max. 4%) or raceme length (max. 2%). For probability of both raceme and seed formation, models had much lower AICc values and higher pseudo R-squared values from early August on. In general, *O. communis* induced leaf damage and abundance explained more variation the closer it was assessed to the flowering time.

Discussion

Our study provides evidence that the level of in-season leaf damage by *O. communis*, in combination with plant volume and site, helps explaining final seed production. Six weeks before flowering, leaf damage by *O. communis* together with plant volume is correlated to pollen production by *A. artemisiifolia* at the end of the season. Explanatory power of models improved over the season. Models including leaf damage had generally higher explanatory

power than models including *O. communa* abundance parameters. For successful raceme formation, experimental sites had a much higher explanatory power than leaf damage, but for seed formation, explanatory power of leaf damage was similar to explanatory power of site, with lower variation. This offers possibilities to use in-season leaf damage for developing impact forecast models, which help informing management whether biological control is likely to successfully reduce seed production of this invasive alien plant species in a given region or year, or whether complementary management interventions should be considered to achieve long-term population decrease.

In-season *Ophraella communa* abundance, damage and plant mortality

The peak in *O. communa* abundance in early August coincides with the expected timing of the fourth and last generation in this region (Mouttet et al. 2018). We did not observe population growth of *O. communa* until late July, probably due to the relatively low densities of the different life stages and considerable variation within censuses. The lack of egg batches from September onwards is most likely due to a photoperiod-induced diapause in reproduction, as described for *O. communa* in Japan (Tanaka and Murata 2017).

While damage increased significantly in August at all sites, there was considerable variation in average leaf damage among sites (Table 2). In particular, average damage in Busto Arsizio increased to > 25% within the first three weeks of the study and was at least 25% higher than the site with the next highest average damage in early August (Magenta; Fig. 2). The high average damage in Busto Arsizio can be explained by the observations that small plants were defoliated quickly and died earlier than the extraordinarily large plants (> 2m) on this ex-arable site, while the large plants also out-shaded the smaller ones (Fig. 3). Intraspecific competition between *A. artemisiifolia* plants has been shown to have a negative effect on leaf area and aboveground biomass (Patracchini et al. 2011), and herbivory could amplify these effects by reinforcing competitive interactions (Crawley 1983).

It should be noted that our study did not cover the very first months of the growing season of *A. artemisiifolia*. In Northern Italy, gravid *O. communa* females that have overwintered start laying eggs on *A. artemisiifolia* seedlings as soon as the temperature is high enough for the beetle to fly (Bosio et al. 2014). At the study sites, first egg batches on *A. artemisiifolia* seedlings were observed from early April onwards (H. Müller-Schärer, unpublished results). Mortality of plants in spring was not covered by our experimental set-up, but is likely to further increase the impact of *O. communa* on *A. artemisiifolia* at the population level (Lommen, unpublished results).

Significant impact on target weed populations are only expected with high densities of biological control agents (Myers and Sarfraz 2017, McEvoy 2018), and the outcome of our experiment supports this notion. Defoliating *A. artemisiifolia* plants up to 90% by clipping leaves did not influence reproductive traits under laboratory conditions (Gard et al. 2013). Similarly, Lommen et al. (2017a) found that 90% defoliation of adult plants by *O. communa* in laboratory experiments did not reduce pollen production, as long as the racemes were not attacked. In our study, average leaf damage by *O. communa* only reached more than 90% in late August, which coincided with increased plant mortality.

Effect of in-season *O. communa* abundance and damage on *A. artemisiifolia* raceme and seed production

With regard to the probability of both raceme and seed formation, *O. communa* leaf damage appears to be a better explanatory variable than *O. communa* abundance, since AICc values were lower for models including damage than those including abundance for all census dates. This could be due to the behaviour of the beetle; *Ophraella communa* adults are highly mobile (Yamanaka et al. 2007) and larvae have been observed to regularly move between plants under laboratory and outdoor conditions (Stéphanie von Bergen, personal communication). So, while leaf damage reflects the cumulative effect of the feeding activity of the beetle over time, beetle abundance represents rather a certain point in time. In weed biocontrol, impact assessments often focus on the abundance of biological control agents that are required to control a certain number of plants. For example, the impact of different life stages of *Zygogramma bicolorata* on *Parthenium hysterophorus* (Shabbir et al. 2016), or the estimation of *O. communa* impact on *A. artemisiifolia* in China (Guo et al. 2011, Chen et al. 2013) were estimated in number of adults per plant. This may be useful information when the herbivore load per plant is augmented by mass-releasing biological control agents, but it remains difficult to estimate absolute abundances of insects in field settings (Fowler and Witter 2017). Furthermore, the per capita impact may vary depending on the plant size (Biere et al. 2017), shading conditions (Muth et al. 2008), nutrient composition of the plant leaves (Zehnder and Hunter 2009), or the amount of induced defence compounds in the plants (Burghardt and Schmitz 2015). Our results suggest that for the leaf beetle *O. communa*, and probably other mobile insect herbivores, in-season damage levels may provide a more robust and easier-to-quantify variable for projecting potential impact on reproductive output of *A. artemisiifolia* at the end of the season. Similarly, early season feeding damage of the bug *Bagrada hilaris* on

broccoli was also found to be a reliable and accurate variable for monitoring this pest in broccoli fields (Palumbo and Carrière 2015).

All but one model in which *O. communa* abundance explained the probability of reproductive organ formation contained an interaction with plant volume. Plant volume influences the response of the plant to abundance of herbivores or herbivore-induced leaf damage. In line with Lommen et al. (2018b), volume was positively correlated with probability of reproductive organ formation (see Figure 4, 5).

In general, we found more adults on bigger plants, probably explained by a positive effect of plant volume on adult beetle abundance, rather than a positive effect of *O. communa* abundance on plant size. Caged experiments with varying plant sizes and number of adults could shed some additional light on the potentially interacting effects of plant volume and *O. communa* impact.

The models for the likelihood of seed formation generally had higher pseudo R-squared values than the models for the likelihood of raceme formation (Tables 1 and 2), indicating a larger amount of variation in the models for successful raceme formation remaining unexplained. Experimental site had a much higher effect size than damage or volume to explain chance of raceme formation, but for the chance of seed formation size effects of site and volume, damage or volume*damage interactions were comparable (see Figure 4,5). This means that the effect of volume and leaf damage could be used to explain successful seed formation, but for successful raceme production, other factors might be more important. We observed that the mortality in between late August (sampling time for racemes) and late September (sampling time for seed formation) increased in the plants that were not treated with insecticides compared to those treated in three of the four sites (Fig.3). We can explain why models for successful seed formation have less unexplained variation than models for raceme formation, if we assume that plant mortality is damage-dependent, but only obvious in late September. In that case, raceme formation would be more influenced by other factors that are not included in our model.

Our study provides evidence that the window of impact by *O. communa* on reproductive output of *A. artemisiifolia* is relatively narrow (see Fig.2). Similarly, two biological control agents released to control *Clematis vitalba* in New Zealand only induced disease symptoms late in the season, resulting in no significant impact on plant growth and only minor reduction of area covered by the invasive weed (Paynter et al. 2006), and impact of two biological control agents of *Parthenium hysterophorus* had different levels of impact between

1996 and 2000 depending on weather conditions (Dhileepan 2003). Thus, in regions or years with less favourable climatic conditions, the population peak of *O. communa* may be delayed or reduced to an extent that the impact of this biological control agent on the reproductive output of *A. artemisiifolia* is considerably impaired.

Arthropod demography is strongly influenced by climate, especially temperature, where an increase often results in quicker population growth. Since overall damage is strongly dependent on the number of generations, and as these are expected to increase with temperature in species with a multivoltine life cycles, damage is also expected to increase in a warming climate in the future (Möller et al. 2017). However, if raising temperatures leads to reduced relative humidity, climate change may also have a negative effect on population build-up of *O. communa*. Laboratory studies revealed that relative humidity of less than 50% during the warmest time of the day significantly reduced egg hatching rates of this biological control agent (Augustinus and Sun et al., 2020). Since biocontrol agent impact is dependent on high population densities (Myers and Sarfraz 2017, McEvoy 2018), both temperature and humidity should be considered for models predicting *O. communa* impact on *A. artemisiifolia* (Augustinus and Sun et al., 2020).

Implications for *A. artemisiifolia* management

Our findings that average leaf damage from mid-June onwards explained a significant amount of variation in the likelihood of seed formation indicates that *O. communa* feeding has a direct detrimental effect on female reproduction in *A. artemisiifolia*. Moreover, while the negative effect of leaf damage on the likelihood of pollen production only was significant in the census made in late July, *O. communa* exclusion experiments conducted in the same area revealed that *O. communa* reduces pollen production per unit area by 82% (Lommen et al. unpublished results). These findings are in line with an observed 80% decrease in airborne ragweed pollen counts in the Milano region since the establishment of *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b), also during the year in which this study was conducted.

Hence, our findings suggest that percent leaf damage in mid-June or early July could be used as an indicator for the likelihood that *O. communa* significantly reduces reproductive output of *A. artemisiifolia* at the end of the season (see Appendix 5). At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. The plants with more than 10% leaf damage in early July that produced seeds were all large plants (91-181cm high in early July) growing at the Busto Arsizio site. At this site, where leaf damage had a less pronounced negative effect on seed and raceme formation,

plants were far taller than at most other sites in Northern Italy. Apparently, plants with such an extraordinarily high volume are able to collect enough reserves to survive and produce seeds successfully before the stark increase of damage by *O. communa* in early to late August, while smaller plants cannot compensate for the damage caused by the beetle. Developing an *O. communa* damage forecasting model based on average leaf damage and plant volume in early summer may be used to support decision making regarding the scheduling of additional common ragweed management interventions. In the case that population build-up is predicted not to be early and high enough to prevent flowering, mass releases through mass-rearing, as practiced in China (Guo et al. 2011, Chen et al. 2013), could be envisaged. Also, as *A. artemisiifolia* can form dense stands on fallow crop fields (Lehoczky et al. 2013, Ottosen et al. 2019), prospective management methods could be additional mowing, ploughing or mulching of the fields just before male flower formation. Decreasing these stands mechanically would result in less pollen production in a direct way, by removing these plants from the flowering part of the *A. artemisiifolia*, and indirectly by forcing *O. communa* to other *A. artemisiifolia* plants that are less accessible for mechanical control. Timing of mowing or herbicide applications along linear transport infrastructures (railways, roads) might also be a cost-efficient means of managing *A. artemisiifolia*, but at least three mowing interventions are needed (Lommen et al. 2018c). Experiments combining mowing regimes and beetle presence on *A. artemisiifolia* are needed to better clarify the joint effect on the control of this species.

Conclusion

This study provides evidence that the level of in-season leaf damage by *O. communa* helps explaining the impact of this biological control agent on seed and – to a lesser extent – pollen production by *A. artemisiifolia* at the end of the season. Leaf damage measured as early as mid-June partially explains, in combination with plant volume, the likelihood of reproductive output of *A. artemisiifolia* at the end of the season. For example, none of the plants with more than 10% leaf damage in mid-June formed seeds at the end of the season. It should be noted, though, that at extreme sites where *A. artemisiifolia* plants grow 2 m and taller (such as at Busto Arsizio), impact of *O. communa* may be largely explained by plant volume, rather than by average leaf damage in early summer. Our results suggest that in-season assessment of leaf damage and plant volume could be used to develop predictive models for *O. communa* impact on *A. artemisiifolia* seed production, similar to the approach used in crop pest forecasting.

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Appendix 1

Study sites

	Latitude	Longitude	Years since agricultural use	<i>Ambrosia artemisiifolia</i> abundance early June	<i>Ambrosia artemisiifolia</i> abundance late September
Busto Arsizio	45.595051	8.864487	1	1001-10000	1001-10000
Magenta	45.459476	8.874251	3	101-500	101-500
Magnago	45.570611	8.785669	Woodland until 4 years before project	>10000	1001-10000
Unito, University of Torino, Grugliasco	45.064812	7.593255	3	101-500	26-100

Appendix 2

Census dates

Site	Census 0	Census 1	Census 2	Census 3	Census 4	Census 5
Busto Arsizio	2016-06-14	2016-07-06	2016-07-19	2016-08-08	2016-08-30	2016-09-22
Magenta	2016-06-15	2016-07-05	2016-07-19	2016-08-08	2016-08-30	2016-09-21
Magnago	2016-06-18	2016-07-06	2016-07-19	2016-08-09	2016-09-01	2016-09-23
Unito	2016-06-13	2016-07-05	2016-07-27	2016-08-06	2016-08-29	2016-09-28

Appendix 4

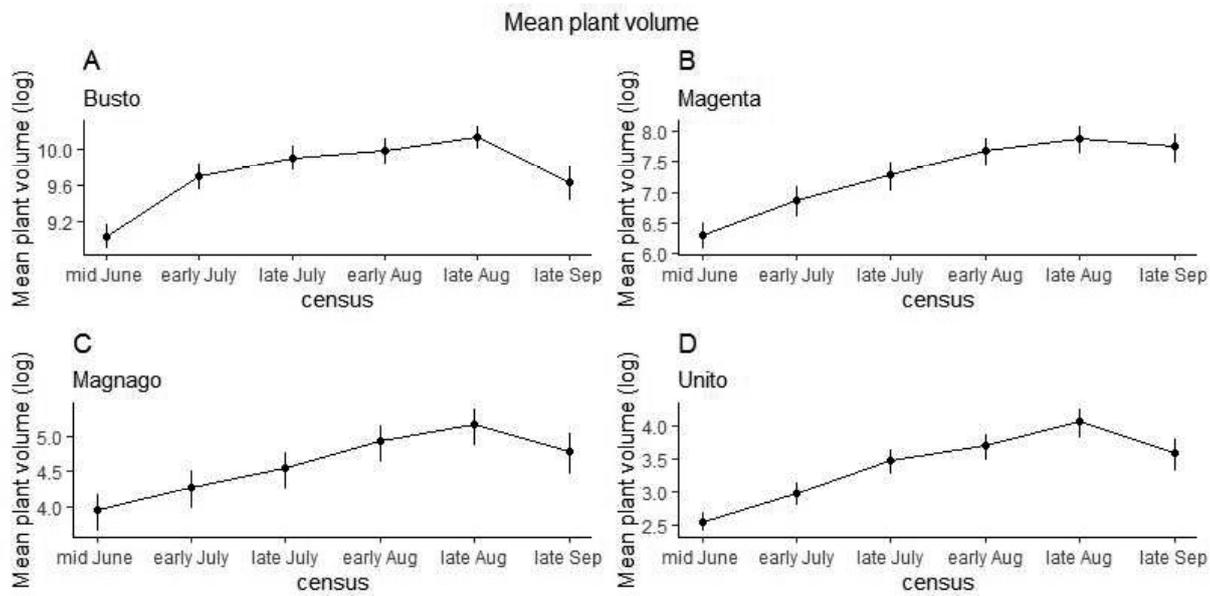


Figure S1: Mean plant volume \pm se of *A. artemisiifolia* plants measured during the experiment in the four experimental sites.

Appendix 5

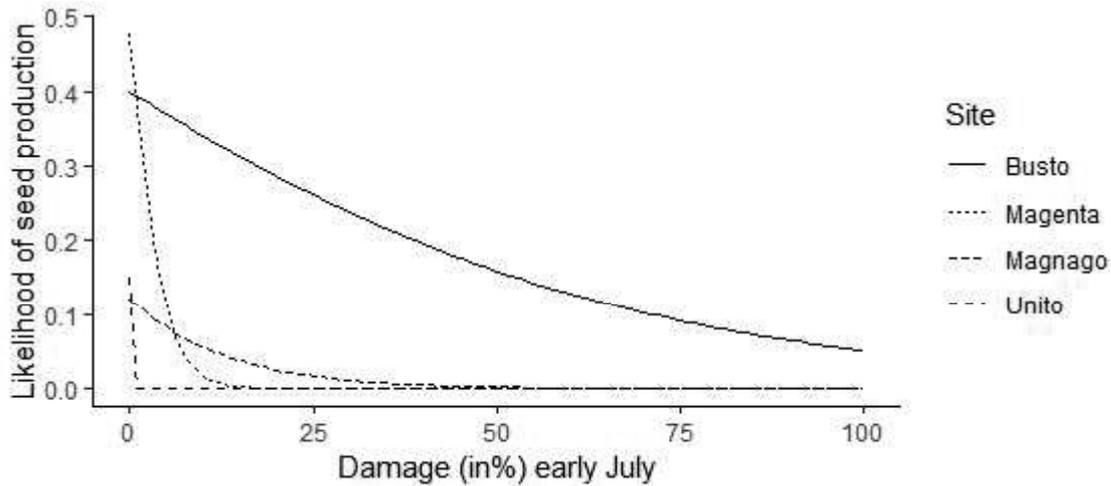


Figure S2.: Likelihood of *A. artemisiifolia* seed formation dependent on *O. communa* leaf damage in early July. The different line types show the different responses between the sites.

CHAPTER 3

Predicting impact of a biocontrol agent: Integrating distribution modelling with climate-dependent vital rates

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Abstract

Species distribution models can predict the suitable climatic range of a potential biological control agent (BCA), but they provide little information on the BCA's potential impact. To predict high population build-up, a pre-requisite of biocontrol impact, studies are needed which assess the effect of environmental factors on vital rates of a BCA across the environmental gradient of the BCA's suitable habitats, especially for the region where the BCA is considered for field release. We extended a published species distribution model with climate-dependent vital rates of *Ophraella communa*, a recently and accidentally introduced potential BCA of common ragweed, *Ambrosia artemisiifolia* in Europe. In field and laboratory experiments, we collected data on climate-dependent parameters assumed to be the most relevant for the population build-up of *O. communa*, i.e., temperature driving the number of generations per year and relative humidity (RH) determining egg hatching success. We found that *O. communa* concluded one generation in 334 cumulative degree days, and that egg hatching success strongly decreased from >80% to <20% when RH drops from 55% to 45% during the day. We used these values to spatially explicitly project population densities across the European range suitable for both *A. artemisiifolia* and the beetle and found that the present distribution of the beetle in Europe is within the range with the highest projected population growth. The highest population density of *O. communa* was predicted for northern Italy and parts of western Russia and western Georgia. Field observations of high impact on *A. artemisiifolia* with records of 80% aerial pollen reduction in the Milano area since the establishment of *O. communa* are in line with these predictions. The relative importance of temperature and RH on the population density of *O. communa* varies considerably across its suitable range in Europe. We propose that the combined statistical and mechanistic approach outlined in this paper helps to more accurately predict the potential impact of a weed BCA than a species distribution model alone. Identifying the factors limiting the population build-up of a BCA across the suitable range allows implementation of more targeted release and management strategies to optimize biocontrol efficacy.

Keywords:

Ambrosia artemisiifolia, bioclimatic range, biocontrol insect, biological invasions, demography, *Ophraella communa*, range prediction, relative humidity, temperature, weed biocontrol

Introduction

Biological invasions by alien plant species can result in devastating impacts on the invaded ecosystems (Vilà et al. 2011). Accordingly, sustainable management of invasive alien plant species is considered one of the biggest challenges conservation biologists will face in the next decades (Kettenring and Adams 2011). Classical biological control of weeds, i.e., the use of specialist natural enemies from the native range to reduce densities of invasive alien plant species below an economic and ecological threshold, is considered as one of the most suitable management options against invasive alien plant species, owing to its effectiveness and relatively high environmental safety (Müller-Schärer and Schaffner 2008, Winston et al.

2014). Predicting the impact of BCAs on the target plant populations remains, however, a challenge in weed biological control (Heimpel and Cock 2018, Paynter et al. 2018, Schwarzländer et al. 2018). Insect population growth is highly dependent on weather, which makes climate suitability an important part of the selection process of weed BCAs (Zalucki and Van Klinken 2006, Robertson et al. 2008). So far, most attention has been given to habitat suitability of a potential biological control candidate (Hoelmer and Kirk 2005, Mukherjee et al. 2011, Sun et al. 2017, Sun et al. 2018). Gassmann (1996), however, argued that the crucial attribute for successful biological control is high population build-up of the BCA, as only high densities of the herbivore insect population are expected to result in significant impact on the populations of the target weed (Myers and Sarfraz 2017, Rand et al. 2017, McEvoy 2018). This is supported by the fact that successful biological control of invasive alien plant species is usually linked to population outbreaks of the natural enemies, during which they may reach densities several orders higher than known from their native range (Gassmann 1996, Müller-Schärer and Schaffner 2008). A better understanding of the factors affecting the population dynamics of weed BCAs will thus be key for improving the prediction of their potential impact across the area invaded by the target plant.

Temperature and relative humidity (RH) are generally considered to be the most important climatic factors affecting the population build-up of insects (Odum 1983). Temperature is an essential climatic factor driving insect population densities and is thus used for pest predictive modelling in the agricultural context (Venette 2015, Johnson et al. 2016, Magarey and Isard 2017, Blum et al. 2018). Besides temperature, RH is also known to strongly influence insect demography, with low RH often resulting in reduced survival or female fitness (Zhou et al. 2010a, Holmes et al. 2012, Wigglesworth 2012). Insect eggs may be particularly sensitive to low RH (Sabelis and Helle 1985, Schausberger 1998) as they are immobile and have a large relative surface area, which makes them prone to desiccation (Krysan 1976, Wigglesworth 2012). Numerous studies have shown that low RH negatively affects egg hatching rate of insects from a wide range of taxa (e.g. Sabelis and Helle 1985, Chaudry and Alikhan 1990, Bethke and Redak 1996, Byrne et al. 2002, Simelane 2007, Lu and Wu 2011). Within the physiological limits, temperature affects insect population density primarily via developmental time, which may prevent a species to complete its life-cycle or reduce the number of generations in multivoltine species (Milbrath et al. 2014, El Iraqui and Hmimina 2016). In contrast, RH is likely to influence population density by affecting the numbers that develop per generation. For example, Byrne et al. (2002) concluded that the reason for the poor establishment and variable impact of a chrysomelid BCA, *Gratiana spadicea* Klug, in the introduced range was more likely drought stress than temperature, since RH dropped below the calculated lethal humidity for eggs each month of the year.

The accuracy of population density estimation depends on detailed and long-term systematic monitoring, which requires considerable efforts (Zeng et al. 2015). As an easier and quicker method, species distribution models (SDMs) have been proposed to predict also population densities (Oliver et al. 2012), since SDMs reflect the extent to which local environments meet the niche requirements of the species and thus a species' overall performance (Thuiller et al. 2014). However, the relationship between suitability predictions and population densities often showed uncertainty and contradicting results (Thuiller et al. 2014). Oliver et

al. (2012) found a significant positive relationship between habitat suitability and population density for both butterflies and birds, while Csergő et al. (2017) showed that the population growth rate of 34 plant species was not correlated with climate suitability.

Keith et al. (2008) and Gallien et al. (2010) suggested combining statistical and mechanistic models as a two-step approach to make reliable and robust predictions. Following this, we start with published SDMs and explicitly incorporate the environmentally (temperature and RH) dependent vital rates (developmental time and hatching success) of a BCA that we experimentally assessed to improve the prediction of the BCA's population density, and ultimately impact within the eco-climatically suitable area. We deliberately kept the mechanistic part simple to provide a quick method to increase the meaningfulness of an SDM. This work is based on the premise that consideration of the effects of temperature and RH on the population build-up of a BCA in areas suitable for both the target weed and the herbivore allows us to make testable and hopefully more accurate predictions regarding the impact of the BCA in the introduced range than estimates based on SDMs only.

We explored the above mentioned effects with the invasive alien plant *Ambrosia artemisiifolia* L. (Asteraceae), or common ragweed, and one of its potential BCAs, the North American leaf beetle *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), which was accidentally introduced and first reported in Europe in 2013 (Müller-Schärer et al. 2014). *Ambrosia artemisiifolia* has particularly raised awareness for invasive alien plant species in Europe (Kettunen et al. 2009). The main issue with *A. artemisiifolia* is its production of a large number of highly allergenic pollen, resulting in huge health costs through decreased quality of life and productivity of the sensitized population (Burbach et al. 2009, Smith et al. 2013, Mouttet et al. 2018, Müller-Schärer et al. 2018). Furthermore, it is a major agricultural weed, especially in spring-sown crops (Burbach et al. 2009, Smith et al. 2013, Müller-Schärer et al. 2018).

Classical biological control has been successfully applied against *A. artemisiifolia* in China, using the accidentally introduced *O. communa* and the deliberately released stem-galling moth *Epiblema strenuana* Walker (Zhou et al. 2014). *Ophraella communa* can reach up to 6-7 generations per year in southern China (Zhou et al. 2014) and 4-5 generations per year in Japan (Yamanaka et al. 2007). The difference is likely due to temperature differences along the latitudinal gradient. In Europe, *O. communa* starts laying eggs on *A. artemisiifolia* as soon as the first seedlings emerge in early April, indicating that temperature might be a main driver for voltinism in this invaded range. Under laboratory conditions, female *O. communa* enter reproductive diapause under short-day conditions (12h daylight) and at a temperature of 20°C (Zhu et al. 2012). Zhou et al. (2010a) showed that a decrease in constant RH from 90% to 60% reduced the survival rate of *O. communa* eggs by approximately 30%, while other life stages were less affected (9% lower larval survival and 2% lower pupal survival at 60% RH compared to 90% RH). In Europe, some areas invaded by *A. artemisiifolia* have RH values which are considerably lower than those studied by Zhou et al. (2010a), e.g. less than 30% during the daytime in central Hungary. Hence, Europe urgently needs better prediction on the relative importance of temperature and RH on the population build-up of *O. communa*.

Here we asked 1) how does temperature affect the development rate of *O. communa* and thus the number of generations per year before the observed initiation of the reproductive diapause in the suitable area in Europe, 2) how does RH affect the egg hatching success of *O. communa* and thus the population build-up within and across generations, 3) what spatially explicit variation in abundances do we expect for *O. communa*, and 4) what is the spatially explicit relative contribution of temperature and RH to population densities of *O. communa* in the environmentally suitable area in Europe? The overall objective of our two-step approach was to improve with limited additional resources the predictiveness of the pre-release impact assessment for weed BCAs and to identify the specific factors limiting their population build-up, which would allow a more targeted release and management strategy.

Material and Methods

Study species

Ambrosia artemisiifolia is a North American Asteracean that has invaded areas outside its native range in all continents except Antarctica. In Europe, the annual plant is considered a weed since the early 1920s (Csontos et al. 2010). It is invasive in more than 30 countries (Essl et al. 2015) and has reached the status of a flagship invader because of its impact on human health (Sheppard et al. 2006). Up to date, *O. communa* has been found in Europe predominantly in and close to the Po plain (Augustinus et al. 2015, Lommen et al. 2017), which is a relatively warm and moist area compared to other parts of Europe invaded by *A. artemisiifolia* (Chapman et al. 2017, Sun et al. 2017). In 2017, *O. communa* has also been recorded from Slovenia and Croatia (M. Cristofaro, pers. observations). *Ophraella communa* overwinters as adults. In northern Italy, the leaf beetle reaches 3-4 generations and females were observed to lay eggs from April to August (Augustinus & Lommen, unpublished data). The cessation of oviposition in northern Italy in early September, when day length is approximately 13h and average temperature starts dropping below 20°C, is in line with the experimental studies on the initiation of reproductive diapause in *O. communa* (Zhu et al. 2012, see above).

Both adults and larvae feed on the green parts of *A. artemisiifolia* plants, which can result in defoliation rates of up to 100% before the monoecious plants form flowers, preventing pollen and seed production (Müller-Schärer et al. 2014). In Northern Italy, *O. communa* has been shown to reduce populations on a local scale (Lommen et al. 2018) and since the arrival of the beetle airborne *A. artemisiifolia* pollen counts have dropped by 80%, which cannot be explained by meteorological factors or land use changes (Bonini et al. 2015a,b).

Effect of temperature and relative humidity on vital rates of *Ophraella communa*

Effect of temperature on developmental time in the field

The temperature-dependent developmental time of *O. communa* from egg to adult was experimentally assessed at five sites along an altitudinal gradient of 1100m in the Southern Alps, approximately 50 km north of the Milan area, in the summer of 2016. We followed two cohorts of egg batches (one starting end of June,

one starting early August). Potted *Ambrosia artemisiifolia* plants with freshly laid eggs were individually caged and brought out to the field sites, which we visited weekly to count the number of eggs (hatched or unhatched), pupae, and adults (see Mouttet et al. (2018) for further details). Temperature-dependent pre-oviposition time of *O. communis* was assessed in an experiment in July 2016 at the same sites as described before. Ten newly emerged females were released together with 10 males in a 1x2x1m cage with 12 *A. artemisiifolia* plants, in two cages per site. We recorded the first date of egg deposition.

To assess the temperature conditions required for a complete generation, we measured temperature and relative humidity data locally in 5-minute intervals inside and outside of the cages with climate data loggers (ELV, TDF 128). In addition, we collected weather data from a local weather station (Arconate, of the Servizio Meteorologico Regionale Lombardia), which is ~7.5km away from the lowest experimental site. The locally collected weather data were considerably different from the weather station data, with more extreme values (higher temperature, lower RH) during the warmest times of sunny days. For the analysis and projection of our climate-dependent data, we chose to use the weather station data, since the available gridded climate data are based on weather station data, rather than on land surface or leaf surface temperatures and RH. We estimated the temperatures for the different sites by reducing the temperatures of the weather station by 0.65°C per 100m altitudinal gain (ICAO 1993), which is well in line with the temperature attenuation per 100m calculated from the measures taken with the climate data loggers (0.69°C).

We estimated the mean generation time by calculating the mean cumulative degree days (CDD) for every replicate by counting the average number of days until adult emergence and added the CDD for the mean pre-oviposition period to it. CDD were calculated using the single sine wave method (Roltsch et al. 1999), with 13.34°C as the minimum development threshold temperature for all immature stages (Zhou et al. 2010b). To calculate the standard error (SE) of the CDD, we first estimated SEs for the developmental period from egg to adult and for the pre-oviposition period using 1,000 bootstrap replicates, respectively. As we found no reports of a relationship between the duration of the two periods of *O. communis* development, we considered them as independent and thus used the sum of the two SEs to represent the SE of the CDD over one generation.

Effect of relative humidity on the egg hatching rate

The effect of RH on egg hatching rate, defined here as the percentage of fertilized eggs that successfully hatch, was determined in a growth-chamber experiment at the University of Fribourg. *Ambrosia artemisiifolia* plants were grown from seeds collected in Busto Arsizio, Italy, in 2015 and 2016. Seeds were germinated in closed Petri-dishes on moist filter paper in a climate chamber at 18-26°C, with a photoperiod of 16:8 (l:d). After germination, seedlings were transplanted in standard potting soil and kept in the biosecurity greenhouse at University of Fribourg, with a light regime of 16:8 (l:d) and temperatures of 13-29°C. *Ophraella communis* were collected in Grugliasco, northern Italy, in September 2016 and reared in the quarantine facility of the Biology Department, University of Fribourg, at 26°C during the day and 17°C during the night, at 60% RH and a daylight regime of 16:8 (l:d).

Three days before treatments were set up, 12 *A. artemisiifolia* plants in the vegetative stage (8-12 leaves) were exposed to *O. communa* adults (20 females and 20 males) of various ages in a cage and daily inspected for newly laid egg batches. To prevent insects from crawling into the soil, we covered the top of the pot with a filter paper disc before exposure to *O. communa* adults. Small leaves were removed to ensure that the egg-infested leaves were suitable for the experiment. Leaves with at least one egg batch consisting of more than 10 eggs were cut off the plant at the leaf petiole with a sharp scalpel blade and randomly assigned to a treatment. Leaves with egg batches with less than 10 eggs were discarded.

50ml Falcon tubes (Fisher Scientific) were filled with water and sealed with parafilm. The parafilm was punctured with a needle and the petiole of a leaf with eggs was inserted into the tube, sealing the hole. The tubes with leaves were put in a holder for 50ml tubes. The holders stood on a plastic plate, of which the borders were lined with double-sided sticky tape to prevent larvae from crawling away. The tubes were placed in two KB 8400 FL incubators (Termaks) with programmable humidity, light and temperature functions.

Each RH treatment was conducted with at least 15 replicates. The driest RH treatment was based on the climate data recorded between mid and end of August from a site in the Pannonian plain (data from the weather station at Hodmezúvasarhely, Hungary), one of the warmest and driest regions of the *A. artemisiifolia* distribution in Europe. The incubators were programmed in order to match the hourly variation in RH and temperature under natural conditions as closely as possible (Appendix S1, Fig. S1 and Fig. S2). The additional RH treatments were set at the RH of the driest treatment plus 7, 14, 17, 20 and 24%, respectively (Appendix S1, Fig. S3), while temperature and light regimes were kept identical among treatments. The light regime was set to mimic the daytime in Hungary in late July (14:10 l:d). We measured egg hatching rate across the six RH treatments, with the treatments randomly assigned to one of the two incubators. Each RH treatment lasted 12 days. Data from one run had to be omitted from the analysis because of technical problems with the incubator.

After assigning the egg batches to a treatment, they were inspected daily and their status (alive, dead, hatched, and unfertilized) recorded. We scored eggs as 'dead' if they turned black, as 'hatched' when a hole was found in the egg and the egg was empty, and as 'unfertilized' when the eggs became flat without turning black. Hatching success was calculated as the percentage of hatched eggs relative to fertilized eggs (sum of hatched and dead eggs). The experiments ran from March through July 2017.

For validation of our treatments, temperature and RH were measured with loggers (ELV TDF 128) in each incubator every 5 minutes. Due to technical limitations of the incubators, we did not get an exactly evenly spaced RH range. To account for this variation, we averaged all measured RH values measured from 14:30 to 15:30 by the data loggers in the middle of the incubator. We fitted generalized linear mixed models (GLMM) using the *lmer* function of the *lme4* package (Bates et al. 2015) using the RH treatments as a fixed factor and incubator and date of start of the replicate as random factors. As response variables we tested hatching time (number of days from egg deposition until first hatching egg of batch), hatching interval (number of days from first to last egg hatching within a given batch) and egg batch size using a Poisson distribution, and hatching success using a binomial distribution. Gompertz models have been used to describe the effect

of environmental factors on egg hatching success (Omkar and Mishra 2010, Quiroz et al. 2015). We fitted a three-parameter modified Gompertz model to the experimental RH data to determine the hatching success with the formula

$$HS_i = f_h(RH_i) = (1 - \alpha) \times e^{(-\beta \times e^{(-\gamma \times RH_i)})} + \alpha, \quad \text{eqn.1}$$

where i refers to the selected month ($i = 4-8$; April to August), α is the baseline of hatching success that is assumed to have a maximum value of 1, β denotes the displacement across the x-axis (RH of the month i) and γ is the rate of increase (Gompertz 1825). The method of least squares was used to fit the Gompertz model and optimal-fit parameter estimates were obtained using the Nelder Mead algorithm in *optim*, a general purpose optimisation function of *stats* package (R Core Team 2018). To estimate the confidence intervals of the three parameters of the Gompertz model, 1000 bootstrap estimates were performed using the *boot* function of the *boot* package (Canty and Ripley 2017). The Akaike Information Criterion (AIC) was used to select the most appropriate model. We found that the Gompertz model results in a lower AIC than a logistic function, with 170.74 and 884.71 for Gompertz and logistic model, respectively. We thus chose the Gompertz model for computing hatching success, as lower AIC values indicate more information preserved in the model and thus greater model performance.

Modelling population density

Number of generations of Ophraella communa

To estimate the number of generations of *O. communa* across its suitable area in Europe, we used the above obtained average CDD, based on a T_{base} of 13.34°C (Zhou et al. 2010b) for the period from the egg stage to adult emergence of *O. communa*, and added the accordingly calculated CDD for pre-oviposition period to achieve the CDD for one generation, since earlier results show a highly significant linear relationship between developmental time and temperature (Zhou et al. 2010b). The number of generations (G) was calculated for every month or the whole growing season, using formula

$$G_i = \frac{\bar{T}_i \times \text{days}}{CDD_{\text{bootstrap}}}, \quad \text{eqn. 2}$$

Where i refers to the selected month ($i = 4-8$; April to August), \bar{T}_i is the average temperature of the month i , $CDD_{\text{bootstrap}}$ refers to the 1000 bootstrap estimates. SE of the generation was therefore calculated based on the above 1000 bootstrap estimates (Appendix S2, Fig. S1).

Relative humidity estimation and hatching rate of Ophraella communa across the European range

We used monthly average RH and monthly minimum and maximum temperature derived from CRU CL2.0 datasets (New et al. 2002) at 10-min spatial resolution (~20 km close to the equator) to estimate the RH in the afternoon of each day, i.e. at 15:00, based on the method proposed by Kriticos et al. (2012). We used the RH at 15:00, which was the driest hour in the field measurements in the Pannonian plain and in the measurements of the weather station in Hodmezúvasarhely, and because it is indicative for the driest 6 hours of the day (Augustinus, personal observation). The climatic data are considered from April to August, i.e. the

egg-laying period of *O. communa* in Europe. The egg hatching rate was therefore calculated based on the above obtained Gompertz formula and estimated RH (Appendix S2, Fig. S2 & S3).

Abundances of Ophraella communa

We calculated the potential maximum population density of *O. communa* in Europe using the average climate of the total egg laying period (April-August) and the average climate per month. For this, we assumed no shortage of the host plants and no effects of natural enemies. To consider monthly variation, population density was calculated for every month separately, using the number of overwintering females or females of the previous month, the number of eggs per female, hatching rate and the number of generations per month. The values for August were then used as the final population density (PD) and thus abundance (model I), using the formula:

$$PD_i = G_i \times \frac{PD_{i-1}}{2} \times 25 \times HS_i, \quad \text{eqn. 3}$$

$$PD_{\text{mod1}} = PD_8, \quad \text{eqn. 4}$$

where i is the selected month ($i = 4-8$; April to August), for the first month of the growth season, April, we considered the number of overwintering females is 1 as a start simulation point. Thus, we assumed that the number of overwintering females was the same across the suitable area in Europe. The average number of eggs laid per female was assumed to be 25, based on previous studies (Yamazaki et al. 2000, Fukano et al. 2016). The relationship between suitability from SDM (using the data from Sun et al. 2017) and population density of *O. communa* calculated from model I was also evaluated.

To explore the relative importance of the average temperature and RH on the population density of *O. communa*, we modeled the population density based on the average temperature and RH from April to August (model II), using the formula:

$$PD_{\text{mod2}} = f_{\text{pd}}(\overline{T_a}, \overline{RH_{1500}}) = \frac{25 \times f_h(\overline{RH_{1500}})}{2} G_{\overline{T_a}}, \quad \text{eqn. 5}$$

where $f_{\text{pd}}(\overline{T_a}, \overline{RH_{1500}})$ refers to the population density function of average temperature and average RH at 15:00, $f_h(\overline{RH_{1500}})$ is the Gompertz function of average RH for hatching success, and $G_{\overline{T_a}}$ is the number of generations calculated based on the average temperature from April to August with $CDD_{\text{bootstrap}}$. SE of the population density (PD_{mod2}) is calculated based on the 1000 bootstrap replicates (Appendix S2, Fig. S4). To test for the agreement between the model I and II, we computed the correlation between the predictions obtained from the two models (see Appendix S2, Fig. S4-S6).

Relative importance of temperature and RH on population density

The relative importance can be derived from the slope of temperature (slope_T) vs. the slope of RH (slope_{RH}), using the following formula (eqn. 6 – eqn. 8):

$$slope_T = \frac{f_{pd}(\overline{T_{av}+1}, \overline{RH_{1500}}) - f_{pd}(\overline{T_{av}-1}, \overline{RH_{1500}})}{2(T_{max} - T_{min})} \quad \text{eqn. 6}$$

$$slope_{RH} = \frac{f_{pd}(\overline{T_{av}}, \overline{RH_{1500}+1}) - f_{pd}(\overline{T_{av}}, \overline{RH_{1500}-1})}{2(RH_{max} - RH_{min})} \quad \text{eqn. 7}$$

$$\text{Relative importance of average temperature (T\%)} = \frac{slope_T}{slope_T + slope_{RH}} \times 100 \quad \text{eqn. 8}$$

The relative importance of average temperature and RH are first presented in a heatmap and then transferred into a spatial map.

All analyses were done within the predicted suitable range of both *A. artemisiifolia* and *O. communa* in Europe, using the raster layer obtained from Sun et al. (2017, Fig. 1B). All analyses were performed using R statistical software, version 3.5.0 (R Development Core Team 2018).

Results

Effect of temperature on the developmental time of *Ophraella communa*

Based on a T_{base} of 13.34°C (Zhou et al. 2010b), *O. communa* developed from egg to adult in 280.93 ± 6.64 CDD and females started laying eggs after 53.11 ± 4.45 CDD, resulting in a complete generation of 334.04 ± 11.09 CDD.

Effect of relative humidity on egg hatching rate of *Ophraella communa*

RH significantly affected the egg hatching success of *O. communa* (generalized mixed model, $p < 0.001$, $z = 8.75$; further details provided in Appendix S3). The curve of egg hatching success starts at a plateau of approximately 20% egg hatching rate below 45% RH, followed by a steep incline from 20-80% egg hatching rate between 45 and 55% RH, and then reaches a plateau of ~90% egg hatching rate from higher than 55% RH (Fig. 1; Table 1). The Gompertz model explains 30% of the variation in hatching rate with RH (Fig. 1).

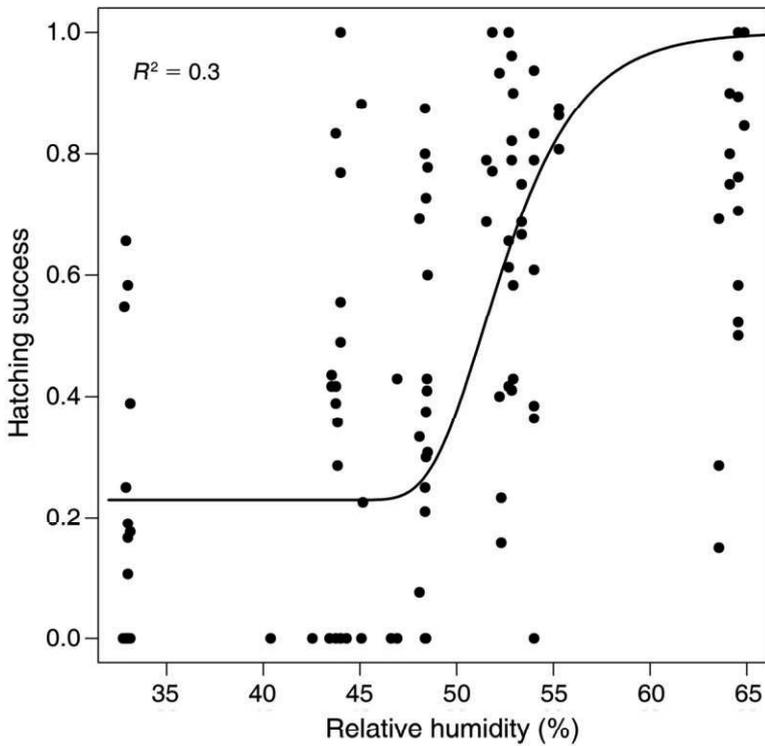


Fig. 1. Gompertz curve (solid line) fitted to the hatching rates determined in the experimental relative humidity study. Each dot represents an egg batch.

In contrast to egg hatching rate, egg hatching time (linear mixed model, $t = -1.495$, $p = 0.536$) or hatching interval (linear mixed model, $t = -1.376$, $p > 0.231$) were not significantly affected by RH. Also, egg batch size did not differ among RH treatments (linear mixed model, $t = -0.019$, $p > 0.979$; see Appendix S3).

Table 1. The optimal parameters of the Gompertz model that fit RH data to determine the hatching rate with their standard errors (SE) and 95% bias-corrected accelerated (BCa) confidence intervals (CI).

confidence intervals (CI).

Parameter	Original	SE	5%BCa-CI	95%BCa-CI
α	0.23	0.1	0.15	0.46
β	1.1×10^8	0.33	1.1×10^8	1.1×10^8
γ	0.36	0.16	-0.06	0.37

Estimated population density of *O. communa* across Europe

The highest average hatching rate of *O. communa* across its suitable area in Europe was found north of the Alps, in northern Italy, Slovenia and along the east coast of the Black Sea in Russia and Georgia, and the highest number of generations was predicted for central and northern Italy, northern Serbia, northern Bulgaria, southern Romania and Georgia (Fig. 2). In general, hatching success was negatively correlated with number of generations ($df = 3165$, $P < 0.001$, $cor = -0.25$).

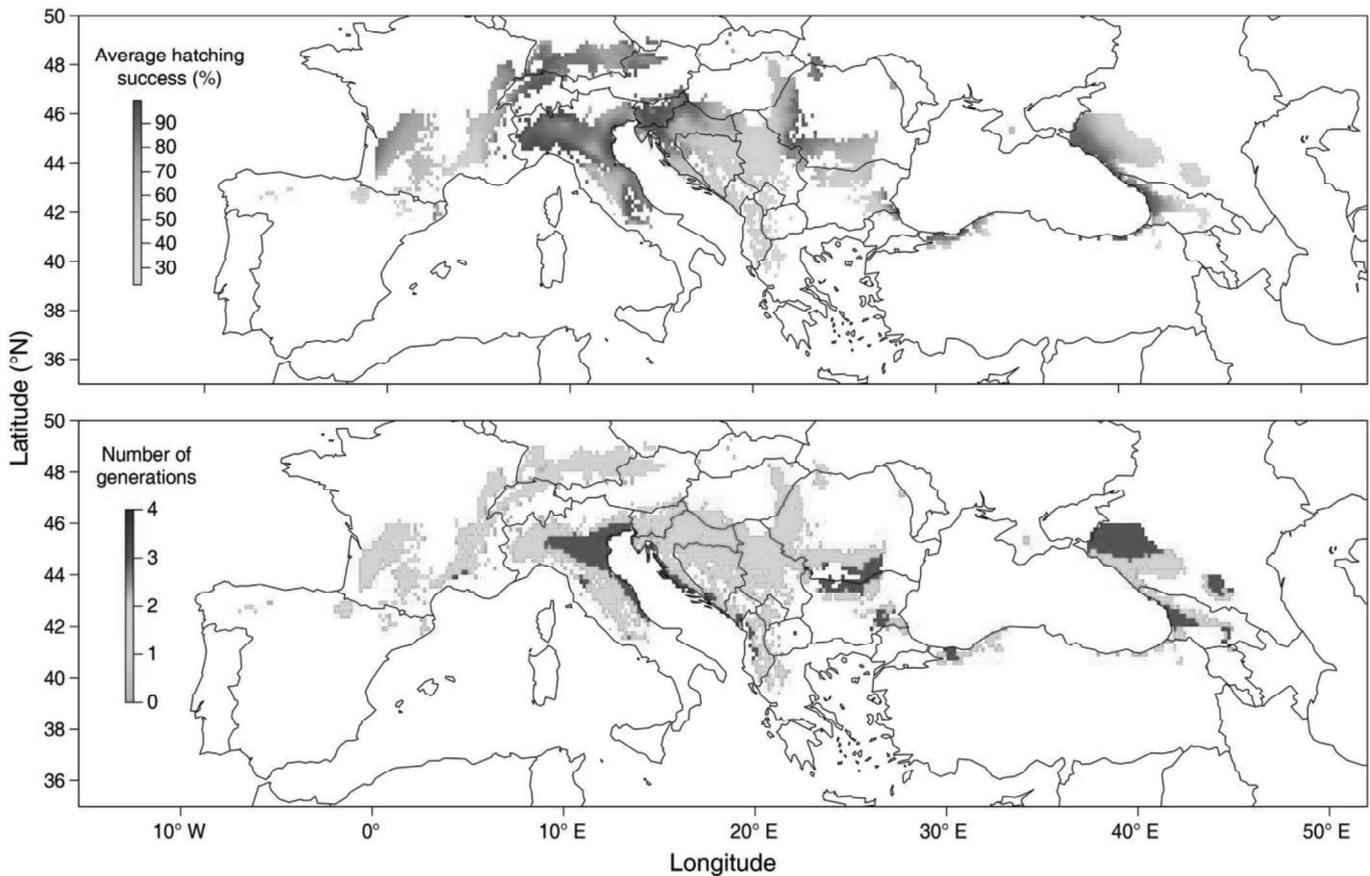
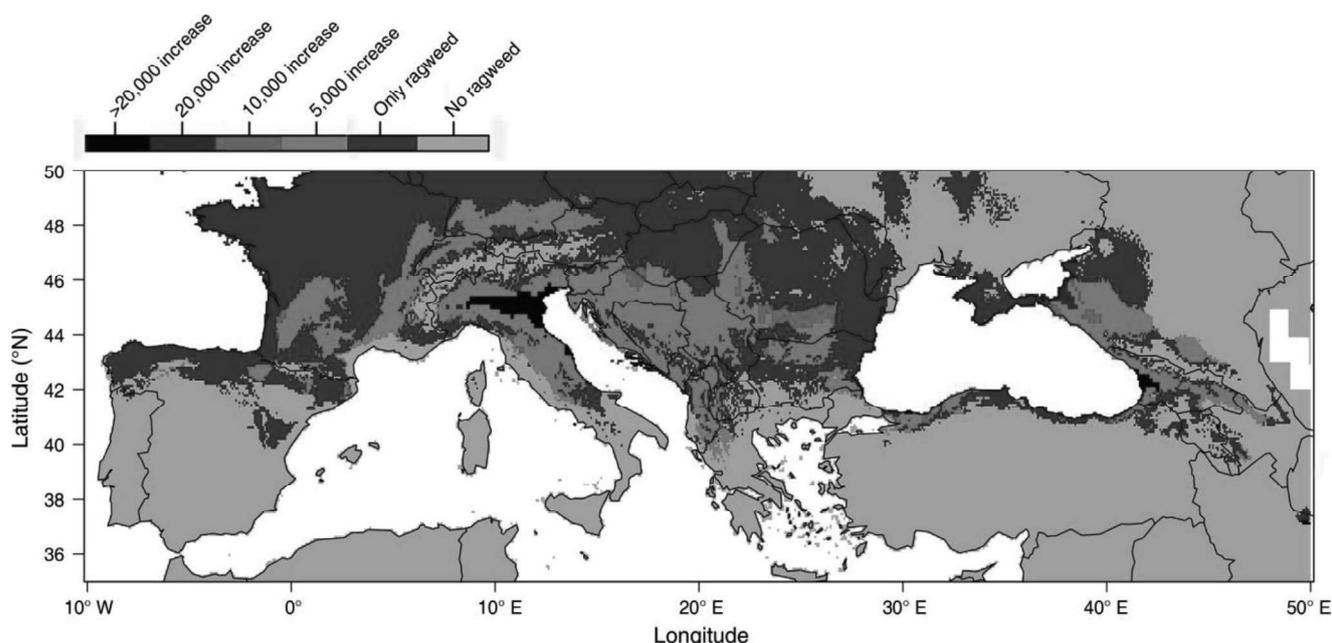


Fig. 2. Average hatching rate of *Ophraella communa* during the growth period from April to August (top), and number of generations of *O. communa* in August (below) across the European range climatically suitable for both *Ambrosia artemisiifolia* and *O. communa*.

The highest population density of *O. communa* was predicted for northern Italy and parts of western Russia and western Georgia (Fig. 3). The predicted population density of *O. communa* based on monthly temperature and monthly RH (model I, eqn. 4) is highly positively correlated with average temperature and average RH (model II, eqn. 5) for the period of April to August ($P < 0.001$, $cor = 0.87$; Appendix S2, Fig. S6). Our results also showed that the suitability level derived from SDM explained a significant, but still very low part of the variation of the population density of *O. communa* ($P < 0.001$, $R^2 = 0.03$; Appendix S2, Fig. S7).

Fig. 3.



Population density of *Ophraella communa* based on average relative humidity and relative temperature from Model II (eqn. 5). Grey color indicates the area not suitable for both *A. artemisiifolia* and *O. communa*; green color indicates the area suitable for *Ambrosia artemisiifolia* but not for *O. communa*; reddish colors indicate the area suitable for both *A. artemisiifolia* and *O. communa*: the darker the reddish color, the higher the density

Average temperature contributed more than RH to population density of *O. communa*, when RH was above 52% at 15:00, while average RH was a key climatic factor when it was below 48% at 15:00 (Fig. 4). We found that the best climatic conditions to achieve a high population density of *O. communa* are 23-25°C (average temperature, April-August) and 53%-65% (average RH, April-August) at 15:00 (Appendix S2, Fig. S8). This information was then used to map the relative importance of the two climatic factors on the population density of *O. communa* within its suitable area in Europe (Fig. 5). Population density of *O. communa* in the Apennines, north of the Alps, north of the Dinaric Alps, in Romania and along the Black Sea coast of Russia and Georgia is shown to be primarily affected by temperature, while population density south of the Massif Central, south of the Dinaric Alps, in Serbia, western Georgia and a small part of western Russia is predicted to be largely affected by RH (Fig. 5).

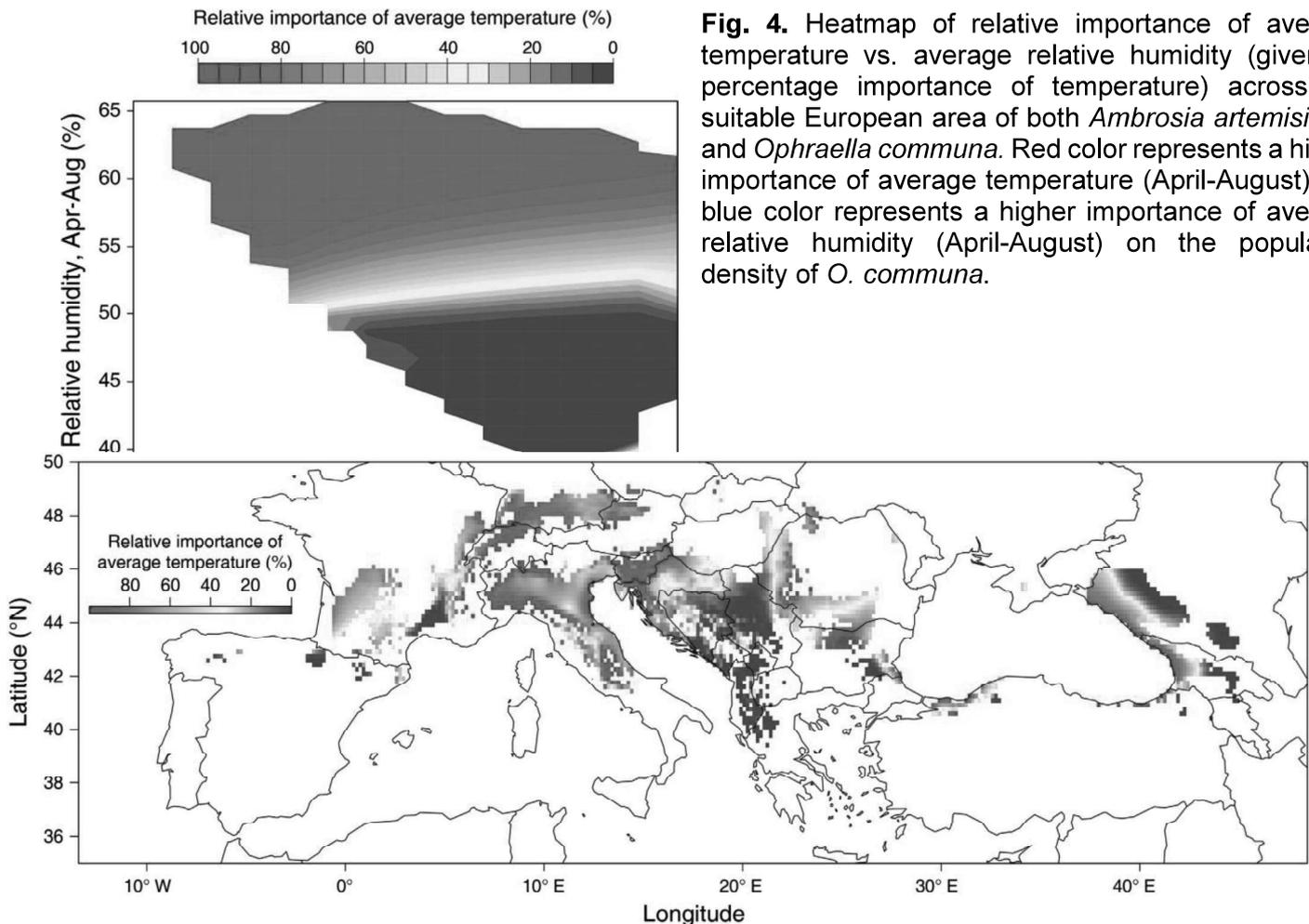


Fig. 4. Heatmap of relative importance of average temperature vs. average relative humidity (given as percentage importance of temperature) across the suitable European area of both *Ambrosia artemisiifolia* and *Ophraella communa*. Red color represents a higher importance of average temperature (April-August) and blue color represents a higher importance of average relative humidity (April-August) on the population density of *O. communa*.

Fig. 5. The relative importance of average temperature vs. average relative humidity (given as percentage importance of temperature) across the suitable European area of both *Ambrosia artemisiifolia* and *Ophraella communa*. Red color represents a higher importance of average temperature (April-August) and blue color a higher importance of average relative humidity (April-August) on the population density of *O. communa*.

Discussion

Our study reveals that both temperature and RH affect vital rates of *O. communa* and that adding physiological-based demographic data to a SDM results in predictions of significant variation in the population density of *O. communa* across the climatically suitable regions in Europe. The currently invaded range by *O. communa* in Northern Italy is predicted to support one of the highest population growth rates in Europe, which is in line with our observation of 80% pollen reduction in this area (Bonini et al. 2015a,b). Other regions that appear particularly suitable for high population densities are the warm and moist areas in western Georgia and the Krasnodar region. Our findings suggest that both temperature and RH affect population dynamics, but that the relative importance of these two environmental variables varies across the suitable range.

Impact of temperature and RH on vital rates of *O. communa*

In our field experiments, *O. communa* completed a generation from egg to reproductive adult in 334.04 ± 11.09 CDD, which is similar to the earlier experiments by Zhou et al (2010b), which suggested it would take 307.20 CDD. The discrepancy in these values could be explained with the nature of the different studies. First, Zhou et al (2010b) did not include the CDD of the pre-oviposition period. Secondly, we describe the

results of field experiments, while the experiments of Zhou et al (2010b) were conducted under constant temperatures in a laboratory environment. In a field experiment with fluctuating temperatures and solar exposure, insects can adjust their body temperatures by their behavior like hiding from the sun or basking (Eigenbrode et al. 2015). The sigmoid form of the fitted curve for the relationship between RH and egg hatching rate showed a strong decrease when RH dropped below 55% during the driest time of the day (see Fig.1). In laboratory experiments using constant RH levels, Zhou et al. (2010a) found that egg hatching success of *O. communa* was significantly higher at 75 and 90% RH, compared to 60% RH. In the for *O. communa* climatically suitable area in Europe, average RH does not reach such high average values, largely because of a significant drop in RH during daytime. In order to approximate field conditions, we included daily variation in our treatments. We found a hatching rate of >85% at RH of 65%, which is considerably higher than that found by Zhou et al (2010a), who report a hatching rate of $55.6 \pm 1.6\%$ at 60% RH and $84.5 \pm 1.3\%$ at 75% RH.

We used weather station data for our estimations since the readily available gridded climate datasets are based on weather station data. The climate data we measured in the field, however, showed more extreme values than the values of nearby weather stations. The shaded data loggers used to obtain these data were placed in between the plants to display the temperature and RH experienced by the insects. Weather stations follow a protocol that provides standardized measurements of temperatures on a global scale, but they measure air temperature, not foliage or land surface temperatures (Mendelsohn et al. 2007). Using land surface or foliage surface temperatures might improve predictions of the insect's demography, as it did for population models of the olive fruit fly (*Bactrocera oleae* Rossi) (Blum et al. 2015) and cotton bollworm, *Helicoverpa armigera* Hübner (Blum et al. 2018). When making predictions for specific areas and population densities, this discrepancy in temperature and RH should be considered.

The effect of RH on egg hatching rate has been estimated for various insect species (Schaber et al. 1975, Bethke and Redak 1996, Simelane 2007, Norhisham et al. 2013), but the demographic consequences have only been assessed in a few cases (Chaudry and Alikhan 1990, Byrne et al. 2002). For example, Lu and Wu (2011) showed that low humidity had a detrimental effect on *Apolygus lucorum* (Meyer-Dür) (Heteroptera:Miridae) egg hatching rate, resulting in a lower intrinsic capacity of increase (r_m) and finite rate of increase (λ). They used this knowledge to explain outbreak events of this pest of cotton, especially after heavy rain. Bearing in mind the considerable effect of RH on predicted *O. communa* population growth, we propose to more often include RH-dependent mortality when studying the demography of insect BCAs. The correlation between estimates for average RH over April-August (model II, eqn. 5) and average RH per separate month (model I, eqn. 4) is relatively high (0.87, see Fig.S4), indicating that trends in relative importance of temperature and RH are stable over the whole period. As *O. communa* was detected in 2018 in the northern parts of the Balkan Peninsula in Slovenia and Croatia (Cristofaro, personal communication), our predictions regarding its population density can probably be tested soon.

To illustrate the approach of combining SDMs with experimental assessments of the climate dependency of vital rates, we focused on the effect of two climatic variables on selected vital rates of *O.*

communa. While the laboratory data by Zhou et al. (2010a,b) suggest that temperature particularly affects the development time and RH primarily the egg hatching rate of *O. communa*, other abiotic or biotic factors may also affect its population dynamics. For example, density-dependent mortality at later developmental stages through competition and predation may compensate for a high egg hatching rate. It should be noted, though, that *O. communa* does not yet have specialized enemies in its introduced range (Müller-Schärer and Schaffner 2008). Nevertheless, as all introduced species interact with resident species across different trophic levels, predictions on population densities of BCAs should also consider consequences of potentially climate- or habitat-dependent biotic factors (Van der Putten et al. 2010).

Predicting climate suitability for biological control agents and their abundances

A common approach to assess habitat suitability of BCAs is to estimate its potential range using SDMs (Trethowan et al. 2011, Sun et al. 2017). SDMs are purely numerical tools, combining species observations with environmental estimates and projecting these over a novel range (Elith and Leathwick 2009). In the case of *O. communa*, habitat suitability level explained a significant but a very low part of the variation in density in our study, suggesting the notion that habitat suitability based on SDMs may be an unreliable proxy for population dynamics (Thuiller et al. 2014). However, as impact of an invasive species, such as a classical BCA, is often described as the product of its distribution, abundance and the per capita effect on the target plant (Parker et al. 1999), abundance is an important factor that needs to be taken into account if one wants to improve predictions on the impact of potential BCAs (Gassmann 1996). Mechanistic models are often used to analyze the mechanisms underlying the spatial pattern of population dynamics (Vinatier et al. 2011). However, only a few studies attempted to link statistical and mechanistic methods (Ehrlén & Morris, 2015). For example, Kearney & Porter (2009) reviewed how physiologically based SDMs can be developed for different organisms in different environmental contexts; and Buckley (2007) showed how geographic variation in traits and life histories influences lizard population densities.

In summary, we propose that our approach allows a simple projection of the population growth at a given site in the suitable area relative to other sites that the BCA is able to colonize, of the region that is considered for field release, or relative to a site where we already know the population growth and/or the impact of the BCA. Furthermore, our approach allows us to analyze the relative importance of changes in temperature and RH affecting demography of the BCA, especially in view of inferring management decisions.

Management implications

Our study revealed that the projected population growth of *O. communa* in its currently colonized area in northern Italy is among the highest in Europe, together with areas in western Georgia and the Krasnodar region, where *A. artemisiifolia* is also highly invasive (Reznik 2009). The high *O. communa* densities observed in Northern Italy since its detection in 2013 and the concomitant 80% reduction in airborne pollen concentrations cannot be explained by meteorological factors (Bonini et al. 2015a,b). Hence, our findings suggest that *O. communa* will be able to drastically reduce pollen production and related health costs also in western Georgia and the Krasnodar region, provided that the beetle is able to actively colonize these regions.

Considering the benefit of *O. communa* in other parts of Europe (Bonini et al. 2015a,b, Mouttet et al. 2018), a risk-assessment of human-assisted dispersal of the beetle in these regions should be considered.

A way to further increase the impact of *O. communa* on *A. artemisiifolia* in Europe is then to adopt a mass rearing program, as it is currently practiced in China (Zhou et al. 2009). Targeted mass releases of beetles could be envisaged in areas where low RH during a certain period in spring may slow down the population growth of *O. communa*. For example, parts of south-eastern France, which harbor high *A. artemisiifolia* densities and are characterized by relatively dry conditions in April, may be potential target areas for mass releases in May.

In other regions, where RH is limiting population establishment and/or population density throughout the season, successful biological control of *A. artemisiifolia* may require introducing a different, more drought-resistant strain of *O. communa*, or of a different BCA, which is less susceptible to low RH. An example for such a region is the Pannonian plain in Hungary, where *A. artemisiifolia* is a particularly problematic weed (Kömíves et al. 2006). For areas predicted to have lower *O. communa* densities and that are more sensitive to temperature, e.g. North of the Alps, one can test for the possibility to develop a cold-tolerant/resistant strain. Zhou et al. (2013) recently studied the potential of *O. communa* to adapt to cold temperatures to assess the possibility of an expansion towards northern China. Their results indicate that cold hardiness of *O. communa* can be promoted by cold acclimation in the previous generation, and that it might counter-balance reduced survival in the next generation, when insects are tracking their host-plants into colder climates.

Conclusions

We propose combining statistically based SDM with the experimental assessment of climate-dependent vital rates for improving the prediction of the potential population growth of BCAs. Our results on *O. communa* show that the relative importance of climatic factors affecting population growth greatly differs spatially across the range that is climatically suitable for both the plant invader and its BCA. From a management perspective, we advocate the proposed combined approach as a step forward to improve predicting the impact in weed biological control programs as well as to increase impact by conducting informed management.

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Supporting Information

Annex 1. Temperature and relative humidity (RH) treatments of the laboratory experiments

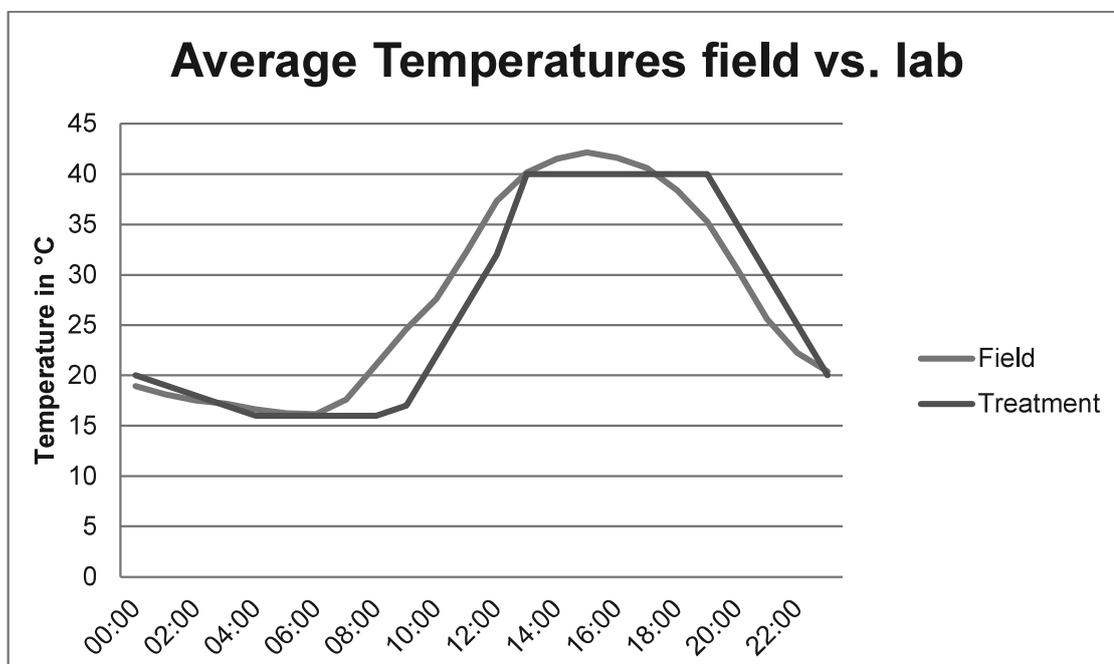


Fig. S1: Hourly average temperature measured in the field in Hodmezúvasarhely (Hungary) and hourly temperature programmed in the laboratory experiment. Field data collected between 12-31 August 2016.

Field

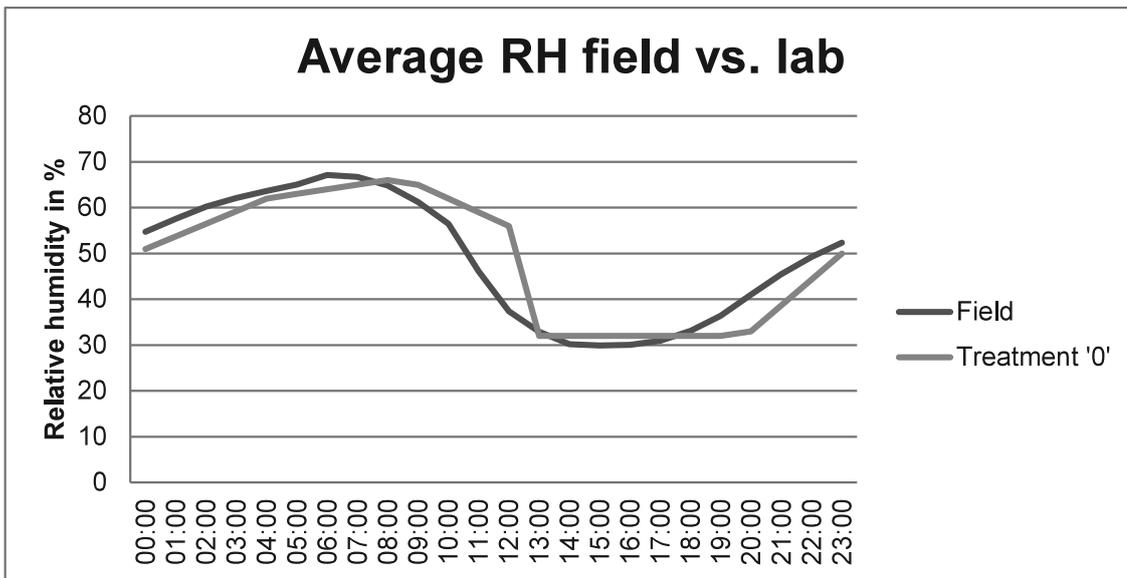


Fig. S2: Hourly average relative humidity (RH) measured in the field in Hodmezvasarhely (Hungary) and hourly RH programmed in the laboratory experiment in Treatment '0' (the driest treatment).

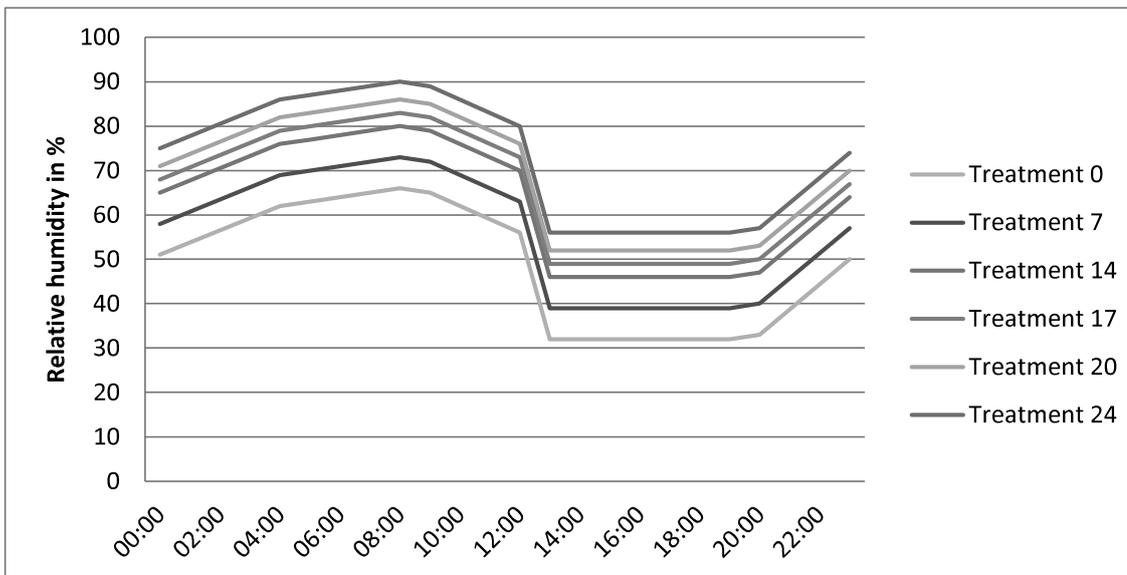


Fig. S3: RH treatments programmed for the laboratory experiments, ranging from "Treatment 0" (see above; Annex 1 Fig. S2) to "Treatment 24" (+24% RH).

Annex 2: Supplementary figures of various models

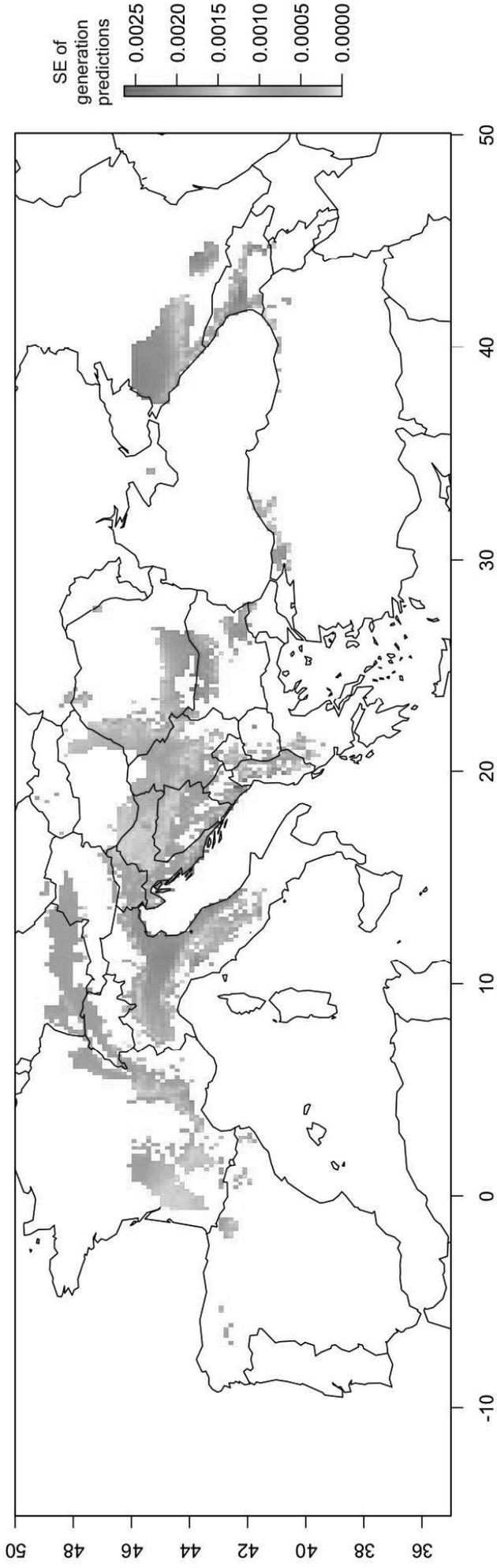


Fig. S1. Standard error (SE) of number of generations of *Ophraella communa*, based on 1000 bootstrap estimates of CDD (eqn. 2), across the European range climatically suitable for both *Ambrosia artemisiifolia* and *O. communa*. Colors represent different levels of SEs.

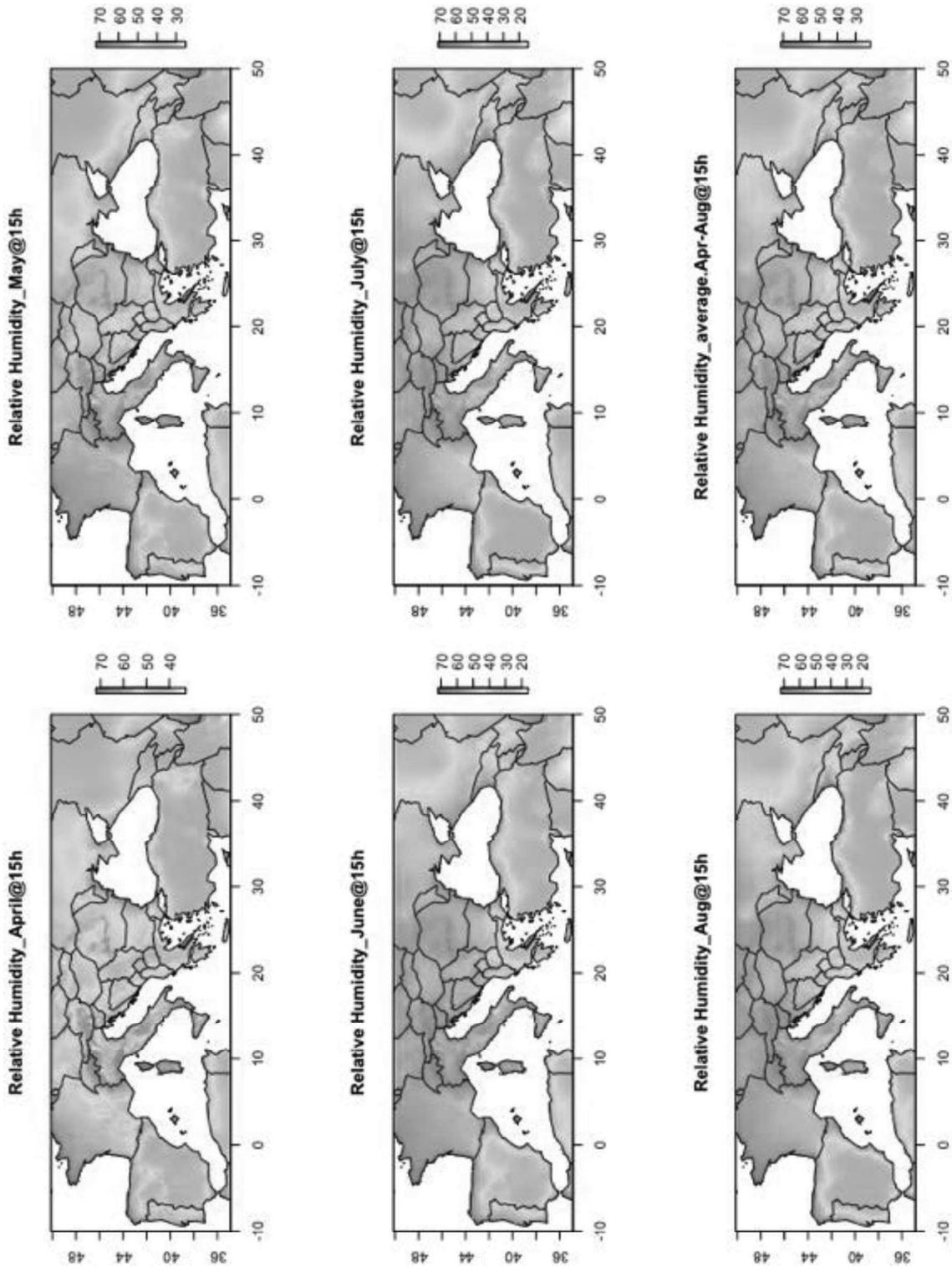


Fig. S2. Monthly relative humidity and average relative humidity (April-August) in Europe. Colors indicate different percent relative humidity (RH).

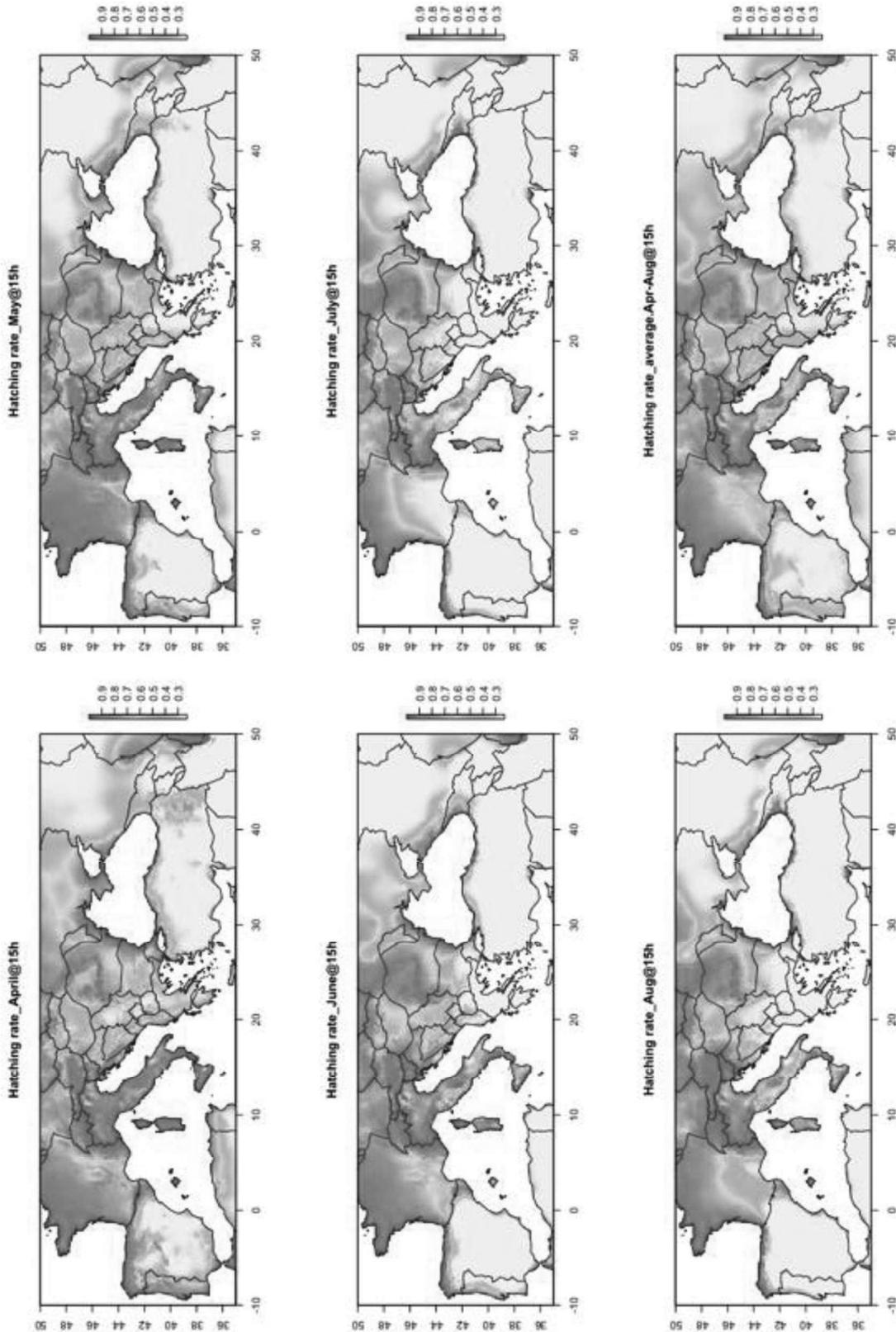


Fig. S3. Monthly hatching success and average hatching success (April-August) in Europe. Colors indicate different hatching rates.

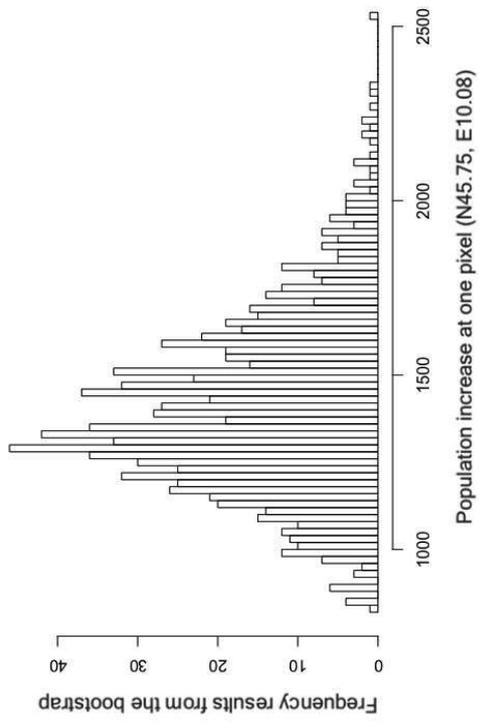
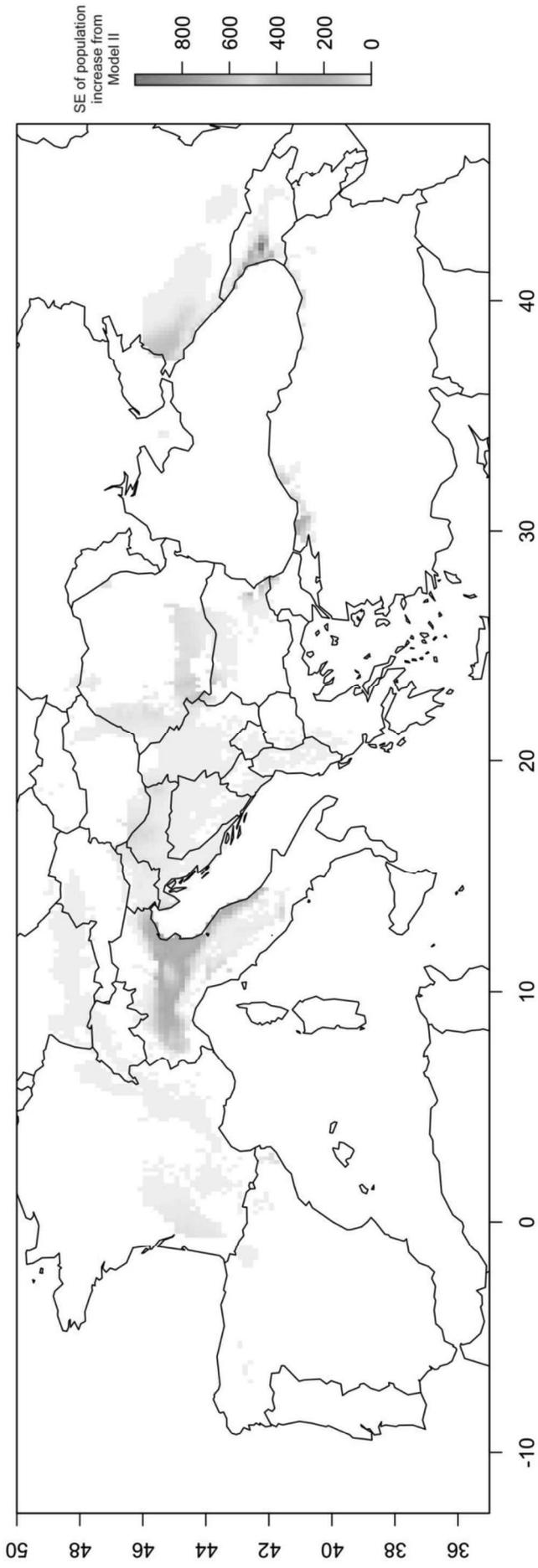


Fig. S4. Above: standard error (SE) of populations density of *Ophraella communa* (based on average relative humidity and monthly relative temperature, Model II, eqn. 5); colors indicate different levels of SEs; below: an example of the 1000 bootstrap replicates of population density predictions at one pixel.

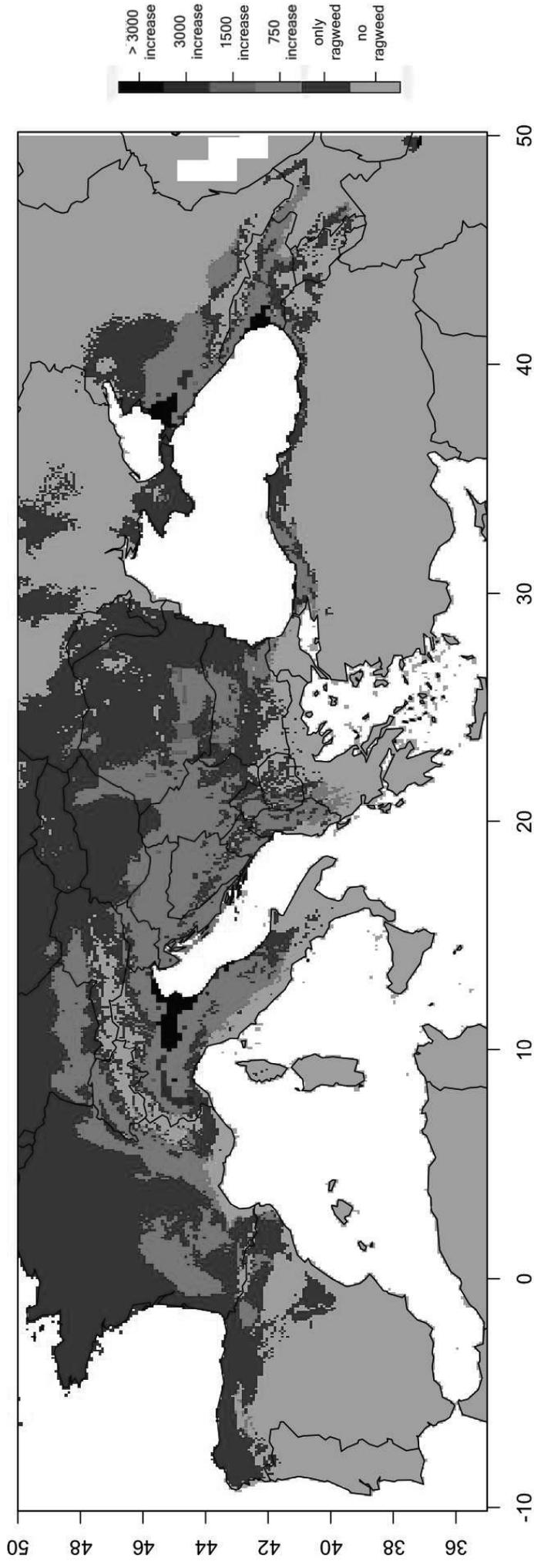


Fig. S5. Populations density of *Ophraella communa* (based on monthly relative humidity and monthly relative temperature, Model I, eqn. 4). Grey color indicates the area not suitable for *A. artemisiifolia* and *O. communa*; green color indicates the area suitable for *Ambrosia artemisiifolia* but not for *O. communa*; reddish colors indicate the area suitable for both *A. artemisiifolia* and *O. communa*: the darker the reddish color, the higher the density of *O. communa*.

Fig. S6.

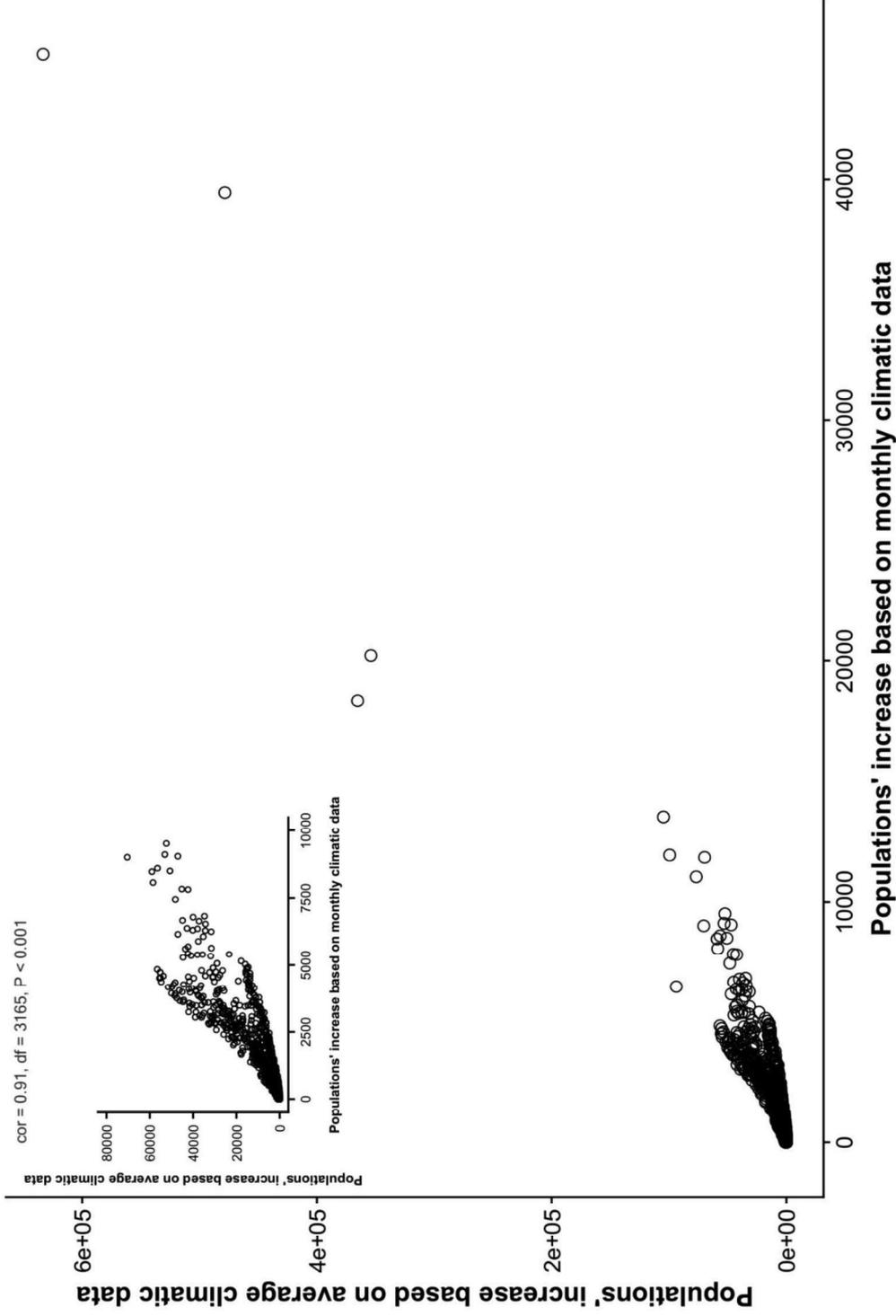


Fig. S6. Correlation between population density *Ophraella communa* calculated based on monthly climatic data (temperature and relative humidity) from Model I (eqn. 4) and based on average climate data from Model II (eqn. 5) for the suitable area of both *Ambrosia artemisiifolia* and *O. communa* in Europe.

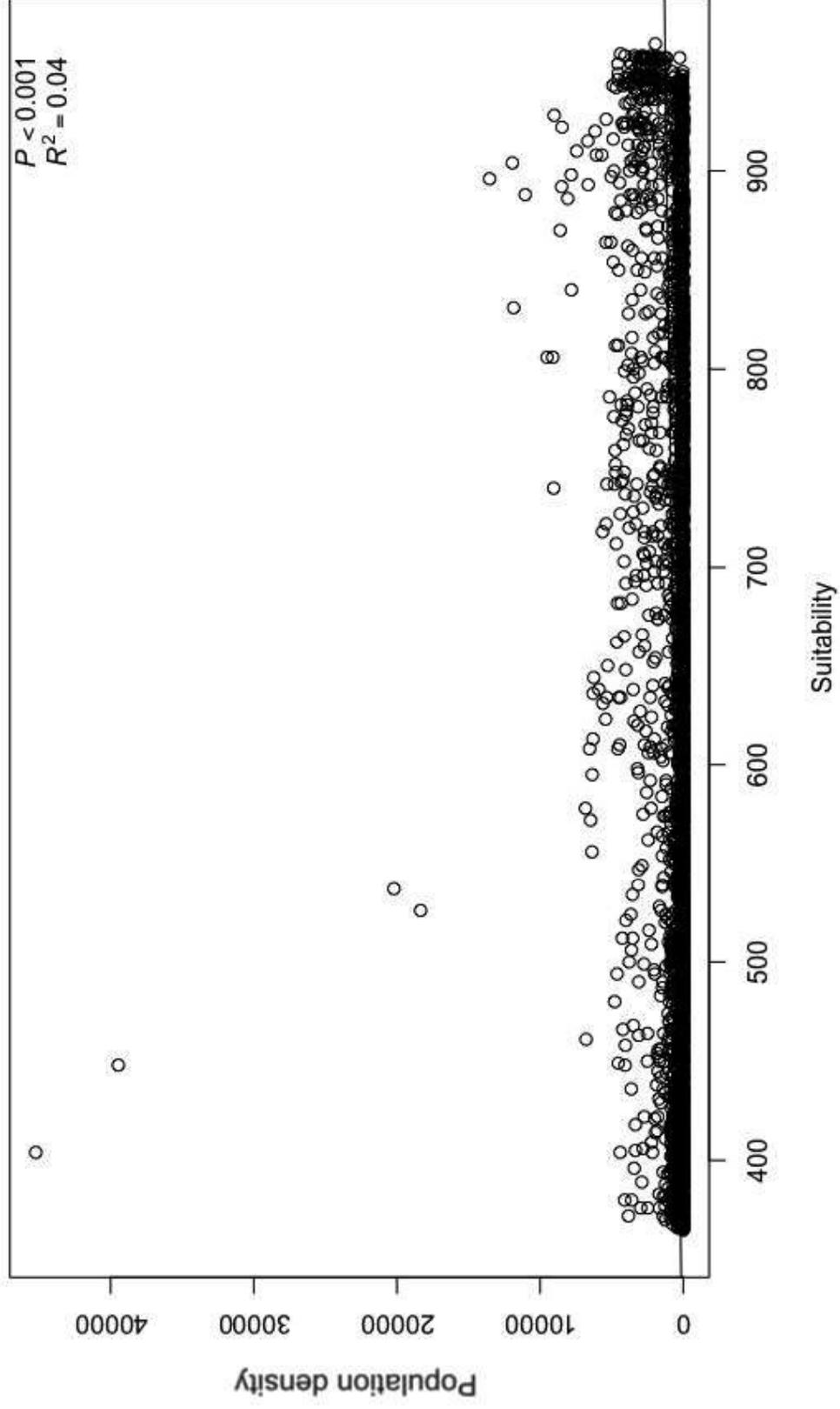


Fig. S7. Correlation between population density of *Ophraella communa* calculated based on monthly climatic data (temperature and relative humidity) from Model I (eqn. 4) and suitability values derived from SDM (Sun et al. 2017) for the suitable area of both *Ambrosia artemisiifolia* and *O. communa* in Europe.

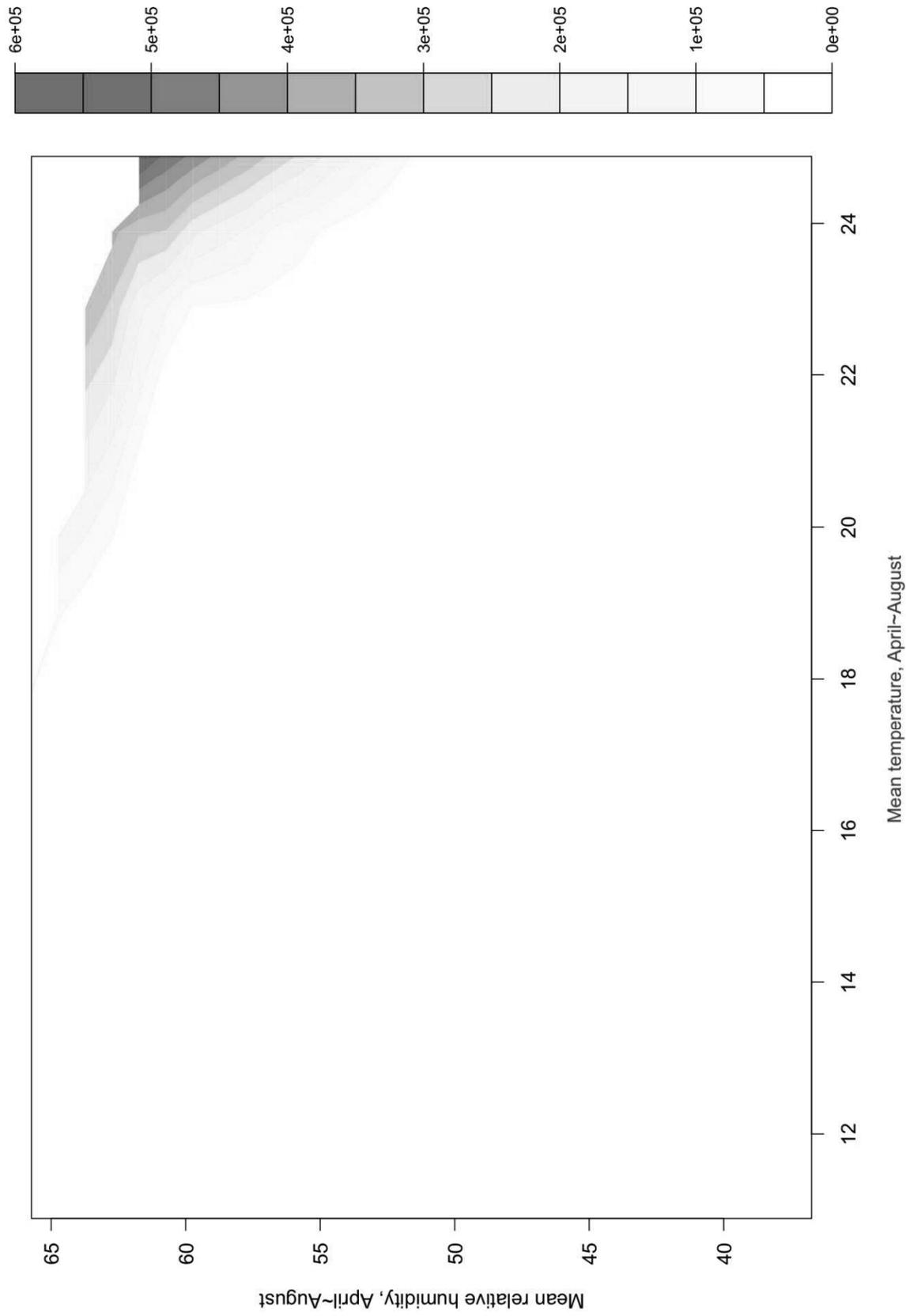


Fig. S8. Heatmap of population density of *Ophraella communa* (calculated based on average climatic data) from Model II (eqn. 5). Colors indicate different levels the population density.

Annex 3: Results of statistical analyses of the laboratory experiments

Outputs of the generalized linear models conducted to assess effect of low relative humidity on egg hatching.

Effect of RH treatment on egg hatching rate:

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: binomial ( logit )
## Formula:
## cbind(Total_hatched, eggs_counted) ~ treatment + (1 | laying_date) +
## (1 | Incubator)
## Data: RH_hatching_all
##
##      AIC      BIC  logLik deviance df.resid
##  839.2    850.5  -415.6   831.2     119
##
## Scaled residuals:
##      Min      1Q  Median      3Q      Max
## -3.1246 -1.6284 -0.0869  0.7128  5.3425
##
## Random effects:
## Groups      Name      Variance Std.Dev.
## laying_date (Intercept) 0.09289  0.3048
## Incubator    (Intercept) 0.01343  0.1159
## Number of obs: 123, groups: laying_date, 23; Incubator, 2
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.697835   0.137211  -12.37  <2e-16 ***
## treatment    0.053169   0.006076   8.75  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr)
## treatment -0.492
```

Effect of RH treatment on time until hatching:

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: poisson ( log )
```

```

## Formula:
## (First_day_hatching) ~ treatment + (1 | Incubator) + (1 | laying_date)
##   Data: RH_hatching_all
##
##      AIC      BIC   logLik deviance df.resid
##   359.9    369.8   -176.0    351.9      83
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.32987 -0.17184  0.05599  0.21842  1.26263
##
## Random effects:
##  Groups      Name          Variance Std.Dev.
##  laying_date (Intercept) 0          0
##  Incubator   (Intercept) 0          0
## Number of obs: 87, groups:  laying_date, 19; Incubator, 2
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  2.059647   0.076428  26.949  <2e-16 ***
## treatment   -0.002870   0.004641  -0.619   0.536
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr)
## treatment -0.859

```

Effect of RH treatment on hatching interval:

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: poisson ( log )
## Formula:
## (hatching_interval) ~ treatment + (1 | Incubator) + (1 | laying_date)
##   Data: RH_hatching_all
##
##      AIC      BIC   logLik deviance df.resid
##   300.4    310.3   -146.2    292.4      84
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.5694 -0.5428 -0.2765  0.4623  3.7584
##

```

```

## Random effects:
## Groups      Name          Variance Std.Dev.
## laying_date (Intercept) 8.016e-02 2.831e-01
## Incubator   (Intercept) 6.929e-10 2.632e-05
## Number of obs: 88, groups: laying_date, 19; Incubator, 2
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  0.62894    0.17927   3.508 0.000451 ***
## treatment   -0.01348    0.01127  -1.197 0.231500
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr)
## treatment -0.760

```

Variation in batch size among RH treatments (to control for a bias among treatments):

```

## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: poisson ( log )
## Formula: (eggs_counted) ~ treatment + (1 | Incubator) + (1 | laying_date)
## Data: RH_hatching_all
##
##      AIC      BIC  logLik deviance df.resid
##  872.1    883.3  -432.0   864.1     119
##
## Scaled residuals:
##      Min      1Q  Median      3Q      Max
## -2.8290 -1.0167 -0.1309  0.8030  4.9139
##
## Random effects:
## Groups      Name          Variance Std.Dev.
## laying_date (Intercept) 0.05908  0.2431
## Incubator   (Intercept) 0.00000  0.0000
## Number of obs: 123, groups: laying_date, 23; Incubator, 2
##
## Fixed effects:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.001e+00  6.389e-02  46.969  <2e-16 ***
## treatment   -7.928e-05  2.952e-03  -0.027   0.979
## ---

```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##           (Intr)
## treatment -0.435
```

GENERAL DISCUSSION

For deliberate releases of a biological control agent of weeds, the study framework generally consists of six stages: target weed ecology, exploration for biological control potential, host-specificity testing, agent release and redistribution, and agent evaluation (Briese 2000, Van Klinken and Raghu 2006, Müller-Schärer and Schaffner 2008). In the case of an accidental introduction, several steps of the framework are omitted, and after the potential biological control agent has been detected, the only available steps of action are host-specificity testing and agent evaluation; which can inform practitioners whether it is recommendable to put effort in acting to either reduce or encourage further spread over the insect's suitable geographic range. Next to a much more compact framework, the large difference between these approaches is that the local impact on the target can be estimated much more precisely and at the same time as non-host target effect in the case of an accidentally introduced potential biological control agent (Louda et al. 2003, Shaw et al. 2016). In conventional programs for classical biological control of weeds, local impact can only be estimated reliably after introduction of the potential biological control agent, which is one step after host-range evaluation.

Here, I report on studies that investigate both host specificity of *O. communa* and its potential impact on *A. artemisiifolia* across its suitable range in Europe. These findings suggest that *O. communa* does not pose a substantial threat to European native plant species. However, *O. communa* can spread over large parts of Europe, and its impact on *A. artemisiifolia* is expected to be extensive in parts of this range.

In the **first chapter**, we investigated feeding by *O. communa* on European native and endangered plant species closely related to *A. artemisiifolia*. We conducted a 2-year field survey, a common-garden and laboratory experiments. We did not find any signs of *O. communa* feeding on any of the 18 populations of the nine non-target plant species, except low collateral damage on a single plant individual. In the common garden experiment, we found considerable *O. communa* feeding damage on three plant species: *Pentanema helveticum* (a species vulnerable to extinction), *Centaurea nigrescens* (a species of lowest concern) and *Dittrichia graveolens* (a neophyte in Western Europe). On *P. helveticum* and *C. nigrescens*, we only observed *O. communa* adults feeding, which we categorize as collateral damage, while we found eggs, larvae and adults on *D. graveolens*. In laboratory experiments, we confirmed *O. communa* oviposits on *D. graveolens*, and that the beetle can complete its life cycle on this (Western European) neophyte. Our results show that it is unlikely that *O.*

communa will cause significant non-target effects on native European plant species in the closely related tribes Inuleae and Coreopsideae. However, to consider field releases of this beetle in other climatically suitable ranges in Europe with high ragweed densities, much more extensive host range testing and field monitoring is necessary.

In the **second chapter**, we investigated how *A. artemisiifolia* reproductive output at the end of the season is affected by *O. communa* abundance or feeding damage earlier in the season. We followed individually labelled *A. artemisiifolia* plants in four locations in Northern Italy during the summer of 2016 and estimated leaf damage and beetle abundance in 3-weekly intervals. Reproductive output of the labelled plants was estimated at the end of the season. While leaf damage together with volume 6 weeks before flowering explained the chance of raceme formation (male reproduction) at the end of the season, difference in site explained far more variation within the plants. However, likelihood of seed formation could be explained with leaf damage and plant volume much earlier (up to 10 weeks) before seed set. Models including leaf damage always explained more variation than models including abundance parameters, suggesting that leaf damage is a better estimator for impact than beetle abundance. Our results show that leaf damage in combination with plant volume could be used to create models to predict chance of seed formation, which could be used for management decisions for the control of *A. artemisiifolia* in regions where *O. communa* is present.

In the **third chapter**, we aimed to predict the potential impact of *O. communa* on *A. artemisiifolia* in Europe. We combined a statistics-based species distribution model with climate-dependent vital rates which we had deduced empirically in field and laboratory experiments. We used temperature to calculate potential developmental time and relative humidity to calculate egg hatching success, since egg hatching success strongly decreases from > 80% to < 20% when RH drops from 55% to 45% during the day. The developed combined model revealed that the current range of *O. communa* in Northern Italy is among the geographic ranges in Europe with the highest potential for population build-up. Other areas with equally high estimates are western Georgia and the Russian Krasnodar region, where *A. artemisiifolia* is also highly invasive. Furthermore, comparing the relative importance of temperature and relative humidity for potential population build-up showed that the importance of these climatic factors greatly differs spatially across the range that is climatically suitable for *O. communa* and *A. artemisiifolia*.

Below, I discuss the implications of these studies for the use of demographic modelling insect herbivores and the assessment of risks and benefits for accidentally introduced biological control agents.

Implications for the use of demographic modelling for weed biological control agents

Schwarzländer et al. (2018) found that from all biological agents of weeds that established, 46,5% of the damage on the target weed was considered less than 'medium'. Being able to predict impact early in the process of selecting a suitable biological control agent could aid in prioritizing candidates that potentially have a large impact on the target weed, which could increase the amount of highly impactful agents. While successful weed biological control agents in the introduced range often stand out because of numbers several orders higher than observed in their native ranges (Kalischuk et al. 2004, Myers and Sarfraz 2017, McEvoy 2018), the timing of the phenology of a weed biological control agent can be of paramount importance for successful control of a target weed as well. For example, *Clematis vitalba* in New Zealand was poorly controlled by two biological control agents since they only induced disease symptoms late in the season (Paynter et al. 2006). Demographic models could be used to describe both phenology and abundance of potential biological control agents of weeds.

Furthermore, ecological suitability for a potential introduction is generally assessed using species distribution models (SDM). SDMs use absence/presence data, analyse the ecological niche and project this niche over a novel range. SDMs are useful tools to evaluate ecological suitability, and therewith chance of establishment in novel ranges, but they do not always predict potential population density in the novel range (Thuiller et al. 2014, Csergő et al. 2017). Demographic models could be used to estimate in which locations potential population density, and therefore biological control agent impact, are expected to be highest. Ideally, a site for introduction of a suitable biological control agent both has a high chance of establishment, and a high chance of large impact of the agent. Combining of mechanistic and statistic models (like SDM) has been suggested to make reliable and robust predictions (Keith et al. 2008, Gallien et al. 2010). In weed biological control, demographic models to predict suitable range with maximal impact and to predict or amend biological control success on a short-term or local scale have been underutilized tool until now (McEvoy 2018). In the following paragraphs I discuss how the findings of this thesis could aid in the development of

demographic models for biological control agents of weeds. I then discuss the applicability of these findings for classical biological control programmes and accidental introductions of potential biological control agents.

In the laboratory experiment of the **third chapter**, we found that relative humidity during the warmest part of the day has a strong negative effect on egg hatching success. It is noteworthy that the range of relative humidity resulting in a >60% decrease in egg hatching success was as narrow as 10%. Pupae of *O. communa* were not or barely affected by the same low relative humidity. Hence, when making predictions, the different responses of different life stages should be taken into consideration. One way to do this is formulating demographic models with a stage structure, considering temperature and humidity effects on every life stage separately.

Additionally, the **third chapter** adds to the body of evidence that humidity has a large effect on insect survival (Schaber et al. 1975, Bethke and Redak 1996, Simelane 2007, Lu and Wu 2011). Therefore, I suggest to include humidity in demographic models developed to predict insect abundance. In addition to the challenges of collecting high-quality humidity data, working with humidity and temperature at the same time can pose technical challenges. For example, relative humidity, a commonly used unit, is inherently correlated to the present temperature since warmer air can hold a higher amount of water:

$$\text{Relative humidity} = \frac{\text{water vapor present in air}}{\text{water vapor the air can hold at the given temperature}} \quad (1)$$

Biological measurements of temperature-based mortality are traditionally taken with constant relative humidity, while biological measurements of humidity-based developmental mortality are traditionally taken with constant temperature (Chaudry and Alikhan 1990, Bethke and Redak 1996, Zhou et al. 2010, Norhisham et al. 2013). The intercorrelation between relative humidity and temperature makes using both simultaneously as drivers for mortality problematic. To prevent this, one could consider mortality from relative humidity and ignore mortality driven by temperature. Another way would be to use absolute humidity (the measure of water vapor in the air regardless of temperature) to predict humidity-dependent mortality.

The **last two chapters** of this thesis clearly highlight the need for thorough ecological understanding when models are interpreted to make predictions in a useful way. For example, there is nearly no information on overwinter mortality of *O. communa* in the field. While Watanabe and Hirai (2004) explored the overwinter host plant use of *O. communa* in Japan,

and Zhou et al (2013) investigated evolvability of cold-hardiness, an accurate estimate of overwinter survival and its dependency on abiotic or biotic factors has not been published so far, to the author's knowledge. This clearly impacts the ability to make long-term predictions on population densities of the beetles with models, since population density at the beginning of the season is based on assumptions. Unpublished field experiments showed that field mortality might not be directly correlated with temperature, but a sound experimental setup to estimate overwinter mortality of this extremely mobile insect can be deemed challenging at least, since caged experiments might constrain adaptive behavior, while open-field experiments on overwinter mortality could require restrictively high numbers of beetles. Furthermore, overwinter experiments need to consider food availability in autumn, which can be highly variable between years.

Another example of ecological understanding is to consider the mobility and host-finding ability of *O. communa*. The relatively high mobility of the beetle is without doubt advantageous for its use as a biological control agent, since beetles can find isolated plants or patches of plants, and move on once a patch of host plants is completely depleted (Yamanaka et al. 2007). However, it creates a challenge for modelling impact, as the **second chapter** illustrated. While biocontrol agent impact is often assessed by releasing a certain amount of adults per plant (Poltavsky and Artokhin 2006, Guo et al. 2011, Shabbir et al. 2016), it might be more suitable to follow leaf damage instead of insect abundance on plants to make predictions in real time, or combining both factors for predictive models. Especially when the insect is mobile, a model for damage instead of abundance might be able to circumvent the problem of abundance estimate error because of the insect's mobility, since the damage is an 'integral' of the total attack. Additionally, a model for leaf damage might be used instead of a growth model for the plant and one for the insect populations, if it contained both leaf growth and insect-caused defoliation.

One issue for making real-time predictions that is exacerbated by the highly mobile behavior of the beetle is the difficulty to estimate local abundance. In pest predictive modelling, where herbivore impact is predicted to inform timely management decisions as well, often biofixes (certain biological events like first trapped individual or first eggs observed) are employed. These biofixes start the model run, and are used to predict when densities might increase to a point that damage is above an economic threshold level, which motivates timing of management intervention (Magarey et al. 2015, Magarey and Isard 2017). These biofixes can be different for different locations, and threshold levels can vary in different contexts. This

means that there is a need to define these biofixes (connected to the desired threshold levels) for different locations, which would be rather laborious.

A modelling approach can also be used to inform potential spread and impact within the novel range, since there is already an estimate for impact of *O. communa* on aerial pollen levels in a suitable region (Bonini et al. 2015a, Bonini et al. 2015b). Using demographic models, we can predict whether populations increase would be bigger or smaller in the novel range compared to its current range, which most likely affects the potential impact of its host range. This approach is illustrated in the **third chapter** of this thesis, but also in Mouttet et al. (2018) and Schaffner et al. (2020, in press). Assuming that equal population densities of *O. communa* will have equal impact on *A. artemisiifolia* in other places in Europe, we used spatial predictions of potential population build-up to predict how high pollen reduction is to be expected in the places where *O. communa* and *A. artemisiifolia* populations overlap.

Demographic modelling can be a useful tool for predicting the impact of a biological control agent. How the models can be parametrized and developed differs between classical biological control programs and accidental introductions of potential biological control agents. One challenge for the accuracy of pre-introduction impact predictions is that the influence of the novel biotic and abiotic environment is only known after introduction of the insect. The experiment that was conducted in Northern Italy, with caged populations of *O. communa* (see **chapter 3**) cannot be conducted in the case of classical biological control programs. In the native range, the populations of the insects are likely to be also controlled by biotic factors (Müller-Schärer and Schaffner 2008). Conducting caged experiments in a novel environment pre-release is not realistic, since the chance of escape of the insect and accidental introduction would be too high. For accidentally introduced potential biological control agents, I advise to use (semi)-field experiments to quantify impact, since this represents the most realistic environment.

If demographic modelling is used for classical biological control experiments, the parametrization will have to depend on field experiments in the native range or on laboratory experiments. Since especially mortality is not only regulated by the abiotic environment in the native range, using data collected in laboratory experiments is the most feasible. However, working with laboratory experiment-derived data has its own suite of challenges. For example, temperatures in laboratory experiments are seldomly as diverse as what the insects are experiencing in the real world. Insects show different responses to variable temperatures in

terms of adaptive physiology, behavior and life history (Colinet et al. 2015), which should be kept in mind when designing laboratory experiments.

Accidental introductions allow researchers to work with more realistic parameters to drive models, but classical biological control programs could still use models to compare potential population density in the introduced range to identify preferable locations for field introductions. Combining species distribution models with demographic models, as proposed by (McEvoy 2018) and illustrated in the **third chapter** of this thesis, could be used for choosing sites for biological control agent release to optimize for chance of establishment and chance of high impact.

Risk and benefits of accidental introductions

In pre-release testing of biological control agents, a point of discussion has been to what extent host-specificity tests assess potential risks towards non-target organisms (McEvoy 1996, Schaffner 2001), since pre-release testing is suitable to test the fundamental host range, but could fail in estimating the ecological host range in the novel environment. Compared to regular biological control programs, host range surveys after accidental introduction can evaluate the ecological host range to a more realistic extent than laboratory and field studies in the native range of the biological control agent. Following this, it is necessary to consider ecological and behavioral traits of the potential biological control agent. For *O. communa*, no precise information on potential spread of adult individuals is known. However, I observed dozens of adult beetles sitting on the outside of the experimental cages in the site with the highest elevation (1250m a.s.l.), while the insects in the cages clearly did not develop a full generation. We expect these beetles to have travelled there by themselves, which indicates that *O. communa* adults can travel at least 5km, covering an altitude difference of ~1000m to find new *A. artemisiifolia* populations. That knowledge on potential (local) spread and host-finding ability was included in the selection of sites for the 2016-2017 field survey in the **first chapter**. I suggest to always consider knowledge of mobility when selecting sites for post-release non-host target attack studies.

As with estimations of life-cycle parameters, accidental introductions give options to conduct more realistic assessments of the chance of non-host target attacks compared to conventional classical biological control programs, since the potential agent is already in the novel range. Field surveys and field experiments in the novel range, as we describe in the **first chapter** of the thesis, are not feasible in the case of classical biological control programs.

In the field surveys, we observed adult beetles feeding on *Centaurea nigrescens* Willd., from the tribe *Cardueae*, which led to including this plant in follow-up field and laboratory experiments. Since *C. nigrescens* is phylogenetically quite far away from the tribe of *A. artemisiifolia* (*Heliantheae*), it would normally not have been included in host range testing. This illustrates how local observations of an accidentally introduced biological control agent can include interactions that would not have been considered in classical biological control programs.

Plant species that were attacked in the field experiment were not attacked in field samplings on natural populations, while nearby *A. artemisiifolia* populations were attacked at high rates. For example, no *O. communis* was found in 24 visits of the four *Pentaneura conyzae* Griess. populations surveyed over two years in the field survey. At the same time, we observed *O. communis* adults damaging *P. conyzae* plants in the field experiment conducted. This illustrates that field experiments should be complemented with field surveys in the case of accidentally introduced biological control agents to assess the chance of non-host target attack more accurately. Another opportunity that accidental introductions of biological control agents offer, especially in the case of *O. communis* or other species that would have not been approved for release, is validating the estimates of the realized ecological host range.

In addition to field surveys and experiments, it might be advisable to conduct non-choice experiments. These experiments can aid categorizing severity of attack (collateral, spill-over or sustained attack). Growing the potential biological control agent on a non-target host plant for several generations could help explore evolvability of the agent's host range. For example, Müller-Schärer et al. (2020) describe an experiment, where *O. communis* individuals from different European populations were grown in replicated experimental cages containing either *A. artemisiifolia* or sunflower (*Helianthus annuus* L., a potential alternative host species) plants. Preliminary analyses of population genomics and phenotyping did not show rapid adaptation of the beetle to different host plants within ~10 generations (Müller-Schärer et al. 2020).

When accidental introductions of potential biological control agents are observed, I suggest to investigate potential impact, positive or negative, as quickly as possible. The impact on the target (or non-target) organism can be predicted more conveniently from an already introduced organism than from one that has not been introduced in the new range yet. Unexpected biological interactions (e.g. parasitism of insects by native generalists, competition) can make predictions for the success and impact of a first-time introduced

biological control agents difficult (Louda et al. 2003). Once an accidentally introduced potential biological control agent has established, it will be very difficult to eradicate. Therefore, the only management option is mitigation of spread. However, the positive impacts can and should be assessed as well. Investing in slowing spread while the potential biological control agent has a positive overall impact is not plausible in the long term. If negative effects outweigh the positive effects, an early reaction might aid in reducing spread of the species. If positive effects outweigh the negative effects, no reaction or assisted dispersal of the prospective biocontrol agent are advisable.

The data obtained for **chapter 3** have been used in two other studies, that estimate the potential economic impact of *O. communa* in the Rhône-Alpes region in France (Mouttet et al. 2018) and in the whole of Europe (Schaffner et al., in press). After no clear risk of *O. communa* feeding on native non-host target feeding has been found, the above-mentioned studies clearly indicate that the potential positive impact of this beetle on human welfare and the decrease in medical costs should motivate European governments to invest in further host-range testing by establishing a European-wide accepted test plant list. I suggest that the potential negative effect of an accidentally introduced biological control agent and the potential positive effects should be weighed carefully before deciding on how to react to this introduction. This was the case for *O. communa* in France: after the French agency for food, environmental and occupational health and safety (ANSES) had issued a request for an expert appraisal for phytosanitary risks of *O. communa* in September 2014, a second request to study its efficacy as a biological control agent of *A. artemisiifolia* was issued in March 2015. The results of this study led to the decision of the French authorities to not interfere should *O. communa* colonize in France. It should be noted, that this recommendable approach is rather unusual in Europe, where biological control of weeds is hardly considered regardless of its ecological and economical potential, although a few projects have started in the recent two decades (Shaw et al. 2016). Furthermore, the countries of the European Union do not have a unified legislative system in place, both for accidental and deliberate introduction of weed biological control agents {Shaw, 2016 #77}. Europe does not only lag behind South Africa and Oceania in clear legislation and adoption rate of biological control of weeds, but assessments of economic benefits of biological control (of weeds and arthropods) have also hardly been conducted in Europe (van Wilgen et al. 2020). We recommend to include estimates of the economic benefits of biological control in future biological control programmes as well, since

they offer sound advice for management decision, as the case described for ANSES and *O. communa* illustrates.

Conclusion

As a consequence of globalization, an increase of the number of introductions of alien species is to be expected. Accordingly, the rate of accidental introductions of potential biological control agents of weeds will also likely increase. While classical biological control of weeds is a well-established field, one should consider the differences when working with accidentally introduced potential biological control agents. Foremost, contrary to classical biological control programs, it is possible to evaluate both non-host target feeding and biological control efficacy at the same time. This improves the accuracy of cost-benefit analyses, which should be part of any evaluation of accidentally introduced potential biological control agents, also because it is very unlikely that establishment of the agent is reversible. While I advocate for weighing positive and negative impacts for each biocontrol program, this approach is particularly important for accidental introductions, since the realistic choice of action is either containment or spread.

The studies described in this thesis demonstrate how field surveys improve the estimated accuracy of likelihood of non-target feeding by refining data from laboratory and field experiments. Furthermore, accidental introductions of potential biological control agents give the chance to measure local impact of the agent on the target. Using demographic models, this provides the opportunity to scale up its impact over the potential geographic range, which is useful information for policy makers.

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APPENDIX 1

Predicting insect herbivore population increase in a biological control field experiment with a demographic model driven by surface temperatures

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Abstract

Herbivorous insects may be seen as agricultural damaging pests or as biological control agents. Predicting the presence and timing of herbivore insect population development can be of paramount importance for agricultural planning and management. Numerical simulation insect population models driven by temperature are often used to predict pest population densities in agriculture. Such simulation models intend to use station-derived temperatures to drive the development of the insect, while this temperature may differ substantially from the experienced by the insect on the plant. Therefore, the use of surface temperature is suggested to replace air temperature data in the model. Here, we use a numerical simulation model of insect pest population dynamics driven by air temperature and land surface temperature derived from satellites to predict the population dynamics of the leaf beetle *Ophraella communa*, a potential biological control agent of *A. artemisiifolia* in Europe. An extensive field experiment that included the establishment of *Ophraella communa* growing cages in five sites along an altitudinal gradient (120-1250 m.a.s.l.) in Northern Italy was conducted during two years (2015 and 2016). We compare our model predictions, with air and surface temperature, to observed beetle populations at different life stages. Model predictions – with both air and surface temperatures – overestimated the observed populations by several orders of magnitude. Yet, they predicted a similar phenology to observed populations. We conclude that the model needs to include mortality factors other than those driven by temperature to make accurate abundance predictions. However, the model can be used to predict the timing and distribution of insect populations under a range of temperature conditions.

Introduction

Insect herbivores are considered to be fundamental for ecosystem function and structure, because of their impact on host performance (Crawley 1983). This impact is particularly significant in the case of agricultural pests or when used as biological control agents of weeds. In agriculture, insect herbivore can cause vast crop losses, and insects like the Colorado potato beetle (*Leptinotarsa decemlineata*), cotton bollworm (*Helicoverpa armigera*) or fall armyworm (*Spodoptera frugiperda*) attract global awareness as crop damaging pests (Liu et al. 2012, Kriticos et al. 2015, Kumar et al. 2018).

As biological control of weeds – i.e. releasing specialist natural enemy to reduce the densities of an invasive alien plant species (Müller-Schärer and Schaffner 2008) – the impact of insect herbivores on plant host is seen as desirable. Insects such as the saltcedar leaf beetle (*Diorhabda elongata*), the mottled water hyacinth weevil (*Neochetina eichhorniae*) or the cochineal *Dactylopius opuntiae* are commonly-used to reduce invasive weeds (Hosking et al. 1994, Ajuonu et al. 2003, Hultine et al. 2010).

The impact on plant performance usually increases with the density of the herbivore insect (Myers and Sarfraz 2017). Therefore, being able to predict insect herbivore population dynamics can be critical to making sound management decisions. In agricultural systems, pest predictive models are used to adjust timing for management decisions such as the timing of application of insecticides (Magarey et al. 2015, Johnson et al. 2016). Models predicting demography of biological control agents can be used to predict biocontrol impact, and inform whether additional management is needed to control a target weed (Augustinus and Lommen et al., in press, Mvandaba et al. 2019).

Demographic models of insects are usually driven by temperature measurements for local predictions, or interpolated datasets of air temperature to make predictions for larger areas. A challenge with air temperatures, however, is to obtain reliable, high-quality spatially continuous data. Air temperatures are generally provided by local weather stations, which can be spread widely, making interpolated estimations inaccurate. An alternative to using interpolated air temperature estimates is the use of satellite information (Blum et al. 2013; Lensky et al. 2018; Lensky and Dayan 2011). Land surface temperatures (LST) derived from satellite measurements may be used to drive insect population models, providing spatially continuous assessments of population dynamics (Blum et al. 2015; Blum et al. 2018). There is a temperature difference between the LST and air temperature (at 2 meters height), where the LST is usually warmer during the day and cooler during the night, with factors such as

vegetation and atmospheric circulation playing important role in the temperature difference (Lensky et al. 2018). The difference is usually larger at day time, resulting in higher LST daily mean temperature than air. This difference needs to be taken into consideration when using LST instead of air temperature as an input in the model (e.g. Blum et al. 2015). In that case, LST may be more adequate than air temperature because it might represent best the temperature experienced by the insect (Suggitt et al. 2011).

Recent agricultural and ecological studies use thermal infrared or microwave measures to determine land surface temperatures to predict insect densities (Muharam et al. 2017), especially in areas where only few or no weather stations exist (Da Silva et al. 2015). A numerical simulation of olive fly (*Bactrocera oleae*) population dynamics driven by tree canopy temperature retrieved from the Moderate resolution imaging spectroradiometer (MODIS) LST showed a high degree of correlation with trapping data over an 11-year observation period (Blum et al. 2015). The same model was successfully used to predict the population dynamics of the polyphagous cotton bollworm (*Helicoverpa armigera*) (Blum et al. 2018).

Here we use the same model described in Blum et al. (2015) and Blum et al. (2018) to predict the population of the beetle *Ophraella communa* (chrysomelidae) LeSage, a potential biological control agent of the invasive common ragweed *Ambrosia artemisiifolia* L. (Asteracea) in Europe. We use both air temperature and MODIS-derived LST to drive the model. The obtained predictions are compared to data of an extensive field experiment conducted in various sites in Italy during 2015 and 2016. The sites differ in term of elevation and temperature conditions, which makes the application of the model more challenging and the evaluation of its results more accurate.

Material and methods

Study species

Ambrosia artemisiifolia L. (Asteracea) is a North-American annual weed that has invaded ranges on all continents except Antarctica. In Europe, it is considered a noxious weed since the 1920s (Csontos et al. 2010). Next to being a costly weed in spring-sown crops, *A. artemisiifolia* has attracted attention because it produces vast amounts of highly allergenic pollen, resulting in high medical costs and decrease in human welfare in the sensitized population (Mouttet et al. 2018, Müller-Schärer et al. 2018, Schaffner et al. in press).

Ophraella communa LeSage (Coleoptera: Chrysomelidae) was found to be a biological control agent of *A. artemisiifolia* after accidentally introducing it in China in 2001 (Meng and Li

2005). It is used as a biological control agent of *A. artemisiifolia* since then (Zhou et al. 2017). In Europe, the beetle was found for the first time in 2013 in Northern Italy and Southern Switzerland (Müller-Schärer et al. 2014) and has expanded its range over Northern Italy and into the Balkans since then (Augustinus et al. 2015, Lommen et al. 2017, Zadavec et al. 2019). *Ophraella communa* overwinter as adult beetles, and can conclude several generations per season, dependent on ambient temperature. In Europe, eggs are laid in batches as soon as the adults encounter host plants in spring. Egg deposition continues until mid-August (Augustinus and Lommen et al, in press), probably cued by a shorter photoperiod and decreasing temperature, as has been show for *O. communa* populations in China (Zhu et al. 2012). After emergence from the egg, all larval stages and the adults feed on the green parts of the host plant. In Europe, the majority of leaf damage is observed in late August, when *O. communa* abundance peaks (Augustinus and Lommen et al., in press).

Since the first observations of high population densities of *O. communa* in Northern Italy, aerial pollen levels have decreased by 80%, which is not explicable meteorologically or by land use change (Bonini et al. 2015a,b). While ecological suitability analyses combined with vital rates suggest that *O. communa* can reach population densities close to those reached in Northern Italy in other parts of Europe (Augustinus and Sun et al. 2019), no demographic models predicting population densities of this beetle have been published yet.

Field experiments

In the summers of 2015 and 2016, we conducted caged semi-field experiments to assess the temperature and RH-dependent developmental rate of *O. communa* on *A. artemisiifolia*. The experiments were conducted in five sites on sun-exposed grassland and private gardens in Northern-Italy, 20-50 km from Milan, Lombardy (Fig. 1, Appendix 1). In order to create a climatic gradient, we selected the sites along an altitudinal gradient from 120 to 1250 m.a.s.l. The experiments were conducted in 1 x 2 x 1 m (l x w x h) aluminium frames with a gauze cage with a zipper per replicate (Diatex SA, France).

Plant material

Ambrosia artemisiifolia plants were grown from seeds collected in Busto Arsizio (Lombardy); plants used in 2015 were grown from seeds collected in September 2014, plants used in 2016 were grown from seeds collected in September 2015. All plants were grown in the greenhouse of Parco Monte Barro- Centro Flora Autoctona in Galbiate (359m a.s.l.). Seedlings were transferred from seed trays to 9cm square pots and used for the experiments when they had 12-15 leaves.

Population build-up experiment

In the beginning of July of 2015 and 2016, we released 20 freshly hatched *O. communa* (10♂, 10♀) per cage. The beetles were individually marked with nail polish dots on the elytra, (colour coded for sex, and patterns for individuals). In every cage we transplanted 12 plants in the soil of the five experimental sites. Per site, we set up two cages (=replicates) per year. We returned to the sites in weekly intervals, and counted eggs which were not black, desiccated or hatched, full pupae and marked (initially released) and unmarked (offspring) adults.

Temperature data

We downloaded the 1 km land surface temperature (LST) product (MYD11A1) from NASA's MODerate-resolution Imaging Spectroradiometer (MODIS) via google earth engine (Gorelick et al. 2017). The LST product was downloaded for day and night (1:30AM/PM). When temperature data were missing due to cloud cover, we estimated the missing values by taking the average temperature of the past 18 years for the given Julian date (Lensky and Dayan 2011). LST was taken from pixels collocated with the experimental sites, except for three sites. In the experimental site of 'CM' (245 m), which included a nearby water body, we used an adjacent pixel instead to prevent overestimations of LST at night and underestimation at day. In the sites 'Fog' (550 m) and 'Ere' (770 m) a nearby adjacent pixel was used because both sites were located within the same pixel area. (Fig1.). Temperature measurement error was taken from the product "LST error flag" per day and site. Air temperatures for the different experimental sites were calculated from two weather stations, which were maximum 10 km of the experimental sites (Arconate SMR and Galbiate DW6022). We used a temperature measurement error of $\pm 0.4^{\circ}\text{C}$. We then downloaded the sounding measurements for the corresponding dates of 1608 LIML weather station in Linate to adjust for temperature differences along the altitudinal gradient per day.

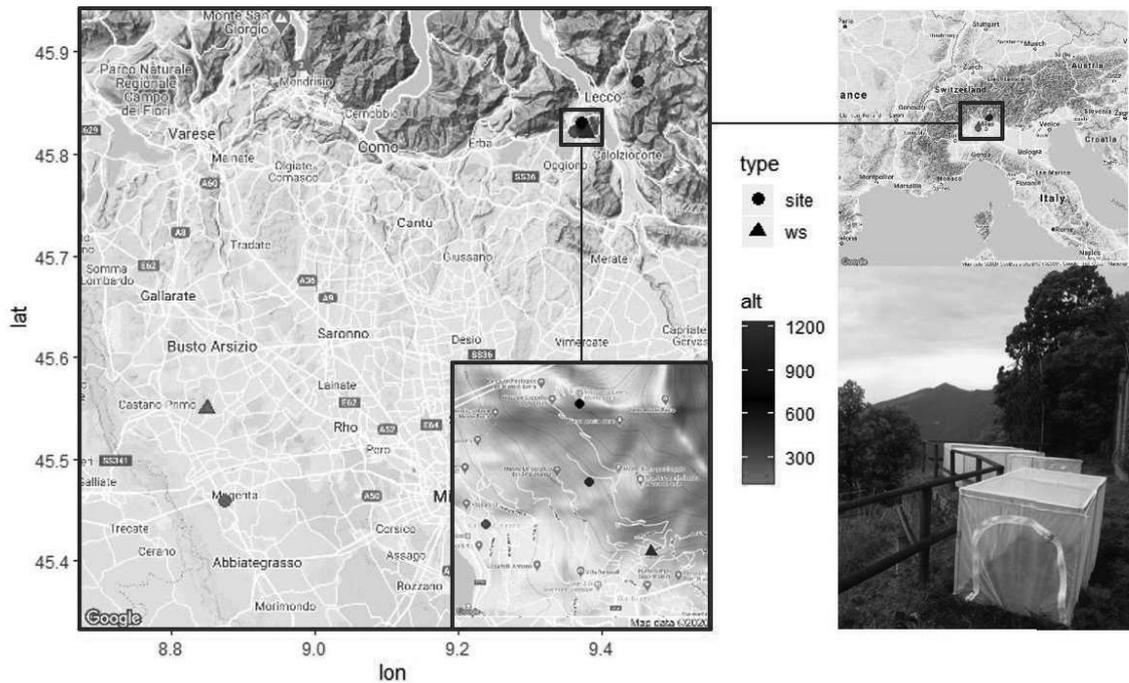


Figure 1: Locations of weather stations (ws, triangles) and experimental sites (sites, circles), with an example picture of one experimental site. The blue boxes show the sites on a smaller geographical scale.

Numerical simulation model of insect pest population dynamics

We used the stage-structured, continuous time-age population model driven by temperature of Blum et al. (2015) and Blum et al. (2015) to simulate the beetle *O. communa* population dynamics. The population model is based on a distribution function defined as a series of sequential differential equations in which temperature is the main driver (Appendix 1). The critical temperature, developmental time and number of age cohorts in the model were taken from Zhou et al. (2010). Mortality and egg production rates were calculated as a function of temperature following Zhou et al. (2010). Pre and post-oviposition adult mortality rates were assumed to be independent of temperature.

Analysis

To compare model fit with the two temperatures (LST and MET), we calculated mean square of the predicted errors (MSPE) per life stage, site and year :

$$MSPE = \Sigma(p - o^2) \quad (1)$$

Where 'p' is the number predicted by the model, and 'o' is the number observed in the experiment at the same date. We then divided this value by the average of the MSPE for the LST and the MET model to obtain the relative mean square predicted error (RMSPE).

Software

Weather data were prepared in R (R 2018), using the packages readxl (Wickham and Bryan 2016), reshape 2 (Wickham 2007) and plyr (Wickham and Wickham 2016) for data transformation and inference. Statistical analysis was conducted in lme4 (Bates et al. 2015), and graphical representation of data in ggplot2 (Wickham 2009), ggmap (Kahle and Wickham 2013), and gridExtra (Auguie et al. 2017). The insect population model was run in IDL (Interactive Data Language Harris Geospatial Solutions).

Results

Our models showed high population abundances, which were several orders of magnitude larger than observed. As expected, the model using LST as an input data predicted higher populations than the model using air temperatures (Fig. 2). The difference was more obvious in the high elevation sites in which a greater temperature difference is expected. Predicted populations increase was much higher than observations across life stages, sites, and years. However, there was one exception in which the predicted number of eggs was less than observed ('PdE' (1250 m) site in 2016) when we used air temperature as a driver for the model (Fig. 2).

Figure 2 shows a two-humped pattern for the egg population, which was also predicted by both models (i.e. with LST and with air temperature). The second peak in the egg population coincides with the emergence of adults from the first offspring generation. Though noted in the observed data, the two-humped pattern was much more pronounced in model predictions, with a second peak being much higher than the first one (see e.g. in the four lowest sites in Fig.2).

Populations were developed earlier in the LST-driven model, particularly for the pupae stage but also for other life stages (Fig. 2). As a result, the MSEF was almost always lower for the model driven by the air temperature (Table 1).

2015

2016

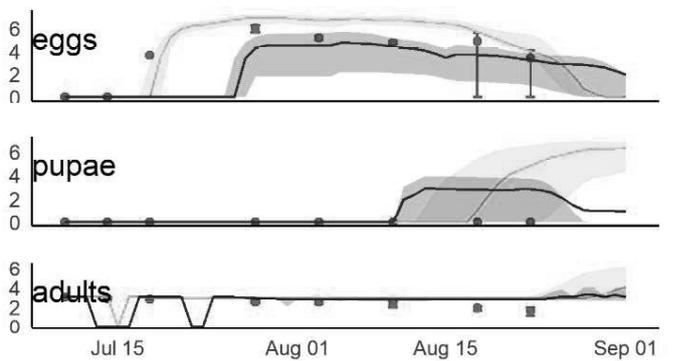
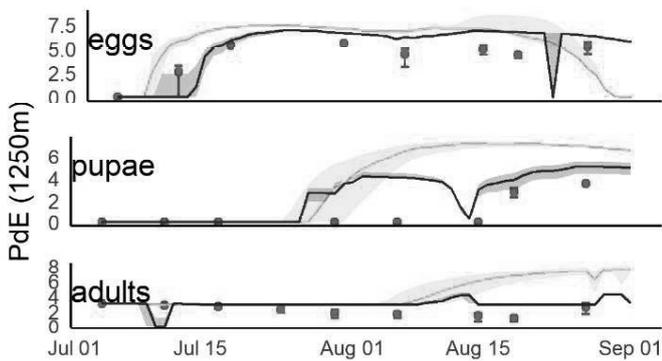
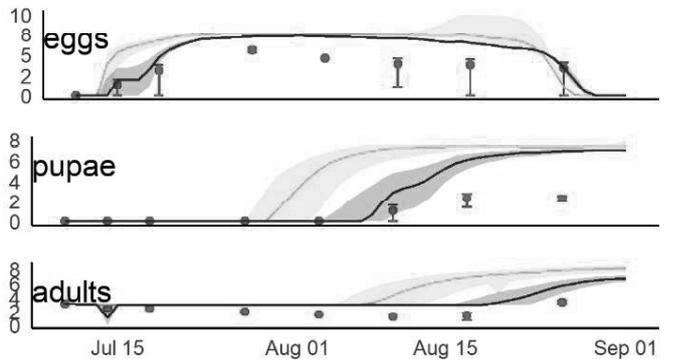
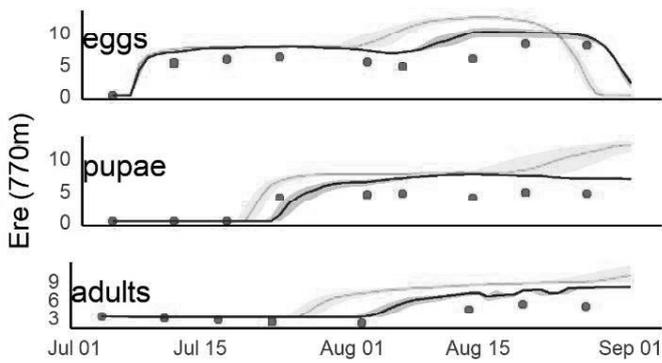
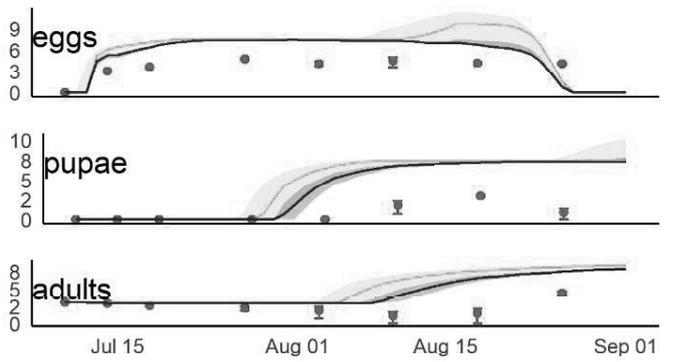
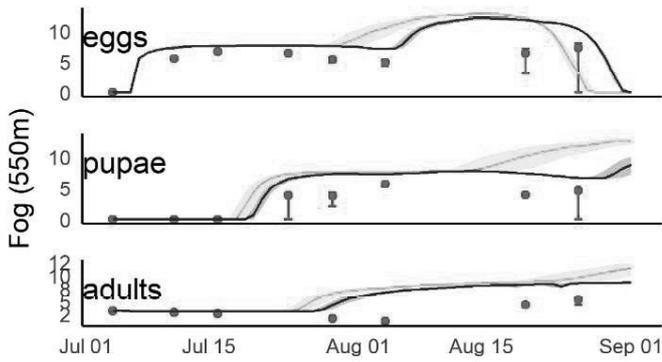
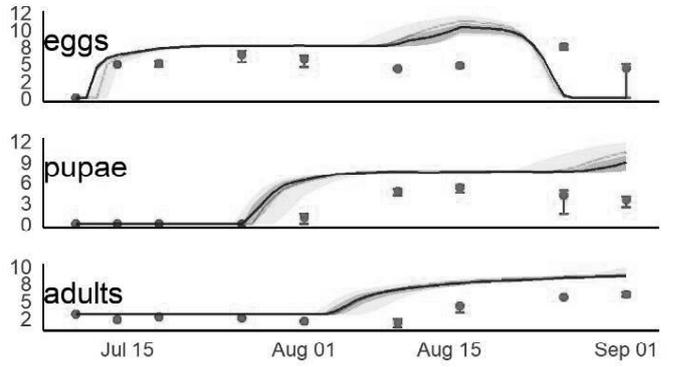
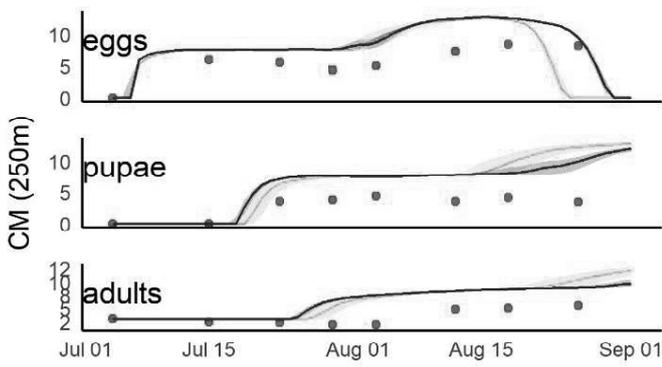
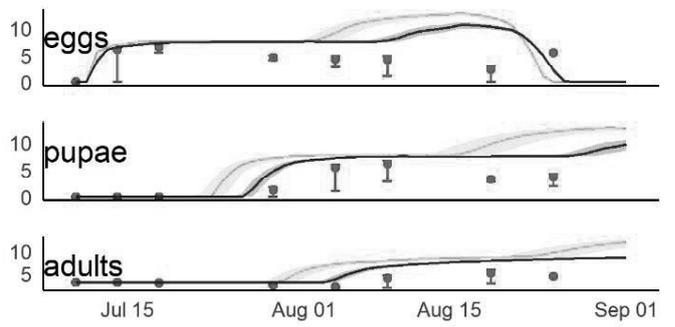
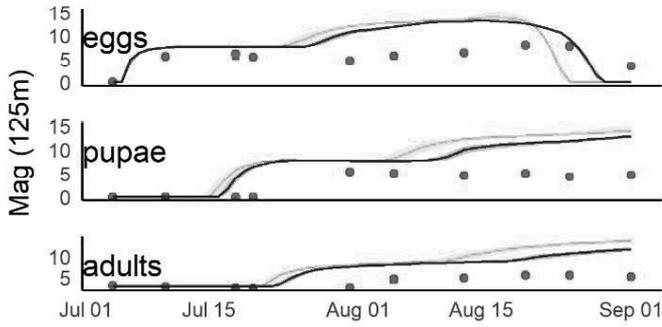


Figure 2: Predicted vs. Observed abundance number of *O. communa* in 2015 (left column) and 2016 (right column). Predictions of the model driven by LST (green) and air temperatures (in blue) with respective temperature error (ribbon). The red points show the validation data, the vertical lines show the variation within the sites of the validation data. All data are displayed on a natural log-transformed y-scale.

Table 1: The relative root mean squared error (r-RMSE) of model vs. observed populations for each site and year. A smaller r-RMSE means a better prediction of the model.

	2015					2016					
	Mag	CM	Fog	Ere	PdE	Mag	CM	Fog	Ere	PdE	
eggs	1.95	1.82	1.71	2.00	2.00	1.86	1.91	1.31	1.57	1.78	LST
	0.05	0.18	0.29	0.00	0.00	0.14	0.09	0.69	0.43	0.22	MET
pupae	1.98	2.00	2.00	2.00	2.00	1.98	1.98	1.14	1.72	1.85	LST
	0.02	0.00	0.00	0.00	0.00	0.02	0.02	0.86	0.28	0.15	MET
adults	1.98	2.00	1.98	2.00	2.00	1.41	1.56	1.34	1.98	1.15	LST
	0.02	0.00	0.02	0.00	0.00	0.59	0.44	0.66	0.02	0.85	MET

Discussion

The model overpredicted the beetle population increase both when driven with LST and air temperatures. However, the model was able to predict the phenology of the insect quite well. We discuss the results from technical, biological, and practical perspectives below.

Technical

Predictions of the model driven by LST resulted in a shorter developmental time and higher population densities compared to the model driven by air temperature. This is not surprising, since LST is overall higher than the air temperature, especially during the daytime (Trigo et al. 2008), and a single critical temperature threshold was used for both models. The higher LST may therefore result in a shorter developmental time of the beetles. The earlier the adults emerge and start laying eggs, the longer they can keep producing offspring before the photoperiod-induced stop of egg deposition sets in. For example, in ‘Ere’ site (770 m), a second

increase in the number of eggs is set earlier in the model driven by LST, which results in a second increase in the pupae and adult populations ('second offspring generation') in mid-August of 2015, which was not observed in the model driven by air temperatures.

Biological

While overprediction of population increase was observed in all sites, the model predictions at the coldest site, which is most likely at the edge of the climatic niche of *O. communa*, were closer to the validation data than in warmer sites. The data used to parametrize the model (Appendix 1) were obtained from studies conducted in constant temperature laboratory experiments (Zhou et al. 2010, see Appendix 2). Our observations, on the other hand, were collected from an open field experiment, under variable temperature conditions. This, might have affected the ability of the model to properly simulate beetle development and growth.

When comparing developmental rates of 29 species of terrestrial insects, Paaijmans et al. (2013) found that fluctuation around low mean temperatures increased fitness, while fluctuation around high mean temperatures decreased fitness compared to the use of parameters derived under constant temperature conditions. This implies that observations from a varying-temperature environment should be closer to model predictions in a colder environment. Furthermore, the data used were collected in incubator experiments without solar radiation, while insects in field environments can use their behavior in the field to increase body temperature (and therefore metabolisms) compared to the air temperature through basking (Eigenbrode et al. 2015).

The general overprediction of the models might be a result of ignoring density-dependent effects. Aggregation pheromones have been found for *O. communa* (Massimo Cristofaro, personal communication), and Cao et al. (2011) found in field observations that the number of eggs per plant increased with damage level. However, resource depletion will eventually have a negative impact on population growth, so higher densities of the beetle will have a negative effect on population growth. We chose to run the model for a shorter time than the whole experiment to avoid this effect, but it is likely that the population density has a negative effect on population growth before plants are completely defoliated. This results in higher number of eggs in the model predictions compared to the validation data.

Practical

Including density-dependent effects could help improve the model fit. However, estimating insect abundance for single species over a larger area in absolute numbers is a

very challenging endeavor. For example, in pest monitoring, most integrated pest management procedures use pest traps to estimate optimal timing, but normally they use an experience-based threshold, and not a trap-based estimation of absolute abundance of pest insects (Adams et al. 2017). These kinds of datasets are not available for *O. communa* populations, partially because studies of overwinter survival, and therefore estimates for population densities, have focused on physiological or evolutionary changes (Zhou et al. 2013, Tanaka and Murata 2016) or host plant utilization (Watanabe and Hirai 2004), but have not given any estimates for overwinter survival. Furthermore, studies on impact of *O. communa* on individual *A. artemisiifolia* plants and populations which included measurements of *O. communa* abundance did not show clear correlation between population density and level of damage to the plant in field experiments (Lommen et al. 2018, Augustinus and Lommen et al, in review). Without dependable methods to estimate *O. communa* density in the field, and without a clear measurement of per capita impact on *A. artemisiifolia* in a heterogeneous environment, we do not think including density-dependence would make the model any more useful.

However, the model can still be used to compare potential *O. communa* population build-up in suitable ranges in Europe to its current range. In Northern Italy, where the beetle is present, the aerial pollen concentrations have decreased by ~80% (Bonini et al. 2015a, Bonini et al. 2015b). Higher beetle population densities are expected to have higher impact on *A. artemisiifolia*. Therefore, numerical simulation models can be used to predict *O. communa* in Europe, with both LST and air temperature–driven models.

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Appendix 1

The insect population model of Blum et al. (2018) is based on a probability density function described by a series of differential equations:

$$\frac{dN_i(t)}{dt} = \frac{k\Delta a}{del} [N_{i-1}(t) - N_i(t)] - \mu_i(t)N_i(t) \quad (\text{eqn.1})$$

where each life stage is calculated by a differential equation in a sequential order. $\mu_i(t)$ in Eq. (1) is the attrition at time t , del is the average developmental time per stage, $N_i(t)$ is stage (i) population density, Δa is age difference, and k is the number of age cohorts. Time and age are measured in degree days (DD), which is calculated using the rectangular method (Arnold 1959). The equation is solved in the model numerically, using the Euler method. The population flux from an age cohort to the next $r(t)$ is described as:

$$r(t) = \frac{k * N(t)}{del} \quad (\text{eqn. 2})$$

Thus, Eq. (1) becomes:

$$\frac{dr(t)_i}{dt} = \frac{k}{del} \left\{ r(t)_{i-1} - \left[1 + \mu(t)_i \frac{del}{k} \right] r(t)_i \right\} \quad (\text{eqn. 3})$$

The model assumes a linear relationship between developmental time and temperature, where b is the slope and a is the intercept of such relationship. And these can define the critical temperature (T_c) and del (Damos and Savopoulou-Sultani, 2012):

$$T_c = -\frac{b}{a} \quad (\text{eqn.4})$$

$$del = \frac{1}{b}$$

(eqn. 5)

Finally, each calendar day is transformed to a physiological time scale (DD) to calculate the variance (*var*) and *k*, as a statistical normal distribution of individuals per age cohort (Vansickle, 1977).

Developmental rates and mortality rates were obtained from published laboratory experiments (Zhou et al. 2010), see Appendix 2

Appendix 2

We took the laboratory results of Zhou et al., 2010) and the results for each stage of *O. communa* for *T_c*, DEL , *k* :

stage	eggs	larva	Pupae	pre-oviposition	adults
<i>T_c</i>	11.10	14.79	6.35	13.04	0.53
DEL	91.01	117.70	145.22	360.30	1264.39
<i>K</i>	42.00	90	119	110	18

Each stage mortality rate (μ) is depend on:

$$\text{Eggs : } \mu(T) = 0.0001T^2 - 0.0067T + 0.0889$$

$$\text{Larva : } \mu(T) = 0.00008T^2 - 0.0042T + 0.0597$$

$$\text{Pupae : } \mu(T) = 0.00002T^2 - 0.0001T + 0.0145$$

Mortality is also a function of relative humidity (RH). data for this dependence were taken from (Zhou et al., 2010):

$$\text{Eggs : } \mu(RH) = -0.0107 * RH + 1.0432$$

$$\text{Larva: } \mu(RH) = -0.0029 * RH + 0.4278$$

$$\text{Pupae: } \mu(RH) = -0.0008 * RH + 0.0842$$

APPENDIX 2

Diffusione di *Ambrosia artemisiifolia* L. e *Ophraella communa* LeSage in Valtellina (Alpi Centrali, Lombardia)

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Contribution: data collection, conceptualization, analysis, writing (first draft)

DIFFUSIONE DI *AMBROSIA ARTEMISIIFOLIA* L. E *OPHRAELLA COMMUNA* LESAGE IN VALTELLINA (ALPI CENTRALI, LOMBARDIA)

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Parole chiave - Distribuzione di ambrosia, gradiente altitudinale, invasioni biologiche

Key words - Altitudinal gradient, biological invasions, ragweed distribution

Riassunto - La presente nota riporta le nuove stazioni della specie esotica invasiva *Ambrosia artemisiifolia* L. (Asteraceae) e del suo nemico naturale *Ophraella communis* LeSage, 1986 (Coleoptera; Chrysomelidae) in Valtellina, Lombardia (Italia settentrionale). Mentre la presenza della pianta in Valtellina era già stata supposta sulla base dei monitoraggi pollinici e da dati bibliografici riferiti alla Provincia di Sondrio (Valchiavenna), la presenza dell'insetto può essere interpretata come segno di una prima graduale colonizzazione di questa valle.

Abstract - Spread of *Ambrosia artemisiifolia* L. and *Ophraella communis* LeSage in Valtellina (Central Alps, Lombardia). In this paper we report for the first time the occurrence of the invasive weed *Ambrosia artemisiifolia* L. (Asteraceae) and a natural enemy, *Ophraella communis* LeSage, 1986 (Coleoptera; Chrysomelidae) in Valtellina, Lombardy (Northern Italy). While the occurrence of the plant had been predicted by pollen monitoring and from bibliographic data referring to the Sondrio Province (Valchiavenna), before, the detection of the insect could be a sign of an early stage of a stepwise colonization of this valley.

INTRODUZIONE

Ambrosia artemisiifolia L. è una specie esotica invasiva presente in tutta Europa, dove si è naturalizzata a partire dal XIX secolo (CHAUVEL *et al.*, 2006). In Italia (BANFI & GALASSO, 2010), la prima stazione della specie fu trovata nei dintorni di Alba (CN) nel 1902, come specie accidentale nel giardino della Scuola Agraria (VIGNOLO-LUTATI, 1934 e 1935). Qualche anno dopo, nel 1907, alcuni individui della pianta furono osservati presso il cementificio di Bressanone (BZ), per diversi anni consecutivi (HEIMERL, 1911). Dopo il 1930, la specie si è progressivamente diffusa lungo tutta la valle del Po e in alcune località dell'Italia centrale (CELESTI-GRAPPOW *et al.*, 2009; GENTILI *et al.*, comunicazione personale). In Lombardia, la prima stazione nota di *A. artemisiifolia* risale al 1941, rinvenuta dal botanico STUCCHI (1942), a Castano Primo (MI), lungo un bordo strada. Per la provincia di Sondrio la specie è riportata per la Valchiavenna (CONSONNI & MAURIZIO, 1999) e quindi indicata come presente nella Flora Alpina di AESCHIMANN *et al.* (2004) e ne *La Flora esotica lombarda* (BANFI & GALASSO, 2010); il recente lavoro di aggiornamento sulla flora esotica effettuato da ARDENGHI & PAROLO (2011), non cita *A. artemisiifolia*.

Questa specie, dai caratteri di pioniera, cresce su suoli di-

sturbati e lungo corridoi lineari, come cantieri edili, cave, margini di strada, aste fluviali ma agisce anche come malerba in campi coltivati, soprattutto a partire dal periodo dell'aratura primaverile; la sua diffusione sembra essere favorita dai cambiamenti climatici (HAMAQUI-LAGUEL *et al.*, 2015; CHAPMAN *et al.*, 2014). La massima preoccupazione riguardo all'espansione di questa specie invasiva è la produzione di polline altamente allergenico che influisce negativamente sul benessere dei soggetti allergici e contribuisce pesantemente alle spese medicosanitarie dei paesi in cui la specie è molto presente (FUMANAL *et al.*, 2007).

Per contrastarne la diffusione, trattamenti con erbicidi e metodi di controllo meccanico, sono stati sviluppati e implementati come misure a breve termine o di pronto effetto (ALLEVA, 2008; BUTTENSCHÖN *et al.*, 2009; GENTILI *et al.*, 2015); tuttavia, non sempre, tali metodi garantiscono un sufficiente controllo della specie nel lungo termine (GERBER *et al.*, 2011; ESSL *et al.*, 2015). Il controllo biologico, ossia l'impiego di nemici naturali specializzati provenienti dalla stessa zona di origine della specie da contrastare, potrebbe essere un potenziale metodo di controllo a lungo termine per limitare *A. artemisiifolia*; tuttavia in Europa non è ancora stato implementato (MÜLLER-SCHÄRER & SCHAFFNER, 2008).

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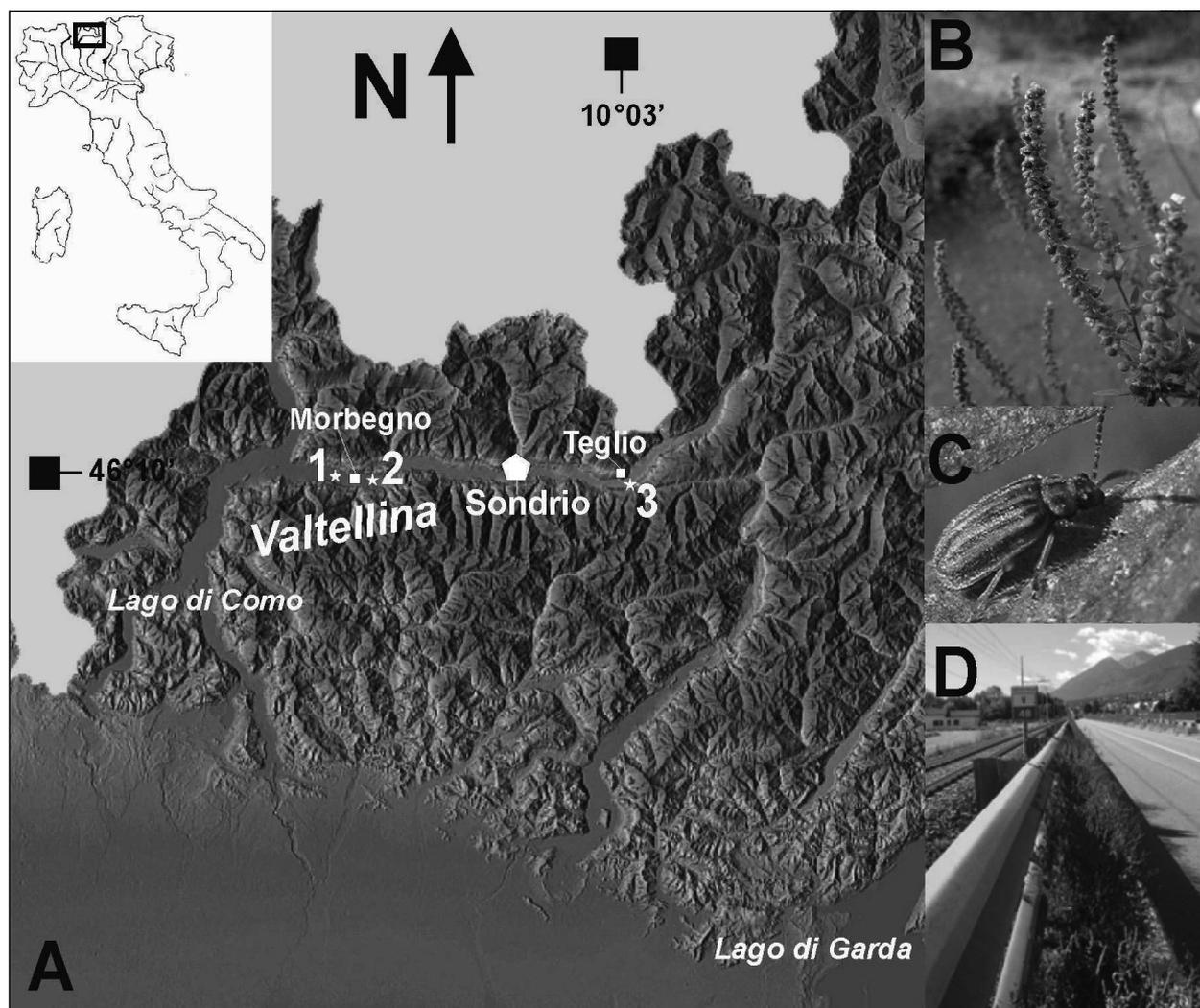


Fig. 1 - A) Mappa dei principali siti di ritrovamento di *Ambrosia artemisiifolia* (Siti 1, 2 e 3, indicati con il simbolo a stella) e *Ophraella communis* (presente solo nel Sito 1). *Ambrosia artemisiifolia* è inoltre presente in modo quasi continuativo lungo la SS38 dello Stelvio, nel tratto compreso tra il Sito 1 e il Sito 3. B) Individuo di ambrosia (infiorescenze maschili). C) Individuo di *Ophraella communis* (foto per gentile concessione di Peter Tóth, Slovak University of Agriculture). D) Sito di crescita 3, lungo la SS38 e nei pressi della linea ferroviaria, a Teglio (SO).

Ophraella communis LeSage, 1986 (Coleoptera; Chrysomelidae), il coleottero di ambrosia, è un efficiente nemico naturale di *A. artemisiifolia* in Giappone e Cina, dove l'insetto è stato accidentalmente introdotto nel 2001 (Guo *et al.*, 2015; ZHOU *et al.*, 2013). In Cina e Giappone, esso è stato riprodotto in massa e utilizzato su *A. artemisiifolia* come agente di biocontrollo (ZHOU *et al.*, 2013, FUKANO & DOI, 2013). In Europa, invece, il primo ritrovamento risale al 2013, nei pressi dell'Aeroporto di Malpensa (BORIANI *et al.*, 2013; MÜLLER-SCHÄRER *et al.*, 2014).

Nel contesto della COST Action europea FA1203 "Sustainable management of *Ambrosia artemisiifolia* in Europe (SMARTER)", un team di ricercatori del CABI (Svizzera), dell'Università di Milano-Bicocca e dell'Università di Friburgo (Svizzera) hanno condotto esperimenti di campo e laboratorio durante l'estate 2015, nei pressi di Milano e Lecco, per esaminare il grado di dipendenza di *A. artemi-*

siifolia e *O. communis* da fattori climatici. Sono state esaminate la *fitness* e la *performance* di entrambe le specie, lungo un gradiente altitudinale di temperatura e umidità relativa, al fine di costruire modelli demografici.

Durante tale progetto, popolazioni naturali di *A. artemisiifolia* a sud delle Alpi sono state oggetto di monitoraggio in campo per verificare la presenza di *O. communis*.

LE NUOVE STAZIONI DI AMBROSIA ARTEMISII-FOLIA E OPHRAELLA COMMUNA

Numerose popolazioni di *A. artemisiifolia* sono state trovate lungo la Strada Statale 38 dello Stelvio e lungo l'adiacente linea ferroviaria. Durante il sopralluogo in campo, nella bassa Valtellina, in data 4 settembre 2015, sono state rinvenute popolazioni di *A. artemisiifolia*, con uova

di *O. communa* presenti sulle piante, rilevate mediante osservazioni visive speditive. Nessun altro stadio di sviluppo dell'insetto è stato osservato sulle stesse piante. Durante il successivo sopralluogo del 20 settembre 2015, ulteriori popolazioni di *A. artemisiifolia* sono state ritrovate risalendo la valle (sino alla media Valtellina), tuttavia non sono state osservate tracce della presenza di *O. communa* sulle piante, mediante stima visiva.

Di seguito si riportano i punti GPS in gradi decimali (WGS84) di alcuni siti di ritrovamento (Fig. 1):

- **Sito 1:** osservati *A. artemisiifolia* e *O. communa* (uova); località: bassa Valtellina, SS38 nei pressi di Pietra Piatta (SO), circa 3 km a ovest di Morbegno; GPS: 46.13482N, 9.51864E;
- **Sito 2:** osservata *A. artemisiifolia* senza tracce dell'insetto; località: bassa Valtellina, SS38 2,5 Km a est del centro abitato di Morbegno (SO); GPS: 46.14069N, 9.60170E;
- **Sito 3:** osservata *A. artemisiifolia* senza tracce dell'insetto; località: media Valtellina, SS38 nei pressi di Teglio (SO), circa 1 Km a ovest del centro abitato di Tresenda (SO); GPS: 46.16056N, 10.07405E.

Si sottolinea che *A. artemisiifolia* è presente in modo pressoché continuativo lungo tutta la tratta di strada statale che va dal Sito 1, prima del centro abitato di Morbegno, sino al Sito 3, nei pressi di Teglio, per circa 40 Km.

DISCUSSIONE E CONCLUSIONI

Sulla base del monitoraggio pollinico effettuato nel 2015 e negli anni precedenti (bollettino pollinico dell'ASL di Sondrio; confronta il sito-web dedicato: <http://www.asl.sondrio.it/>), e da dati bibliografici di presenza/assenza, riferiti alla provincia di Sondrio (AESCHIMANN et al. 2004; BANFI & GALASSO, 2010) la presenza di *A. artemisiifolia* in Valtellina era stata supposta. Questi nuovi dati stazionali, direttamente osservati, confermano quindi le

indagini polliniche e inoltre evidenziano come estese popolazioni di questa pianta invasiva e pericolosa per la salute umana, possano essere trascurate, se non soggette a monitoraggi mirati.

Anche l'insetto *O. communa* non era ancora stato segnalato in Valtellina, benché durante i sopralluoghi del mese di settembre 2015 esso sia stato osservato solo allo stadio di uova. In altre località lombarde, con clima simile a quello della bassa Valtellina (es. lecchese), dove popolazioni stabili dell'insetto sono state riscontrate, non sono state trovate uova della specie a metà settembre. Tuttavia, la densità delle popolazioni di *O. communa* era più alta in questi altri siti e più stadi vitali erano presenti sugli individui di *A. artemisiifolia*, che spesso erano danneggiati o morti. La bassa presenza di *O. communa*, ritrovata in uno solo sito in bassa Valtellina, potrebbe indicare che siamo di fronte ad una prima fase di colonizzazione della valle da parte dell'insetto. In tal caso, osservazioni future in media e alta Valtellina potrebbero dare importanti informazioni circa il pattern di colonizzazione di questo potenziale agente di biocontrollo dell'ambrosia.

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APPENDIX 3

Biological weed control to relieve millions of allergy sufferers in Europe

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Contributions: Data collection and analysis of demographic rates

Abstract

Invasive alien species (IAS) can substantially affect ecosystem services and human well-being. However, quantitative assessments of their impact on human health are rare and the benefits of implementing sustainable IAS management likely to be underestimated. In this study, we (1) quantify the effects of the allergenic plant *Ambrosia artemisiifolia* on public health in Europe and (2) assess the potential impact of the accidentally introduced leaf beetle *Ophraella communa* on the number of patients and healthcare. We find that, prior to the establishment of *O. communa* in 2013, some 13.5 million persons suffered from *Ambrosia*-induced allergies in Europe, causing economic costs of approximately Euro 7.4 billion annually. Our projections reveal that biological control of *A. artemisiifolia* will reduce the number of patients by approximately 2.3 million and the health costs by Euro 1.1 billion per year. Our conservative calculations indicate that currently discussed economic costs of IAS underestimate the real costs and thus also the benefits from biological control.

Introduction

As a consequence of globalisation, the number of biological invasions has substantially increased over the past decades, and new introductions do not appear to be slowing down¹. IAS can have multiple effects on biodiversity and ecosystem services² and incur significant economic costs³⁻⁵. However, while alien parasites, invertebrates and plants are known to cause public health problems⁶⁻⁷, only a few studies have attempted to quantify their impact on human well-being^{4,5,8}. Moreover, the studies published so far are considered to underestimate the real costs because they are regionally focused, disparate or not grounded in verifiable data⁴⁻⁵. Yet, accurate information of policy and management about the impact of IAS on human health and the potential savings due to the implementation of mitigation measures is essential to ensure that reasonable resources are invested and actions coordinated in IAS management⁹.

We applied an interdisciplinary approach to quantify the effects of allergenic pollen produced by common ragweed, *Ambrosia artemisiifolia* L., on human health in Europe. Common ragweed, a native to North America, has invaded different parts of the world¹⁰. In Europe, it is considered invasive in more than 30 countries¹⁰ and its spread and impact are likely to increase with changing climate¹¹⁻¹³. Here we show that, prior to the establishment of

the biological control agent *O. communa* in 2013, some 13.5 (95% confidence interval (CI) 10.9-14.8) million persons suffered from *Ambrosia*-induced allergies in Europe, causing economic costs of approximately Euro 7.4 (CI 5.4-8.6) billion annually. Field studies in Italy proved evidence that *O. communa* can reduce *A. artemisiifolia* pollen production by 82%. By modelling the number of generations of *O. communa* across its suitable habitat range in Europe, we project that biological control of *A. artemisiifolia* will, once the leaf beetle has colonized its environmental niche, reduce the number of patients to approximately 11.2 (CI 8.6-12.9) million and the health costs to Euro 6.4 (CI 4.4-7.5) billion per year. Our estimates of the costs of *A. artemisiifolia* for public health are considerably higher than what has been reported previously, suggesting that the actual costs of IAS in Europe and the benefits from their management are underestimated.

Results

Based on information from the European pollen monitoring programme, we mapped seasonal total ragweed pollen integrals in Europe during the period 2004 to 2012, i.e. before the accidental introduction of the North American leaf beetle *Ophraella communa* LeSage. We interpolated data from 296 European pollen monitoring sites to a 10 x 10 km grid and extracted them for the European Union (EU) member states and the non-EU member states that are located within the boundaries of the EU (Fig. 1; Supplementary Note 1; Fig. S1, Table S1).

Pollen allergens produce clinical symptoms only among previously sensitised persons. Therefore, we mapped ragweed sensitisation rates in Europe by combining data on (a) the overall sensitisation rate among the general population, and (b) the ragweed sensitisation rates among the sensitised population (Supplementary Note S2, Table S2). The interpolated map of ragweed sensitisation rates in the European population (Fig. 2) corresponds to that of total seasonal ragweed pollen integrals, with both highest pollen integrals and highest ragweed sensitisation rates found in the Pannonian plain, the Po plain and the Rhône valley. By multiplying the interpolated ragweed sensitisation rates with the European population at a 10 x 10 km grid cell size¹⁴, we estimated that currently 23.2 million persons in Europe are ragweed sensitised (Supplementary Tables S3, S4). Taking into account that not all persons with a positive allergy test develop symptoms upon contact with the ragweed allergen, we corrected these numbers for clinical relevance¹⁵, resulting in an estimated 15.8 million persons in Europe with clinically relevant ragweed sensitisation (Supplementary Table S4). Our estimate is 37% lower than that published by Lake *et al.*¹², which is largely due to our more

accurate approach using interpolation of ragweed sensitisation rates based on a large number of geo-referenced locations compared to the region-based approach by Lake *et al.*¹². We then determined the population with clinically relevant sensitisation that was exposed to ragweed pollen prior to the establishment of *O. communa* (Fig. 1). This approach resulted in a total of approximately 13.5 (CI 10.9-14.8) million persons in Europe, which likely suffered from seasonal ragweed pollen allergy prior to the arrival of *O. communa* (Supplementary Table S5).

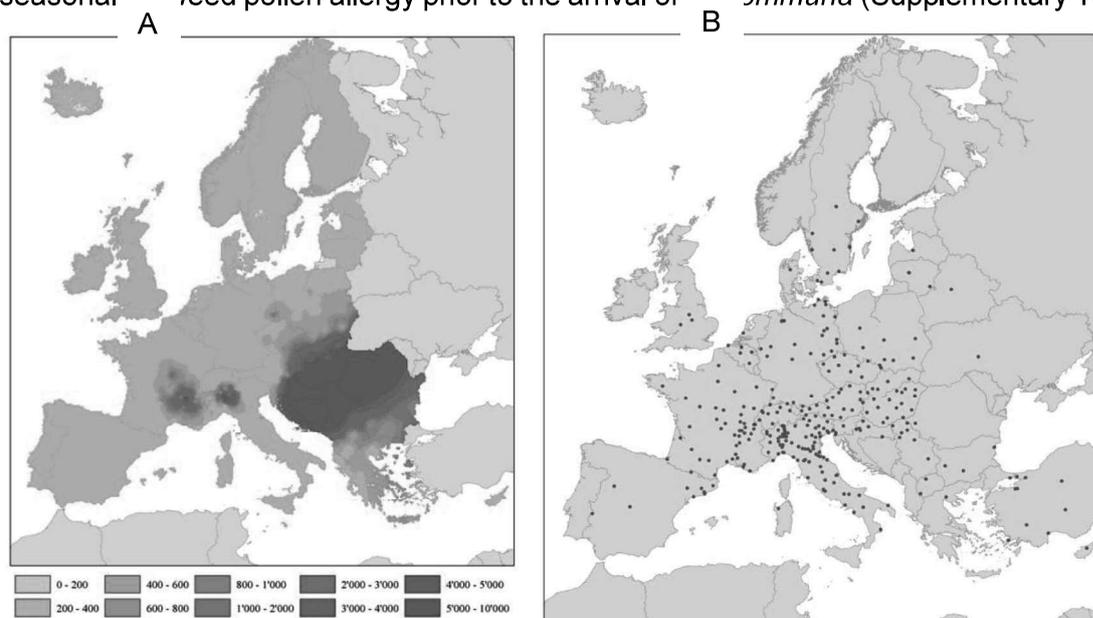


Figure 1. (A) Interpolated seasonal pollen integrals for ragweed (number of grains per cubic meter of air) across Europe before the establishment of *Ophraella communa* (data from 2004-2012). (B) Seasonal pollen integrals from 296 pollen grid monitoring stations were used to interpolate ragweed pollen exposure to a 10 x 10 km grid.

To validate the estimated number of patients suffering from ragweed pollen allergy, we compared our European-wide assessment with detailed healthcare data from the Rhône-Alpes region in southeastern France¹⁶ (www.auvergne-rhone-alpes.ars.sante.fr/; Supplementary Table S6). For each of the region's 313 communities, we calculated the average number of persons which got reimbursed for purchasing anti-allergy or anti-asthma medication during the ragweed flowering season and related them to the average seasonal total ragweed pollen integrals in the communities (the period from 2007 to 2015; Supplementary Figs. S3, S4). We found that seasonal total ragweed pollen counts were significantly correlated with the number of patients, with a 10% decrease in the seasonal total ragweed pollen counts resulting in an 8.4% reduction in the number of patients (Supplementary Fig. S5). With our interpolation approach used at the European level, we

estimated that the average proportion of affected patients in the 313 communities in the Rhône-Alpes region was 3.2% (SD 2.2). This figure is similar to the proportion of the population in the communities receiving reimbursements for anti-allergy or anti-asthma medication (2.9%, SD 1.0). Thus, our approach to estimate the number of persons suffering from ragweed pollen allergy appears to be reasonably accurate.

To estimate the European-wide economic costs due to ragweed pollen allergy prior to the establishment of *O. communa*, we estimated European-wide treatment costs per patient and year based on the cost estimates summarized by Bullock *et al.*¹⁷ for nine European countries. The annual treatment costs varied between Euro 8.30 (for antihistamines in the Czech Republic) and Euro 8,060 (for treatment of asthma in Switzerland), with median treatment costs of Euro 565 per patient and year (Supplementary Note S3). To also account for socio-economic costs, we used the ratio between medical expenses and absence from work calculated for the Rhône-Alpes region (18.5%, Supplementary Table S6). This resulted in estimated annual costs of Euro 670 per patient, a lower and thus a more conservative estimate than the median costs for seasonal allergic rhinitis in Europe (Euro 964 per patient and year¹⁸). By weighting the treatment and lost work time costs at the country level using purchasing power parity (PPP)-adjusted health expenditures per capita for 2015, we found that the overall economic costs amount to approximately Euro 7.4 (CI 5.4-8.6) billion per year in Europe (Supplementary Note S3; Supplementary Table S7). Our cost estimates are approximately 8-fold higher than those presented by Bullock *et al.*¹⁷, which is only partly explained by their lower estimate of the medical treatment costs per patient (Euro 303). More importantly, Bullock *et al.*'s¹⁷ estimates of the number of people affected by ragweed allergy in Europe (between 0.84 and 4.18 million). Our calculations rely on an extensive data set of observed pollen integrals and on a geospatial approach for calculating total number of patients in Europe using a large data set of observed sensitisation rates. This suggest that our approach is more accurate concerning exposure and that previous numbers of patients have been substantially underestimated. This is supported by the fact that the healthcare data from the Rhône-Alpes region indicate that in this region alone some 200,000 persons per year received reimbursement for purchasing anti-allergy or anti-asthma medication during the ragweed flowering season¹⁶.

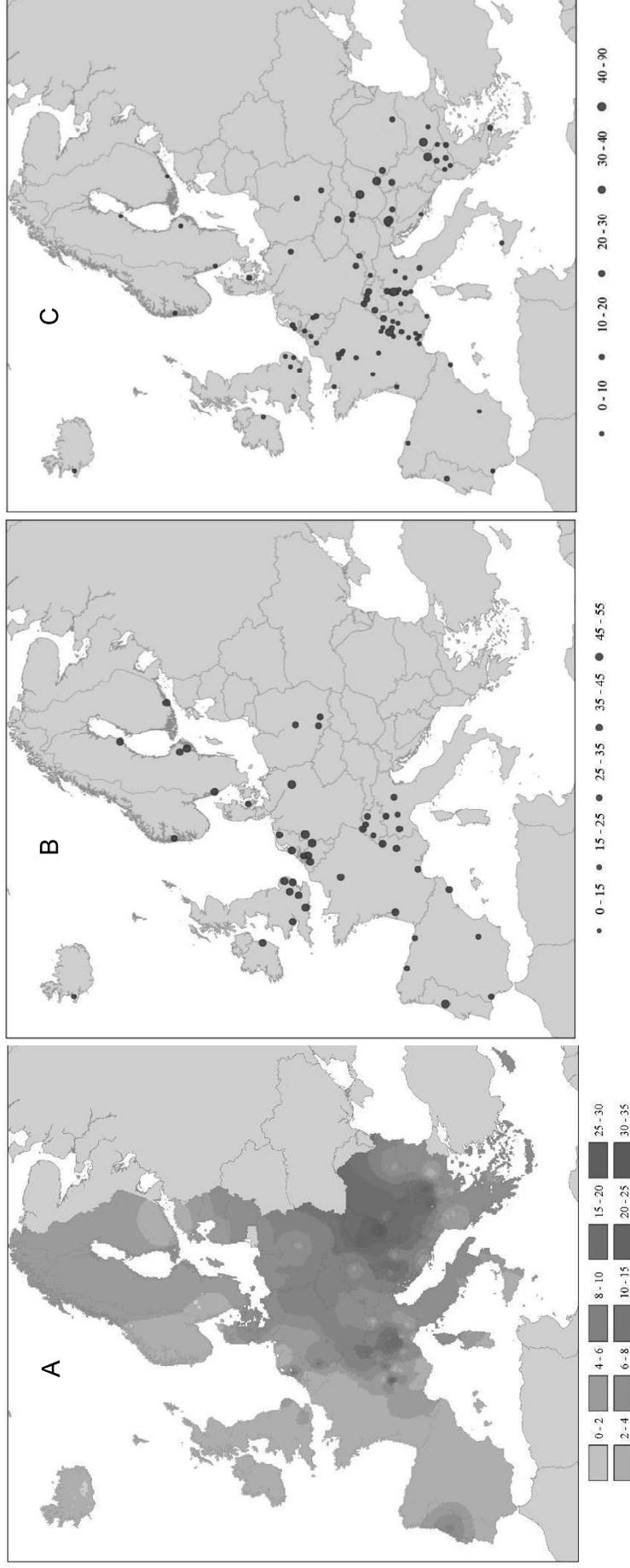


Figure 2. (A) Interpolated percentage of ragweed sensitised persons in the European population. (B) Geographic distribution of studies assessing overall sensitisation rates (%) among the general population in Europe. Size of points indicates overall sensitisation rates (%) among the persons tested. (C) Geographic distribution of studies assessing ragweed sensitisation rates (%) among the sensitised persons tested. Size of points indicates ragweed sensitisation rates (%) among the sensitised persons tested. Studies are based on ragweed specific skin prick tests or circulating ragweed specific IgE tests.

The establishment of *O. communa* in Europe in 2013 raised the question whether this leaf beetle, which is mass-reared and actively distributed in China for biological control of *A. artemisiifolia*¹⁹, might also contribute to the sustainable management of this plant invader in Europe²⁰. In Northern Italy, where the beetle was first detected, up to 100% of *A. artemisiifolia* plants are attacked with damage levels high enough to completely prevent flowering of most ragweed plants²⁰. Pollen monitoring studies in the Milan area revealed that the substantial drop in airborne ragweed pollen concentrations since the establishment of *O. communa* cannot be explained by meteorological factors²¹.

To assess whether the reduction in airborne *Ambrosia* pollen concentrations in Northern Italy indeed reflects the impact of *O. communa* on pollen production of *A. artemisiifolia* plants, we conducted a field experiment in the Po plain during which *O. communa* was excluded in replicated plots at two of three study sites. We found that *O. communa* reduced pollen production on average by 82% (73-100%; Fig. 3), which well corresponds to the drop in airborne pollen concentrations recorded in the Milano area since the establishment of this beetle²¹ (Supplementary Fig. S6). Hence, our findings support the notion that the observed drop in *Ambrosia* pollen integrals in Northern Italy is caused by *O. communa*.

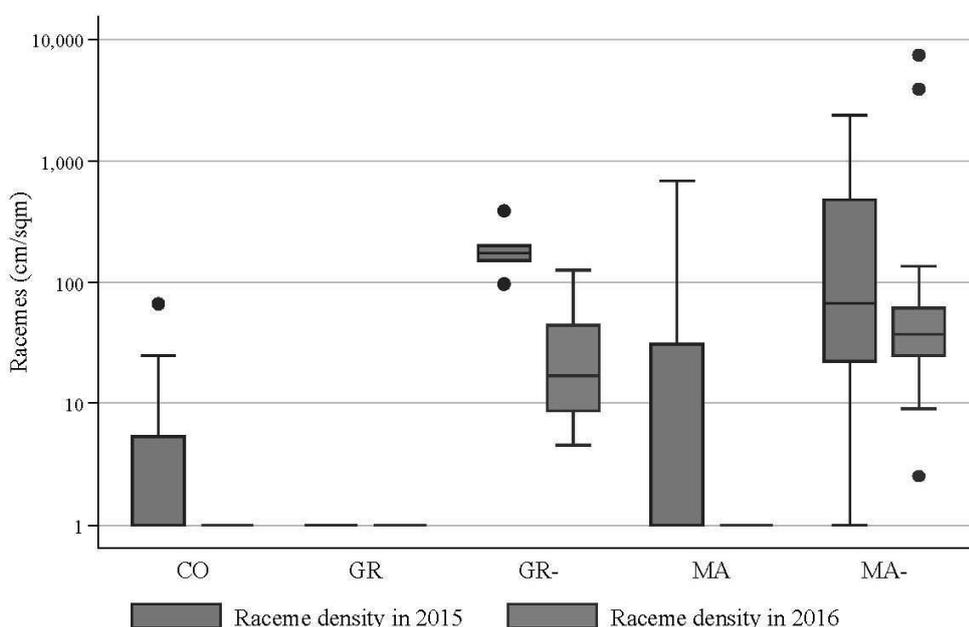
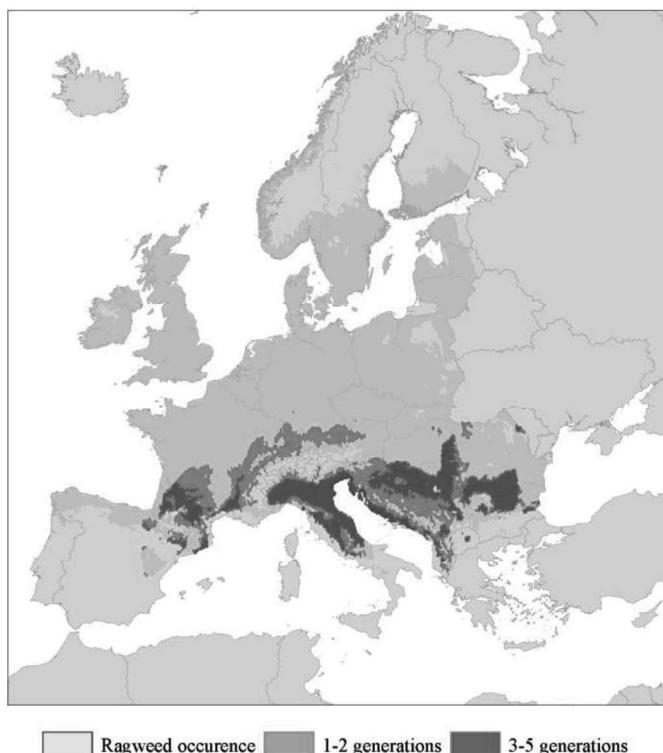


Figure 3. Estimated *Ambrosia artemisiifolia* raceme density per year at three study sites in Northern Italy in the presence and absence of *Ophraella communa*. CO = Corbetta; GR = Grugliasco; MA = Magnago. GR- and MA- indicate plots

from which *O. communa* was excluded by spraying insecticides. At Corbetta permission for insecticide application was not obtained. Boxplots represent the variation across plots within treatment and site, with the thick line as the median, the boxes representing the quartiles, the whiskers 1.5 times the interquartile range and dots data points outside the whiskers.

To estimate the potential impact of *O. communa* on ragweed pollen integrals at the European level, we developed species distribution models for *A. artemisiifolia* and *O. communa* based on worldwide occurrences and bioclimatic variables (Fig. 4; Supplementary Fig. S7). We conducted a field cage experiment along an altitudinal gradient in Northern Italy and determined an average growing degree days (GDD) value of 288.7 (Supplementary Table S8) for one generation of *O. communa*. This value was incorporated into the species distribution model to map the number of generations which *O. communa* is likely to complete across its environmental niche in Europe (Fig. 4). We then quantified the potential impact of *O. communa* on ragweed pollen integrals by matching the number of generations of the beetle with the interpolated seasonal pollen integrals (Supplementary Note S1). Based on the association between seasonal total pollen integrals and relative number of patients determined for the Rhône-Alpes region (Supplementary Fig. S5), we projected *O. communa* to reduce the number of patients to approximately 11.2 (CI 8.6-12.9) million once it will have colonized its entire environmental niche in Europe. This corresponds to an average reduction in the number of patients by 2.3 million (16.9%; Supplementary Fig. S8, Table S5). Correspondingly, the approximated yearly economic costs are, when accounting for PPP among countries, projected to drop to Euro 6.4 (CI 4.4-7.5) billion. This reduction results in



economic savings of approximately Euro 1.1 billion annually (Supplementary Table S7). Besides Northern Italy, where the beetle has already significantly reduced airborne pollen concentrations, our projections suggest that people in countries of the Balkan Peninsula will benefit most from the establishment of *O. communa* (Figs. 2 and 4; Supplementary Table S5).

Figure 4. Modeled distribution range of *Ambrosia artemisiifolia* and the *Ophraella communa* (including the expected number of generations) in Europe.

Discussion

Our study provides evidence that the impacts of common ragweed on human health and the economy are so far highly underestimated, but that biological control by *O. communa* might mitigate these impacts in parts of Europe. So far, host specificity studies with closely related crops, ornamentals and native endangered species indicate no significant negative impact of *O. communa* on non-target plants under field conditions^{20,22}. The results of our interdisciplinary study justify a comprehensive risk:benefit assessment of *O. communa*, also regarding a possible deliberate distribution of this leaf beetle across the climatically suitable areas in Europe.

Estimates of the economic impact of biological invasions have provided important input in policy and management at the national and international level^{3,23}. Our estimate of the costs incurred by *A. artemisiifolia* is in a similar range as the currently discussed overall economic costs of IAS in Europe²⁴ (Euro 12 billion per year). We propose that future assessments of economic impacts of IAS should more thoroughly consider costs related to human health.

Methods

Ragweed pollen exposure map

The estimates of ragweed pollen exposure in Europe are based on the best available information on long-term exposure to *Ambrosia* pollen for the period from 2004 to 2012. The data were obtained from published work of the mean seasonal total *Ambrosia* pollen integrals from 296 observational sites found in most large urban regions of Europe (Fig. 1B). We incorporated data from all sites where seasonal pollen integrals cover at least five years in order to secure sufficient data points near and outside the main invasion fronts of *A. artemisiifolia* (Supplementary Note S1). The final data set with 296 calibration points for *Ambrosia* pollen exposure both within and outside the EU was interpolated to a 10 x 10km grid using the common European Geographical Reference System (GCS_ETRS_1989). The gridded data were then extracted for the study area which covers 42 countries within Europe and includes EU27 and the bordering non-EU member countries Albania, Serbia, Macedonia, Bosnia-Herzegovina, Kosovo, Montenegro, Iceland, Norway and Switzerland (Supplementary Fig. S1).

Map of ragweed sensitisation among the European population

The map of ragweed sensitisation rates among the population in Europe is based on a combination of two types of published studies: a) overall sensitisation rates among the general population obtained from medical centres and b) ragweed sensitisation rates among sensitised patients collected from medical centres. A thorough review of all European studies was conducted and all peer-reviewed studies based on skin prick and circulating specific Immunoglobulin E (IgE) testing were included, while questionnaire studies were excluded. The review provided 50 data sets on the sensitisation rate among the general population and 80 data sets on ragweed sensitisation among the sensitised population (Figs. 2B and 2C; Supplementary Note S2; Supplementary Table S2).

The two data sets (50 areas and 102 areas) were then interpolated to a 10 x 10 km grid using the common European Geographical reference system (GCS_ETRS_1989). After that, the gridded data were extracted for the study area. Resulting *Ambrosia* sensitisation rates among the European population were then calculated by multiplying the gridded overall sensitisation rates among the population with the ragweed sensitisation rates among sensitised persons. We used natural breaks in the data to classify the exposure and population data (Figs. 1 and 2). Considering that only a proportion of patients with positive skin prick test reactions express symptoms, we calculated the clinically relevant sensitised population by multiplying the ragweed sensitised population with the clinically relevant ragweed sensitisation rates at the country level¹⁵. When determining the population with clinically relevant sensitisation that was exposed to ragweed pollen prior to the establishment of *O. communa*, we excluded all locations with very low seasonal ragweed pollen estimates (<10 grains per cubic meter during the pollen season¹²).

Relationship between seasonal *Ambrosia* pollen integrals and patient numbers

The relationship between the seasonal *Ambrosia* pollen integrals and the number of patients was calculated with information from the database compiled by the 'Agence régionale de santé Auvergne-Rhône-Alpes' (www.auvergne-rhone-alpes.ars.sante.fr/) on health costs related to common ragweed allergies in the Rhône-Alpes region. The Rhône-Alpes region, which is since 1 January 2016 part of the new region Auvergne-Rhône-Alpes, lies in south-eastern France and covers an area of 43'700 km² with approximately 6.5 million inhabitants. The Rhône Valley, which runs north-south through the Rhône-Alpes region, reports the highest common ragweed infestation rates in France¹⁰ and, together with the Pannonian plain and Northern Italy, also the highest rates in Europe²⁵.

Since 2007, the Regional Health Agency, in association with the National Aerobiological Monitoring Network, has been collecting data on the annual health costs of ragweed allergies in the Rhône-Alpes region²⁶. These cost calculations are based on the consumption of medical care and medical goods by persons affiliated to the general health insurance scheme and cover 98.9% of all patients. The included costs relate to allergy medication, doctor consultations, allergy tests, oral desensitization treatments and sick leave when linked to the prescription of anti-allergy drugs during ragweed flowering season (see Supplementary Table S3 for further explanations). The relationship between total seasonal ragweed pollen integrals and number of patients was calculated for the period 2007-2015 using data from all 313 communities in the Rhône-Alpes region (Supplementary Fig. S5). The number of patients is defined as the number of persons receiving reimbursement through the general health insurance scheme for purchasing anti-allergy or anti-asthma medication to treat allergies during the ragweed pollen season from July 7 to October 21²⁶.

We interpolated the community-level pollen exposure from pollen data for 62 monitoring sites in France (Supplementary Fig. S3), using the same approach as for the calculation at the European level. Instead of interpolating the annual total pollen integrals at the grid-cell level, we used the community centroid as the central point for interpolation. We found the highest exposure in the area around Lyon and the area on both sides of the river Rhone which is consistent with the habitat preferences of the plant²⁷ (see Supplementary Fig. S4).

The method for estimating the number of patients is likely to represent the lower bound of the overall effect since only those persons were included which were reimbursed for the consumption of allergy-related medication.

Besides *A. artemisiifolia*, some other plant species flowering in late summer or autumn also possess allergenic pollen, which may theoretically pose some challenges for allergy epidemiological studies. However, in southeastern France, the two other ragweed species (*Ambrosia trifida* L. and *A. psilostachya* DC.) are rare. Moreover, peak flowering of allergenic mugwort species, such as *Artemisia vulgaris* L., *A. absinthium* L. and *A. verlotiorum* Lamotte, is before and after the peak flowering season of common ragweed, and the season of airborne *Artemisia* pollen hardly overlaps with the season of airborne *Ambrosia* pollen²⁸. Moreover, seasonal pollen integrals for *Artemisia* pollen are far lower in southeastern France (Supplementary Fig. S4B) and northern Italy (Supplementary Fig. S6D) than those for *A.*

artemisiifolia and thus only marginally contribute to the consumption of allergy-related medication in these regions (Supplementary Note S3).

To obtain an estimate of the treatment effect, we averaged the total seasonal pollen integrals and the number of patients for each community and the period 2007 to 2015. We then log-transformed the data and estimated the relationship without a constant using the linear least squares approach. This approach forces the regression to go through the origin which is a necessary condition for modelling the causal relationship between pollen counts and patient numbers. Overall, the specified regression model fits the data well and explains a significant share of variation (Supplementary Fig. S5).

Impact of *O. communa* on pollen production

In 2014, we selected three sites in northern Italy where *A. artemisiifolia* and *O. communa* co-occurred (Corbetta: N45.4709 E8.9368, Magnago: N45.5707 E8.7855, Grugliasco: N45.0654 E7.5923). Half of each site was assigned to experimental exclusion of *O. communa* in an experimental block design, except for the site in Corbetta for which we did not obtain permission to apply insecticides. In each site-treatment combination, 14 permanent plots of 0.5 x 0.5 m were installed covering the range of *Ambrosia* densities in June 2014 after ragweed had successfully established, and ensuring similar densities across treatments within sites. Starting in July 2014, beetles were experimentally excluded from the blocks assigned by the biweekly application of insecticides while control blocks in these sites were treated with water only. In 2015 and 2016, treatments started in May (corresponding to the period where the first beetle generation of offspring from the overwintering adults develops) and lasted until *O. communa* disappeared from the sites in autumn. The insecticide treatment constituted of spraying three insecticides (including two contact insecticides and a systemic insecticide; for further details see Lommen et al.²⁹). This method has no direct effects on seed and pollen output or pollen allergenicity of *A. artemisiifolia*²⁹.

As a proxy for the yearly pollen production per plot³⁰, we estimated the total raceme length r per plot in 2015 and 2016 following $r = n * m * e^{v*\beta+\alpha}$, where n is the total number of *A. artemisiifolia* plants in the plot at the end of the season in September (excluding the new seedlings that had just appeared in Magnago in 2015 and which did not produce pollen anymore), m is the fraction of these *A. artemisiifolia* that had matured per site-treatment combination (as a proxy for the fraction of plants that had produced racemes), v is the mean log-transformed volume of the matured *A. artemisiifolia* in the plot (based on all or a sample

of ca. 10 plants measured in the plot (N=47 plots), or the site-treatment mean if no plants in the specific plot had been measured (N= 23 plots) , and α and β are the intercept and the slope, respectively, of the linear relationship between the log-transformed total raceme length and the log-transformed volume of individual matured *A. artemisiifolia*, as assessed by a sample of 21 plants taken outside of the plots per site-treatment combination. The analysis excluded plots that contained no *A. artemisiifolia* plants in 2014 (as these may represent the absence of a soil seed bank), and all plots in one block in Grugliasco where grasses dominated and suppressed ragweed occurrence after 2014. As too few mature plants were available in Corbetta and Magnago in 2016, the respective raceme-volume relationships from 2015 were used instead. The estimated raceme lengths per plot were rescaled to represent racemes per square meter.

Since many plots were estimated to produce a raceme length of 0 (i.e. no racemes), we took a hurdle-model approach for the statistical analysis of the effect of treatment on the raceme length per plot. As in 2016 none of the control plots and all of the insecticide-treated plots produced racemes, we could only do this for the data from 2015. We first assessed the effect of treatment on the probability of a plot producing racemes in 2015 by a generalised linear mixed effect model (GLMER) with treatment as a fixed effect, site as a random effect, and a binomial error distribution, tested against the corresponding null-model. For those plots producing racemes in 2015, we then similarly assessed the effect of treatment on the total length of racemes produced by a GLMER with treatment as a fixed effect, site as a random effect, and a Poisson error distribution with a log-link, which was tested against the corresponding null-model. The models were then used to obtain fitted estimates of the probability to produce racemes and the total length of racemes produced for each treatment, and their product was an estimate of the density of racemes produced (in cm per m²).

In 2015 the insecticide treatment increased the probability of a plot to produce racemes from 0.26 (95% CI=0.14-0.42) to 0.84 (95% CI=0.37-0.98; $\chi_1^2=17.496$, $p<0.001$). When considering plots with raceme production, insecticide-treated plots produced significantly higher densities of racemes than control plots (fitted average 905 cm/m² versus 260 cm/m², respectively; $\chi_1^2= 6573$, $p<0.001$). As mentioned above, in 2016 only insecticide-treated plots produced racemes (Fig. 3).

Species distribution models for *A. artemisiifolia* and *O. communa*

We collected occurrence data for *A. artemisiifolia* and *O. communa* worldwide from the literature, from online resources and from additional occurrence sources (see Appendix S2 in

Sun et al.¹³). In summary, we used WORLDCLIM current climate data (developed by Hijmans et al.³¹) at 5-min spatial resolution to derive a set of meaningful predictors that are considered critical to plant or insect physiological function and survival of both species. The final model for *A. artemisiifolia* included annual mean temperature, isothermality, temperature seasonality, max temperature of warmest month, min temperature of coldest month, temperature annual range, mean temperature of driest quarter and an approximation of growing degree days based on monthly average temperatures (Thom's formula). The final model for *O. communa* included max temperature of warmest month, temperature annual range, mean temperature of driest quarter, mean temperature of coldest quarter, precipitation of wettest month, precipitation of wettest quarter, precipitation of driest quarter, precipitation of coldest quarter and standard insect growing degree days as described in Sun *et al.*¹³. We modelled the current potential distribution for each species in Europe using the integrative modelling framework Biomod2 R-package³². The combination of different modelling techniques generating an ensemble of predictions is proposed as an optimal solution for dealing with the uncertainties of extrapolation³³. Generalized linear models (GLM), generalized boosting models (GBM), random forest (RF), and maximum entropy (MAXENT) were therefore calibrated on a random sample of the initial data (80%) and tested on the remaining data sets (20%) with both the receiver-operating characteristic curve (ROC) and the true skill statistic (TSS). We then estimated their area under the curve (AUC) that evaluates the discriminatory power of model predictions. Above techniques were chosen because they have proven to presently be among the most effective species distribution models³⁴. Duplicated presences within a raster pixel were removed. As only occurrences were available, random pseudo-absences were generated³⁵ to fill the absence component of models. The entire training-evaluation procedure was repeated 100 times (25 times for each model), using a different set of calibrated presences and absences within each iteration to ensure robustness of the predictions and provide uncertainty estimates³⁶. A stacked assemblage of predictions across individual models including mean, inferior confidence interval, superior confidence interval of both *A. artemisiifolia* and *O. communa* were generated. The suitability of species distributions of two species were then binary-transformed using species-specific thresholds maximizing the rate of the number of corrected predicted presences to number of false absences (i.e., to transform the probabilities of presence into presence and absence). Based on these thresholds, we also binary-transformed all confidence interval predictions (Supplementary Fig.S7), which were later used for uncertainty estimation. In our models, AUC ranged from 0.87 to 0.96 across the

two species and four model types, which provided useful information for an analysis of climate suitability through modelling of the species distributions. Finally, the overlap maps of *O. communa* on its host plant *A. artemisiifolia* that we used for later computations were produced based on their binary mean distribution maps, binary lower confidence interval maps and binary upper confidence interval maps (for more details see Sun et al.¹³).

The number of generations of *O. communa* across the potential distribution in Europe was estimated by experimentally assessing the temperature-dependent developmental time of *O. communa* along an altitudinal gradient in the Southern Alps, approximately 50 km north of the Milan area. The five field sites were set up on sun-exposed grasslands or in private gardens at 130 m (in the Po plain), 250 m, 480 m, 700 m and 1230 m. Greenhouse-reared, potted *A. artemisiifolia* plants were exposed to female *O. communa* in a field cage set up at the lowest elevation. Plants with freshly laid egg batches were individually covered with a gauze bag, firmly attached to the pot with an elastic ribbon, and transferred within 48 hours after oviposition to one of the five field sites in a randomised order. At the field site, *A. artemisiifolia* plants were placed within a 1 x 1 x 2 m gauze cage, dug together with the pots into the soil and regularly watered. Two cohorts of 5-8 plants each were transferred to the five field sites, with the exception of the highest site, which only received plants from the second cohort. The first cohort was set up between 29 June and 2 July 2016 and the second cohort between 8 and 10 August 2016. Each field site was visited at least once a week to record the presence of beetle life stages on each *A. artemisiifolia* plant. Based on the field study, we calculated an average GDD $> 13.3^{0.37}$ of 288.7 for the period from the egg stage to adult emergence of *O. communa* (Supplementary Table S8). This value closely corresponds to the GDD reported for *O. communa* by Zhou et al.³⁷ which was based on growth chamber experiments with constant temperature regimes (307.2). We then used the GDD calculated from our field experiment to map the number of generations of *O. communa* in relation to the climatic conditions within its potential distribution range in Europe.

Impact of *O. communa* on airborne *Ambrosia* pollen concentration in Europe

To quantify the potential impact of *O. communa* on seasonal pollen integrals in Europe, we exploited retrospective data from northern Italy. We used data on ragweed pollen concentration (number of pollen per cubic meter of air) with a daily resolution for three monitoring sites in the Milan area for the period 2004 to 2018. The sites are located in proximity (less than 30 km) to the Malpensa airport, where the beetle was initially detected in 2013. Because common ragweed was already established in all potential habitats before the beetle's

arrival, we can calculate the pre-treatment exposure and compare it with the post-treatment exposure. For this purpose, we first obtained a measure of the average daily pollen integral for each station during the two periods and then calculated the average of pollen integrals over the three monitoring sites. Supplementary Fig. S6 shows the average impact of *O. communa* on daily pollen integrals in the Milan metropolitan area. The average impact of the beetle on ragweed pollen exposure is 86.3%, which implies that the average pollen integrals dropped from 46.2 to 7.5 pollen grains per cubic meter of air. We used this information to estimate the empirical reduction function of pollen concentration due to *O. communa*. For this purpose, we cumulated the daily pollen integrals before the arrival (2004-2012) and calculated the reduction in pollen concentration at the respective level of pollen concentration in the pre-arrival period. The impact of the beetle is stronger for the lower range of annual pollen concentration (above 90%) and levels out at 83.7% for the higher range (Supplementary Fig. S6B). In terms of human exposure to ragweed pollen, this resulted in a reduction of the number of days with ragweed pollen and reduced pollen integral during days with ragweed pollen (Supplementary Fig. S6C).

In Northern Italy, the population density of *O. communa* and the feeding damage increased significantly in August, which corresponds with the end of the beetle's third generation. We thus assumed that all projected areas with at least three beetle generations face the same reduction in total seasonal ragweed pollen integrals as in the Milan area (86%; Supplementary Figs. S6A and S8). For areas with one or two generations we assumed a reduction in seasonal *Ambrosia* pollen integrals by 30%, due to a likewise reduction in plant densities by early feeding damage that is not compensated for during the growing season (Lommen, unpubl. results). For all other areas, we assumed no effect of *O. communa* on seasonal *Ambrosia* pollen integrals.

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APPENDIX 4

Estimating economic benefits of biological control of *Ambrosia artemisiifolia* by *Ophraella communa* in southeastern France

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Contributions: Data collection and analysis of demographic rates



Estimating economic benefits of biological control of *Ambrosia artemisiifolia* by *Ophraella communa* in southeastern France

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Abstract

The North American common ragweed, *Ambrosia artemisiifolia*, which produces highly allergenic pollen, is invasive in different parts of the world, including Europe. In 2013, common ragweed in northern Italy was found attacked by another accidentally introduced species, the North American leaf beetle *Ophraella communa*, which is used for biological control of common ragweed in China. Since the establishment of *O. communa*, ragweed pollen concentrations in northern Italy have significantly dropped. Here we set out to estimate the potential economic benefits of establishment of *O. communa* in the Rhône-Alpes region in south-eastern France, where detailed data on the economic impact of common ragweed are available. Extrapolating from the change in airborne ragweed pollen concentrations in the Milan area, we estimated that establishment of *O. communa* in the Rhône-Alpes region will reduce the number of days with ragweed pollen concentrations at which sensitive people express symptoms by 50% and the medical costs due to common ragweed by 5.2–6.8 M € annually. Our findings suggest

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that investments of public funds are justified to conduct a complete assessment of the potential risks and benefits associated with the accidental establishment of *O. communa* in Europe.

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Keywords: *Ambrosia artemisiifolia*; Biological control; Economics; Health

Introduction

Invasive alien plant species (IAPS) not only cause significant harm to biological diversity and ecosystem functioning (Vilà et al. 2011), they also induce direct economic impacts by affecting ecosystem services such as livestock production, water availability or human health (Pejchar & Mooney 2009). To address a growing pressure for delivering sustainable IAPS management solutions, targeted management solutions need to be elaborated and their environmental and economic consequences assessed and communicated to decision-makers and other stakeholders (Hulme 2006; Müller-Schärer, Schaffner, & the COST-SMARTER Task Force Ophraella 2017).

When selecting suitable IAPS management options, it is worth distinguishing between the likelihood of success of a control programme and its potential impact (Cock et al. 2015). The former addresses the implementation of the steps of an IAPS control programme and the anticipated reduction of the target weed, while the latter addresses the socio-economic and environmental benefits of successful IAPS control. A key aspect regarding the impact of a sustainable IAPS management strategy is the relationship between the economic investment in the implementation of the management strategy and the expected economic benefits in case of a successful control of the target weed (Culliney 2005; Jetter 2005; Page & Lacey 2006).

Classical biological control aims to reduce the IAPS' abundance below an economic or ecological threshold and/or slow down its spread by the introduction of one or a few specialist natural enemies from the weed's native range (Müller-Schärer & Schaffner 2008). Usually, the introduction of specialist biological control agents is based on a decision process by competent authorities after careful evaluation of pre-release studies assessing the likelihood of non-target effects and impact of the agent, but in times of increased biological invasions (Seebens et al. 2017), more and more potential biological control agents are also accidentally introduced into regions where IAPS cause problems (Shaw et al. 2018). There is growing evidence that classical biological control, if successful, can play a major role in the sustainable IAPS management (Seastedt 2014) and that it is highly cost-effective compared to other methods (Page & Lacey 2006; De Lange & van Wilgen 2010; van Wilgen et al. 2012). Most of this evidence comes from retrospective studies, or ex-post studies, that compared actual data on the costs of implementing the program, the impact of the IAPS as well

as the reduction in impact due to classical biological control, and therefore tend to provide relatively accurate estimates of the cost-effectiveness of this approach (Culliney 2005).

In contrast to chemical or mechanical weed control, which often benefit directly those who supply them, the implementation of classical biological weed programs often requires a significant initial investment of public funds (Pannell 1994). Therefore, attempts have also been made to evaluate the likely benefits of a classical biological control program ex-ante, i.e. before or at an early stage of a biological control program, in order to assess whether the funding required for its implementation is likely to be justified. The few ex-ante studies that have been conducted so far also suggest that biological control programs have favourable benefit:cost ratios (Gurr & Wratten 2000; Perrings, Williamson, & Dalmazzone 2000; Culliney 2005; Jetter 2005). However, because their results depend on the accuracy of the various assumptions made a priori, they are usually subject to considerable uncertainty (Jetter 2005). This uncertainty may be reduced when the likelihood of success of the biological control approach can be extrapolated from experiences made in other parts of the invaded range, and when the weed's impact – and hence the potential savings – can be attributed to specific costs, e.g. to health costs that can be allocated to the target IAPS.

The North American plant species *Ambrosia artemisiifolia* L., common ragweed, was first recorded in Europe in the 19th century and started spreading in the late 1920s (Essl et al. 2015). Today it is widely distributed in Europe, with invasion hotspots in the Pannonian plain, the Balkans, northern Italy and around the Rhône valley in France (Šikoparija et al. 2016). While *A. artemisiifolia* is also an agricultural weed in Eastern Europe, the main concern regarding its economic impact in Europe is its large production of highly allergenic pollen that causes rates of sensitisation among Europeans in the invaded regions ranging from 10% to 60% (Burbach et al. 2009). Exposure to ragweed pollen induces allergic rhinitis and severe asthma, generating significant medical costs and reduced quality of life among the allergic population (D'Amato et al. 1998; Thibaudon, Hamberger, Guilloux, & Massot 2010). Climate change is likely to increase spread and impact of *A. artemisiifolia*, posing a significant risk to society, even in countries presently not yet affected (Hamaoui-Laguel et al. 2015).

In 2013, the North American leaf beetle *Ophraella communa* LeSage, 1986 (Coleoptera, Chrysomelidae) was found to have accidentally established in northern Italy and southern Switzerland (Müller-Schärer et al. 2014). This beetle is

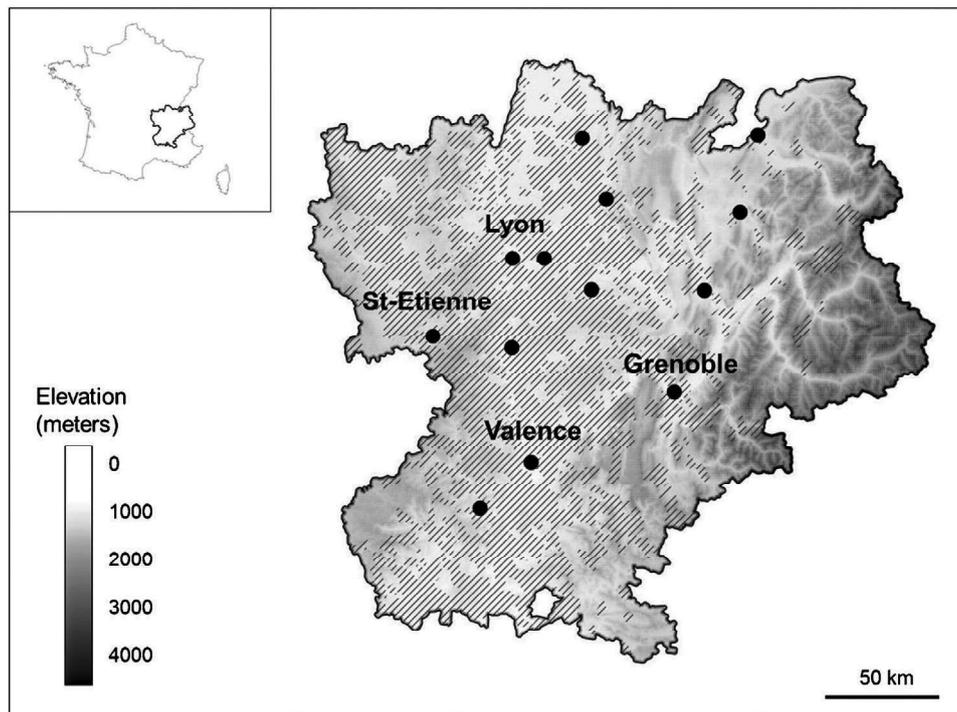


Fig. 1. The Rhône-Alpes region with location of pollen stations, distribution of ragweed at the municipal level (source: Observatoire des ambrosiées), and elevation (source: Institut Géographique National, France). Black dots correspond to the thirteen pollen stations and hatching to the municipalities with confirmed presence of *A. artemisiifolia*.

an oligophagous beetle that is being used as a biological control agent against *A. artemisiifolia* in China (Guo et al. 2011), although no-choice tests under laboratory conditions revealed that it can complete its life-cycle on sunflower, *Helianthus annuus* L. (Palmer & Goeden 1991). At sites in northern Italy where the beetle was present, up to 100% of the common ragweed plants were attacked, with damage levels high enough to cause complete defoliation and to prevent flowering and seed set in most ragweed plants (Müller-Schärer et al. 2014). 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). 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. 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. 2017a), 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*. By 2016, the beetle had colonized almost the entire area in northern Italy invaded by common ragweed (Müller-Schärer et al. 2017).

To inform science-based decision processes about how to respond to a likely upcoming establishment of this accidentally introduced insect in other parts of Europe, risk

assessments (Müller-Schärer et al. 2017; Lommen, Jolidon, Sun, Bustamante Eduardo, & Müller-Schärer 2017b) should be combined with ex-ante studies to evaluate both the potential risks and benefits of an establishment of *O. communa*. Here we set out to estimate the potential effect of an establishment of *O. communa* on human health costs in the Rhône-Alpes region in France, which is bordering the Italian region invaded by *O. communa*. Capitalizing on the observed impact of *O. communa* on airborne ragweed pollen concentration in the Milan area and on the well-documented health costs related to common ragweed in the Rhône-Alpes region, we estimated to what extent a potential establishment of *O. communa* in the Rhône-Alpes region might reduce the health risks for the inhabitants of this region and hence the associated human health costs.

Material and methods

Study area

The Rhône-Alpes region lies in south-eastern France, covering an area of 43,700 km² with approximately 6.5 million inhabitants. Its topography consists of two areas of high elevation (the Alps in the eastern part and the Massif Central mountains in the western part) divided by the Rhône Valley, which runs north–south (Fig. 1). The climate is mainly continental, characterized by cold winters and hot summers. The Rhône valley areas have the highest common ragweed

infestation in France (Thibaudon, Šikoparija, Oliver, Smith, & Skjøth 2014) and, together with the Pannonian plain and northern Italy, one of the highest common ragweed infestations in Europe (Šikoparija et al. 2016).

Estimating the impact of *O. communa* on airborne *Ambrosia* pollen concentrations

Daily ragweed pollen concentrations for the Rhône-Alpes region for the period 2008–2015 were provided by the French National Aerobiological Monitoring Network (RNSA) from thirteen pollen stations (Ambérieu, Annecy, Annemasse, Bourg-en-Bresse, Bourgoin-Jallieu, Chambéry, Coux, Genas, Grenoble, Lyon, Roussillon, Saint-Etienne, Valence) located across the Rhône-Alpes region (Fig. 1). For the Milan region, daily ragweed pollen concentrations for the period from 2008 to 2012 (before the detection of *O. communa*) and from 2013 to 2015 (when high densities of *O. communa* were recorded) were provided by the Local Health Authority of Milan 1 (now Local Health Authority of Milan Città Metropolitana) from three pollen stations (Legnano, Magenta and Rho) located in the north-western area of the Province of Milan in northern Italy. In both regions, atmospheric concentrations of *Ambrosia* pollen are monitored using volumetric spore traps of the Hirst design (Hirst 1952). Each year, the period covered by the data ran from July 15 to October 15. The amount of airborne pollen was expressed as the number of ragweed pollen grains per cubic meter of air and day.

The potential impact of *O. communa* on airborne *Ambrosia* pollen concentrations in the Rhône-Alpes region was assessed by transferring the observed relative change in airborne *Ambrosia* pollen concentration in the Milan area from years before to years after establishment of *O. communa* to the conditions in the Rhône-Alpes region. Specifically, we estimated the pollen reduction in the Milan area by calculating the relative difference between the two time-integrated pollen load curves averaged over the three Italian stations for the periods 2008–2012 and 2013–2015. The Milan area is mainly situated below 200 m where *O. communa* can complete 3–4 generations per year. We thus adjusted the expected impact in the Rhône-Alpes region by calculating the percentage of *Ambrosia* populations that grow at an altitude that allows *O. communa* to complete at least 3 generations per year. Information on the altitudinal threshold in southern Europe below which *O. communa* can complete at least 3 generations per year was collected from a field cage experiment set up along an altitudinal gradient in the southern Alps (see Supplementary Appendix A: Fig. 1). The altitudinal distribution of common ragweed in the Rhône-Alpes region was assessed from all geo-referenced *Ambrosia* records collected by the French National Federation of Botanical Conservatories (FCBN). As the impact of *O. communa* on common ragweed mainly builds up during the third generation of *O. communa* in late summer, we predicted that *Ambrosia* popu-

lations below the altitudinal threshold determined in the field experiment will experience the same impact as populations in the Milan area, while populations above a certain altitudinal threshold are expected to escape from significant impact by *O. communa*.

Estimating the impact of *O. communa* on allergic risk

RNSA defined an allergic risk index (RAEP; ‘risque allergique d’exposition aux pollens’; allergy risk when being exposed to pollen) with six risk levels according to the daily ragweed pollen exposure (see Supplementary Appendix A: Table 1; Thibaudon, Oliver, & Cheynel 2008). The pollen risk threshold $RAEP=3$ (daily abundance of ≥ 6 grains $\times m^{-3} \times day^{-1}$) corresponds to the level of pollen concentration at which 100% of ragweed sensitized people express symptoms in the Rhône-Alpes region (ORS Rhône-Alpes 2015). As such, the number of days with $RAEP \geq 3$ provides an estimate of the allergenic risk of common ragweed over the season. Daily airborne pollen data from the Rhône-Alpes region from 2008 to 2015 were therefore converted to the number of days with $RAEP \geq 3$ in order to assess the allergic risk in the actual situation and in a scenario where *O. communa* is present.

Estimating the impact of *O. communa* on health costs

Since 2007, the Regional Health Agency, in association with the National Aerobiological Monitoring Network, has been collecting data on the annual health costs of ragweed allergy in the Rhône-Alpes region (ORS Rhône-Alpes 2011, 2012, 2013, 2015). The costs are based on the consumption of medical care and medical goods by persons affiliated to the general health insurance scheme and include costs related to allergy medication, doctor consultation, allergy tests used, oral desensitization treatments and on sick leave when linked to the prescription of anti-allergic drugs (see Supplementary Appendix A: Table 2 for further explanations). These assessments include a low-cost estimate which accounts for the population that is most likely affected by ragweed allergy as well as a high-cost estimate accounting for the whole regional population (ORS Rhône-Alpes 2012). The relation between the annual allergic risk (expressed as the cumulated number of days with $RAEP \geq 3$ over the 13 stations) and the estimated annual health costs in the observed scenario were examined by a linear regression model with a logarithmic transformation of the estimated health costs. This model was used to estimate annual health costs in the scenario with a decrease in pollen emission after the establishment of *O. communa*. All analyses were done using software R version 3.2.3 (R Core Team 2015).

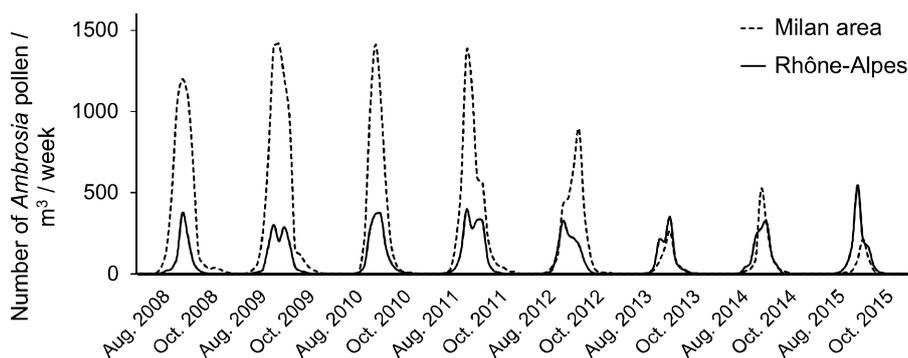


Fig. 2. Comparative trends of ragweed airborne pollen concentration in the Milan area (mean over three pollen stations; data provided by the Local Health Authority of Milan Città Metropolitana) and in the Rhône-Alpes region (mean over thirteen pollen stations; data provided by French National Aerobiological Monitoring Network) between 2008 and 2015. Given are weekly numbers of *Ambrosia* pollen per m^3 during the pollen season (August–October) of each year.

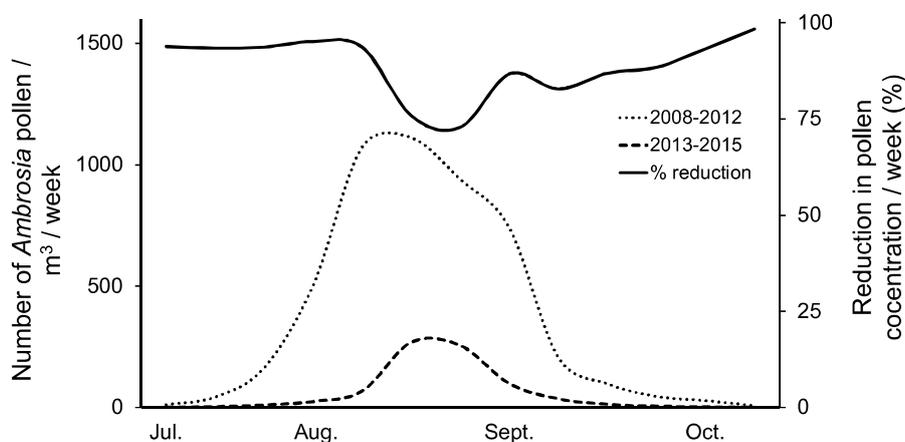


Fig. 3. Phenology of airborne pollen concentration from 2008 to 2012 (before the detection of *O. communa*) and 2013 to 2015 in the Milan area and percent reduction in airborne pollen concentration after the detection of *O. communa*. Curves (smoothed with moving average functionality) of airborne pollen concentrations are based on mean \pm SE weekly numbers of *Ambrosia* pollen per m^3 during the years before and after detection of *O. communa*, respectively. Data were provided by the Local Health Authority of Milan Città Metropolitana.

Results

Estimating the impact of *O. communa* on airborne *Ambrosia* pollen concentrations

Seasonal appearance of airborne ragweed pollen is similar in the Rhône-Alpes region and the Milan area, but the pollen concentration before the establishment of *O. communa* in the Milan area was higher than in the Rhône-Alpes region (Fig. 2). In both cases, the observed aerial pollen concentration dynamics follows the flowering phenology of *A. artemisiifolia* and known patterns of pollen emission in other regions with high *Ambrosia* infestations (Šikoparija et al. 2016).

In the Milan area, an 85.9% decrease in pollen emission was observed between the periods 2008–2012 and 2013–2015 (Fig. 3). No such reduction was detected in the Rhône-Alpes region or in any other part of Europe (Šikoparija et al. 2016), where the biological control agent was absent, suggesting that the reductions in Milan area were due to the

biological control agent. After an examination of airborne pollen data from the Milan area, a constant reduction in airborne pollen concentration by 85.9% across time and space was chosen to extrapolate the reduction observed in northern Italy to the study area. This constant reduction level was chosen given that (i) it corresponds to the average reduction in airborne pollen concentration observed from the period 2008–2012 to 2013–2015 in the Milan area (Bonini et al. 2015, 2016), that (ii) the reduction in airborne *Ambrosia* pollen concentration was relatively consistent across the pollen season (Fig. 3) and assuming that (iii) *O. communa*, once it will have established in the Rhône-Alpes region, will exhibit similar population dynamics (and impact on *A. artemisiifolia*) as in northern Italy.

The field study set up along an altitudinal gradient north of the Milan area in Italy (see Supplementary material for a description of methods and results) revealed that time from oviposition to adult emergence increased from approximately 17 days in the Milano area to approximately 20 days at 250 m a.s.l., approximately 23 days at 480 and 700 m a.s.l., and

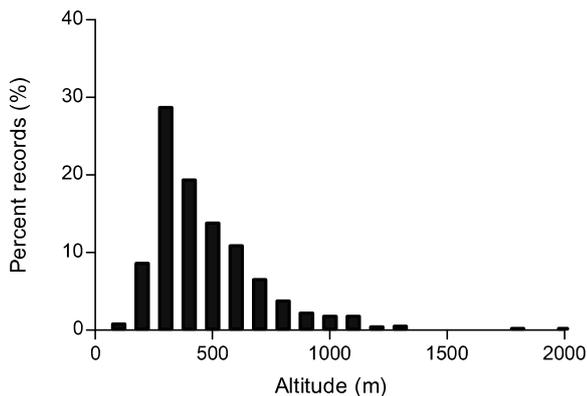


Fig. 4. Altitudinal distribution of common ragweed occurrence data in the Rhône-Alpes region ($n = 1340$; data from the French Network of Aerobiological Monitoring).

37 days at 1,230 m a.s.l. (see Supplementary Appendix A: Fig. 1). Based on the development threshold and cumulative degree days determined in this field study (Augustinus, unpubl. results) and from the literature (Zhou, Guo, Chen, & Wan 2010), these results indicate that *O. communa* can complete at least three generations up to 700 m above sea level in the Rhône-Alpes region. Based on the altitudinal distribution of 1340 records of common ragweed populations in the Rhône-Alpes region (Fig. 4), 88.7% of the common ragweed populations found in the Rhône-Alpes region are found ≤ 700 m a.s.l. and are therefore expected to be damaged by *O. communa* to a similar extent as those in the Milan area. Considering the altitudinal distribution of *Ambrosia* populations in the Rhône-Alpes region, the reduction in airborne ragweed pollen concentration due to *O. communa* is estimated at $85.9\% \times 0.887 = 76.2\%$.

Estimating the impact of *O. communa* on allergic risk

Between 2008 and 2015, there were on average 24 days per year in the Rhône-Alpes region with $RAEP \geq 3$. A 76.2% decrease in airborne ragweed pollen concentrations would reduce the number of days with $RAEP \geq 3$ –12 days (50% reduction; Fig. 5).

Estimating the impact of *O. communa* on health costs

In the Rhône-Alpes region, the annual number of days with $RAEP \geq 3$ proved to be a significant predictor of the log-transformed low and high estimates of annual health costs, but the model fit was relatively modest (low-cost estimate: adjusted r -squared 0.504, $p = 0.0292$; high-cost estimate: adjusted r -squared = 0.417, $p = 0.0498$; see Supplementary Appendix: Table 3). Based on this relationship, we estimated annual health costs in the scenario with a 76.2% decrease in pollen emission and compared it to the actual health costs

measured. In the observed situation, the mean annual health costs in the period from 2008 to 2015 were estimated to range from €9.70 million (low estimate) to €14.04 million (high estimate), while they were estimated to range from €4.47 million to €7.21 million in a situation with a 76.2% decrease in airborne pollen concentrations (Fig. 6). Thus, the establishment of *O. communa* in the Rhône-Alpes region is expected to lead to annual savings in health costs of €5.23 million (low estimate) to €6.83 million (high estimate) in this region.

Discussion

Economic benefits of an establishment of *O. communa* in the Rhône-Alpes region

The estimated reduction in airborne pollen concentration is based on the assumption that most of the airborne pollen in the Rhône-Alpes region is produced locally or regionally and that *O. communa* will exhibit a similar impact on ragweed pollen production in areas below 700 m a.s.l. as in the Milan region. In the absence of wind, pollen collection at the regional pollen stations is between 06:00 and 14:00, suggesting that the pollen are of local origin (Laaidi & Laaidi 1999). However, the Rhône Valley is characterized by sometimes strong winds from the south and also from the north. The area invaded by common ragweed that is situated south of the Rhône-Alpes region also appears suitable for *O. communa* (Sun et al. 2017), suggesting that the amount of pollen entering the Rhône-Alpes region from the south will also drop once *O. communa* has established in south-eastern France. The climatic conditions north of the Rhône-Alpes region appear to be less suitable for *O. communa* to complete at least 3 generations (Sun et al. 2017), but common ragweed densities and airborne pollen concentrations are lower than in or south of the Rhône-Alpes region (Škoparija et al. 2016).

Our field studies in the Milan region revealed that temperature and air humidity are key abiotic factors affecting within-season population build-up of *O. communa*, as they influence egg hatching rate, larval development rate and generation length (Augustinus, unpubl. results). We also found that significant impact on ragweed pollen and seed production in the Milan region only becomes apparent from the third generation onwards (Lommen et al. 2017a). We acknowledge that all our estimates of the current impact of *O. communa* on ragweed pollen concentration in the Milan region and on its predicted impact on ragweed pollen concentration and on the allergic risk in the Rhône-Alpes region are associated with a level of uncertainty. For example, while our field studies suggest that *O. communa* can complete three generations in the lower parts of the Rhône-Alpes region, it remains to be shown whether the third generation of *O. communa* will defoliate the plants before they start producing male flowers. Nevertheless, we argue that the uncertainty of our predictions regarding the impact of biological control is

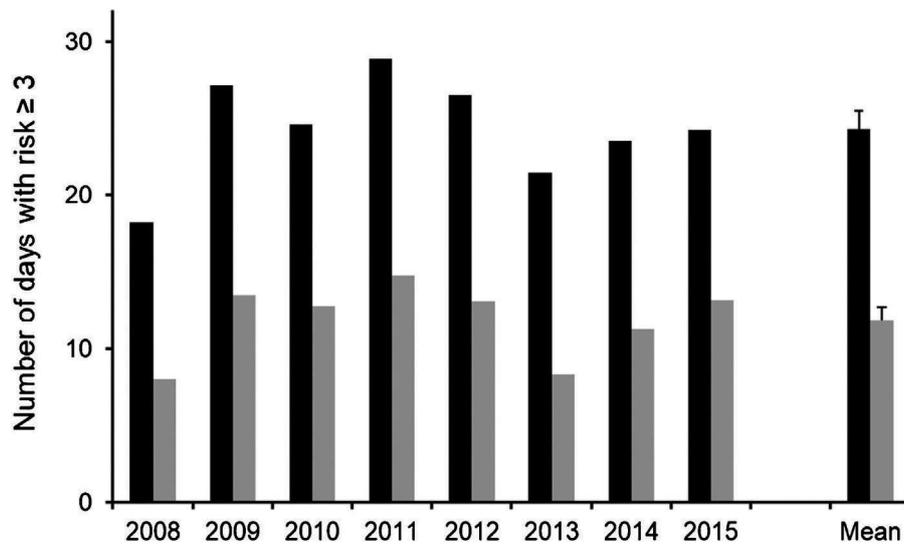


Fig. 5. Allergic risk index (expressed as the annual number of days with ‘risque allergique d’exposition aux pollens’; RAEP) ≥ 3 for the period from 2008 to 2015, based on the actual aerial pollen concentrations measured (black) and on a scenario with a 76.2% decrease in pollen concentrations (grey). Average allergic risks for the whole period are expressed as mean \pm SE.

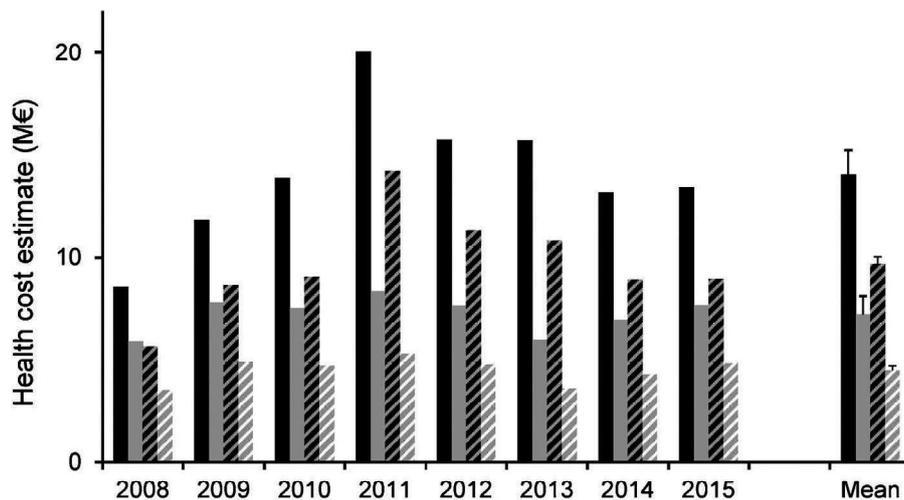


Fig. 6. Health costs estimates (expressed in M€) in the Rhône-Alpes region from 2008 to 2015 in the actual situation (black) and in a scenario with a 76.2% reduction in airborne pollen concentrations (grey). The high cost estimates (solid histograms) account for the whole regional human population, while the low cost estimates (hatched histograms) account for the population that is most likely affected by ragweed allergy (ORS Rhône-Alpes, 2012). Average health costs estimates for the whole period are expressed as mean \pm SE.

relatively low, compared to other ex-ante studies evaluating the likely impacts of a classical biological control programs (Gurr & Wratten 2000; Perrings et al. 2000; Culliney 2005; Jetter 2005). The number of days with pollen risk ≥ 3 , i.e. the number of days where ragweed sensitized people express symptoms, is a significant explanatory variable of the health costs related to ragweed allergies in the Rhône-Alpes region (ORS Rhône-Alpes 2015), but the model fit (approx. 50%) is relatively modest. Our estimates of the health costs caused by common ragweed in the Rhône-Alpes region are based on detailed data collected from the general health insurance scheme, but not all costs may be due to ragweed-caused allergies, nor are all health impacts caused by common ragweed included in these numbers. Anti-allergic drugs sold during

the common ragweed flowering period might have been used for purposes other than ragweed allergies, which would lead to an overestimation of the costs related to ragweed allergy. However, in order to link the health costs as closely to ragweed allergy as possible, patients were selected on the basis of reimbursements of anti-allergic medicaments for which a peak of consumption was observed in September, i.e. during the peak of airborne *Ambrosia* pollen concentration. In southern Europe, very few plant species with allergenic pollen flower at the same time as common ragweed, and the two other ragweed species (*Ambrosia trifida* and *A. psilostachya*) are absent or very rare in this area. For example, the flowering phenology of mugwort species, such as *Artemisia vulgaris* L., is approximately 3 weeks earlier than that of common rag-

weed, and the season of airborne *Artemisia* pollen ends during the second half of August (D'Amato et al. 1998). Accordingly, in the Milan area mugwort monosensitized patients have symptoms before monosensitized ragweed patients and they stop suffering before monosensitized ragweed patients do (Bottero et al., unpubl. results).

On the other hand, persons suffering from ragweed allergy might not have been included in the costs because they may have taken precautions or got a prescription for an anti-allergen earlier in the year, which would underestimate the actual costs inflicted by common ragweed. Also, sufferers may not have sought medical help and just waited for the symptoms to pass. Thus, there would have been no medical costs, but potentially costs in work time lost. We are therefore confident that selecting patients on the basis of reimbursements of anti-allergic medicaments for which consumption peaks in September is a realistic approach to estimate health costs linked to common ragweed.

Based on the data collected by RNSA, ragweed allergies affected in 2013 some 204,186 people in the Rhône-Alpes region, with a total cost of €15.7 million or an average costs of approximately €77 per patient (ORS Rhône-Alpes 2015). This average cost estimate is at the lower end of what is reported in the literature. Reported medical costs of *A. artemisiifolia* in Europe range from €8 to €8000 (median €565) per patient and year, depending on the type of medication and the country (Bullock et al. 2012). Medication costs vary considerably across countries in Europe, but the costs documented for the Rhône-Alpes region are also at the lower end of the range reported from France (€26–€386 reimbursement; Bullock et al. 2012). In a recent review of European studies on the costs associated with allergic respiratory diseases, median direct (treatment) and indirect (primarily due to absenteeism) costs for seasonal allergic rhinitis were calculated to amount to €791 per patient and year (Linneberg et al. 2016). This suggests that the estimates of a reduction in health costs in the Rhône-Alpes region due to a potential establishment of *O. communa* are conservative and that the actual savings may be considerably higher. We therefore propose that a potential establishment of the accidentally introduced *O. communa* may indeed generate significant economic benefits in terms of savings in health costs.

Assessment of the benefits and costs of biological control of common ragweed in Europe

In an economic impact assessment of weed biological control undertaken in Australia between 1903 and 2005, Page and Lacey (2006) estimated a reduction of approximately 350,000 people suffering from common ragweed-induced hay-fever and an economic benefit of approximately AUS \$23.85 per patient (expenditure on medicine) due to biological control of common ragweed. With an estimated cost of AUS \$625,000 for implementing the biological control pro-

gram, this resulted in a benefit:cost ratio of 103.7:1 for the biological control of common ragweed in Australia by 2005. It should be noted though that the common ragweed project benefited from releases made in the frame of the biological control program against *Parthenium hysterophorus* L., a close relative of common ragweed that also produces highly allergenic pollen. The biological control program against *P. hysterophorus* in Australia began in 1977 and exploration and new releases finished in 2004. In total, it is estimated to have cost AUS\$11.0 million (Page & Lacey 2006). By 2005, key outcomes achieved from the *Parthenium* biocontrol program included annual savings of AUS\$380,000 productivity benefit in sown pasture, AUS\$986,000 productivity benefit in native pasture, and AUS\$8.0 million benefit in reduced medical expenses. Despite the higher project costs, the benefit:cost ratio of the *Parthenium* biocontrol program was already 7.2:1 by 2005 (Page & Lacey 2006) and continues to increase.

The limited number of studies estimating the benefit:cost ratio of classical biological weed control ex-ante, i.e. before the project is launched or at an early stage of the project, show quite favourable results (Gurr & Wratten 2000; Culliney 2005). However, most ex-ante studies rely on some estimates of the probable success of the proposed program (Gurr & Wratten 2000). This is particularly problematic in those cases where the biological control programs are based on new introductions of biological control agents that have not been used anywhere else (Gurr & Wratten 2000). In the case of *O. communa*, both scenarios that facilitate the estimation of probable success are given; it is already used as a biological control agent of common ragweed in China (from where unfortunately no economic benefit:cost analyses are available), and it has been accidentally introduced into Europe, the region where biological control should be implemented. The fact that the establishment of *O. communa* in Northern Italy allows for an assessment of its population dynamics and impact under similar biotic and abiotic conditions as in the target region in the Rhône-Alpes region is expected to increase the robustness of the predictions of our ex-ante study.

To estimate the benefit:cost ratio of biological control of *A. artemisiifolia* by *O. communa* for the Rhône-Alpes region, we assumed that the overall costs for conducting the risk assessment for *O. communa* (see below) are comparable to those of the common ragweed program in Australia (AUS\$625,000 in 2005 = approx. €380,000; Page & Lacey 2006), which also benefited from previous biocontrol programs (see above). On this basis, five years with high *O. communa* densities would already lead to a benefit:cost ratio of 69:1 using the low estimates (€5.23 million × 5/€0.38 million) and of 90:1 using the high estimates for annual health costs (€6.83 million × 5/€0.38 million). The predicted benefit:cost ratio of a biological control program against *A. artemisiifolia* using *O. communa* in the Rhône-Alpes region is comparable to that calculated for Australia (Page & Lacey 2006), despite the fact that this French region is approximately five times smaller than the area invaded in Australia (Julien, McFadyen, & Cullen 2012). Considering that *A. artemisiifolia* has a much

broader distribution in Europe (Šikoparija et al. 2016), the European-wide economic benefits of implementing biological control against common ragweed would be significantly higher than in Australia.

Using accidentally introduced natural enemies for biological control of invasive plant species

As a consequence of globalization, biological invasions have greatly increased over the past decades, and there is no evidence for a slowing down of new introductions (Seebens et al. 2017). In this process, an increase of accidentally introduced natural enemies of IAPS has been reported, including species that are used or considered for use as biological control agents elsewhere (Shaw et al. 2018). In general, the handling and relocation of an accidentally introduced exotic organism, be it a biocontrol agent or not, requires permission from national competent authorities, but such organisms usually lack a rigorous risk analysis for the target region. Hence, biological control programs assessing the potential risks and benefits of accidentally introduced biological control candidates also require public funding to collect all information needed for a science-based decision on whether introduction of such organisms should be considered a fortunate coincidence or a threat (Müller-Schärer et al. 2014, 2017). The costs tend to be smaller since the host-range testing can build on previous studies done for other regions where the organism was considered for biological control (in the case of *O. communa*, e.g. Palmer & Goeden 1991; Dernovici, Teshler, & Watson 2006; Zhou et al. 2011).

Risk assessment studies with *O. communa* in Europe started within the frame of a European project (EU-COST Action on Sustainable Management of *A. artemisiifolia* in Europe, 2012–2016), and first results indicate that this beetle poses little risk to commercially grown sunflowers, which are harvested in late summer, and to native endangered plant species (Lommen et al. 2017b; Müller-Schärer et al. 2017). However, adult feeding may well occur on sunflower grown as ornamentals or used as green manure which still grow late in the year (Müller-Schärer et al. 2017). Based on the assessment of the potential benefits of biological control of common ragweed in the Rhône-Alpes, we propose that investments of public funds to finalize the risk assessment of *O. communa* for Europe and, provided the non-target risks are acceptable, to redistribute the biological control candidate across the heavily infested regions in Europe are economically justified. Moreover, investments in an inundative biological control approach, which builds on mass-rearing and mass-release of *O. communa* as practiced in China (Zhou et al. 2014), should also be considered, particularly in regions that are heavily invaded by common ragweed, but that are climatically less suitable for a rapid population build-up of *O. communa* in early summer.

Conclusions

Biological control is a key strategy to manage widely established IAPS, given the scale of the infestation and the costs of applying other management options such as chemical or mechanical control (Culliney 2005). When IAPS impact human well-being directly, biological control programs are particularly likely to remain cost-effective (Seastedt 2014). Biological control of common ragweed has been considered in Europe since the 1960s, and various biological control candidates have been prioritized over the years (Gerber et al. 2011). Because *O. communa* can complete its life-cycle on sunflower under contained conditions, this species was neither proposed for field release in Australia (Palmer & Goeden 1991), nor was it listed as a prioritized biological control candidate for Europe (Gerber et al. 2011). Yet, our findings suggest that *O. communa* has the potential to significantly reduce economic costs incurred by common ragweed, and that it is justified to invest in thorough risk assessment studies to assess its suitability for biological control of common ragweed in Europe. Provided that no action is taken to slow down the spread and population build-up of *O. communa* (ANSES 2015), the calculations made in this paper should become soon verifiable.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.baae.2018.08.002>.

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APPENDIX 5

Direct effects of insecticides on common ragweed-implications for natural enemy exclusion trials

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Direct effects of insecticides on common ragweed - implications for natural enemy exclusion trials

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Experimentally applying pesticides is an important method to assess the efficacy of weed biocontrol agents, but potential direct effects of the chemicals on plant performance are controversial or unknown. We assessed how three broad-spectrum insecticides applied in combination affect the performance of the widely invasive, crop-yield reducing, allergenic common ragweed (*Ambrosia artemisiifolia* L.) in an insect-free environment. Spraying insecticides had no significant effects on aboveground dry weight, seed and pollen output or pollen allergenicity, and only explained 1-8% of variation in these parameters. Our insecticide treatment can hence be applied to assess biocontrol impact on biomass and reproductive output of common ragweed. As our insecticide treatment delayed senescence, however, other methods of insect exclusion should be preferred when studying common ragweed phenology.

Keywords: biological weed control, growth promotor, insect exclusion, invasive plant, plant performance, phytotoxicity.

Electronic supplementary material: The online version of this article contains supplementary material, which is available at <http://www.jstage.jst.go.jp/browse/jpestics/>.

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Introduction

Biological control is an important method to manage noxious plants, as shown by an increasing number of targeted plants and new biocontrol agents released.¹⁾ This is especially useful when traditional control methods (e.g. herbicides, cutting) cannot be applied due to environmental or economic constraints, such as in semi-natural habitat types or organic farming and for widely dispersed invasive plants. To test the efficacy of the intended biocontrol agents, the performance of the target plant population is ideally assessed in parallel in the absence and in the presence of these antagonists.²⁾ The application of insecticides is one frequently used method to eliminate insects in order to assess the efficacy of the natural insect community (in conservation biocontrol), or the augmented or the introduced insects (in augmentative and classical biocontrol, respectively).^{3), 4)} The underlying assumption is that insecticides have no direct effects on the plants. This should, however, be tested for the specific system under study, since literature dealing with direct interactions between insecticides and plant performance are scarce, and so far published results report mixed effects.

Plants can be negatively affected in their growth by insecticides through phytotoxicity or interference with the plants' physiology causing growth inhibition, precocious tissue differentiation, and flower abortion.⁵⁾ For example, the application of phorate reduced root and shoot growth and yield of tomato and pearl millet.^{6), 7)} Some organophosphate insecticides such as dimethoate and chlorpyrifos also caused phytotoxicity to several (non-target) weed species (i.e. annual forbs, *Poa* spp.), in particular by inhibiting or slowing weed seed germination.⁸⁾ Other studies, in contrast, found positive effects of insecticides through enhanced root development, plant growth, or physiological activity, sometimes resulting in increased yield.^{9), 10)} The application of carbofuran, a systemic carbamate, increased yields and biomass of several crops including watermelon, pea, tobacco and corn, because the metabolites of this molecule promoted crop growth and inhibited the activity of indole acetic acid oxidase.⁵⁾ Treatments with chlordimeform, a formamidine insecticide, and imidacloprid, a neonicotinoid insecticide had similar positive direct effects on cotton growth.^{11), 12)} Other molecules did not show any effect on tested crops, such as flonicamid applied to okra.¹³⁾ The specific effect, therefore, likely depends both on the insecticide class applied and the treated plant species.⁹⁾ This highlights the relevance of assessing these effects when using insecticides in insect-exclusion studies, especially when the experimental frequency of application is higher than when applied to crops.

We used three broad-spectrum insecticides in exclusion experiments in natural populations of the invasive common ragweed, *Ambrosia artemisiifolia* L. (Asteraceae), in northern Italy, to assess the potential impact of the accidentally established ragweed leaf beetle *Ophraella communa* L. (Chrysomelidae).¹⁴⁾ Common ragweed originates from central USA where it is a major weed of field crops.¹⁵⁾ It has become invasive on many other continents¹⁶⁾, causing crop yield reductions (overview for Europe in ¹⁷⁾) and producing allergenic pollen that have increased allergenicity-related symptoms in human populations.¹⁸⁾ *Ophraella communa* is a candidate biocontrol agent which has already proven successful in reducing common ragweed densities after mass-release in crop fields in Canada¹⁹⁾, and more recently in an inoculative approach in ruderal areas in China where the species had earlier

been accidentally introduced.²⁰⁻²²⁾ The beetle has also accidentally been introduced into Japan, where it feeds on several invasive species of *Ambrosia*.²³⁾ Assessments of its potential impact in Europe have been prioritized.²⁴⁾

It is unknown how insecticides affect common ragweed performance or the allergenicity of its pollen. The allergenicity of pollen of this species is known to be altered by other abiotic stressors, such as drought, aerial pollutants and elevated levels of CO₂, NO₂ and O₃.²⁵⁻²⁹⁾ We therefore specifically tested whether the application of the three selected broad-spectrum insecticides has direct effects on i) plant traits, ii) seed and pollen numbers, and iii) the allergenicity of pollen of common ragweed.

Materials and Methods

1. Selection of insecticides and application design

Since no insecticide is used specifically against our target species *O. communa*, we chose broad-spectrum pesticides widely applied in crops or horticulture. Neonicotinoids are systemic insecticides that have become the most used class of insecticides worldwide.^{30), 31)} Their translaminar activity acts as agonist on nAChRs opening cation channels inducing excitation of the neuronal membranes, causing paralyzes. We selected acetamiprid (Epik®, 5 g ai 100 g⁻¹, SIPCAM, via Sempione 195, 20016 Pero, Italy), which is one of the most used neonicotinoids and applied on about 60 crops. We applied this every four weeks, and due to its long-lasting activity in the plant, it hence provided continuous protection. Insects sequester the chemical by feeding on the plant, but since the substance is only toxic at high doses, it does not provide instantaneous control. Some studies have reported positive effects (increased plant height and flowering, enhanced seed vigor, and higher chlorophyll content) as well as negative effects (lower total soluble protein) on treated corn plants, while others found no effects on treated plants.³²⁾

To complement the systemic insecticide we selected two contact insecticides to instantaneously suppress high densities of insects that can occur in the field (e.g. through immigration of adults). Pyrethroids are synthetic analogous of pyrethrins that affect insect nerve fibers. This class of insecticides has been widely used since 1970 to control a wide spectrum of agricultural and public health pests, such as thrips, aphids, beetles, flies, mosquitos and beetles.^{30), 33)} We chose deltamethrin (Decis® Evo, 2.42 g ai 100 g⁻¹, Bayer CropScience Italia, Viale Certosa 130, 20156 Milan, Italy) and lambda-cyhalothrin (Karate® Zeon 1.5, 1.47 g ai 100 g⁻¹, Syngenta Italia, Via Gallarate 139, 20151 Milan, Italy), two of the most widely used pyrethroids.³⁰⁾ Deltamethrin was reported to have negative effects on the growth of maize compared to untreated plants³⁴⁾, but no such effects are known for lambda-cyhalothrin. As adults of the targeted biocontrol agent are highly mobile, they can easily move between ragweed populations. We therefore chose a biweekly (thus frequent) application of contact insecticides, but alternated the two chemicals to reduce the development of resistance.

2. Experimental design

Seeds were originally collected from about 30 plants from a natural population of common ragweed along the roadsides at the campus of DISAFA in Grugliasco, Italy in 2014, and were

stored under dry and cool conditions. In mid May 2016, pots with one litre of local silt-loam soil were sown with five of these seeds each in a greenhouse, and emerged seedlings were thinned to one seedling per pot after two weeks. The experiment commenced when seedlings had developed four true leaves and were ca. 5 cm tall. Half of a total of 72 undamaged potted seedlings was randomly picked and assigned to the control treatment, while the rest was assigned to the insecticide treatment. Pots were arranged in trays, such that each tray contained three pots of the same treatment (i.e. 12 trays * 3 plants per treatment). The 24 trays were then randomly placed in the centre of an insect-free experimental mesh cage of 2m by 2m by 2m outdoors at the same campus, and were surrounded by a line of potted common ragweed plants serving as a buffer (Supplemental Fig. S1). The maximum height and largest diameter of each experimental seedling was recorded prior to the treatment application.

All experimental plants were treated biweekly by spraying the trays with an equivalent of 1000 L ha⁻¹ outside the cage. In the insecticide treatment we alternated a mix of acetamiprid (100 g ai ha⁻¹) and deltamethrin (20 g ai ha⁻¹) with lambda-cyhalothrin (20 g ai ha⁻¹) only (doses corresponded to the maximum amount allowed in crops or horticultural products). The control plants were sprayed with an equal amount of water. To avoid microclimatic effects, we randomly changed the position of the trays within the cage after each application. A total of 6 applications was made.

At the end of August, when male flowers were flowering, we collected mature pollen in Eppendorf tubes by gently tapping the racemes (Supplemental Fig. S2). Because pollen of individual plants was often insufficient for analyses and could not always be collected separately, we pooled pollen from the three plants per tray (i.e. 12 tubes per treatment). To assess the allergenicity of the pollen samples, we evaluated the immunoreactivity of pollen extracts to a pool of sera from ragweed allergic patients³⁵) using a slot-blot technique. For each pollen sample, four subsamples of 5µl were taken, and their reactivity signal was determined with image analyses by the integrated optical density (IOD) of the immunoreactive spots relative to the IOD of a standard protein extract from commercial ragweed pollen (Allergon®, Allergon Thermo Fisher Scientific, Vålingevägen 309 SE-262 92 Ängelholm, Sweden).

Treatments continued until the plants had set seed in September. At that moment, we assessed individual plants for survival, senescence and damage, recorded again their maximum height and width, measured the total length of the racemes (a proxy for the amount of pollen produced), and counted individual seeds (including flowers and unripe seeds). We then harvested the above-ground biomass of each plant and determined its dry weight.

3. Statistics

We assessed the effect of treatment on log-transformed dry weight, number of seeds and total raceme length by linear mixed effect models using log-transformed initial volume of the seedling (calculated as $volume = height * \pi * (0.25 * width)^2$) as a covariate and tray as a random effect. For each response variable we created a set of models including all relevant combinations of treatment, seedling volume and their interaction, or no factor (the null-model) as fixed effects, and fitted them with Maximum Likelihood to allow model comparison. We then selected a subset of credible models based on the values of the conditional Akaike Information Criterion (AICc). We always selected the model with the

lowest AICc value (the "best" model), and subsequently included models with higher AIC values up to a difference of 6 compared to the best model ³⁶⁾ if these had fewer parameters (consistent with the principle of parsimony). This subset was used to create a weighted average model, whose factors were considered significant if the 95% confidence interval of their parameter estimates did not overlap zero. As the random effect of tray never explained a significant amount of variation, the proportion of variance explained by the treatment was then calculated by an Analysis of Variance (ANOVA) with treatment and seedling volume as fixed factors. To test differences in allergenicity between the treatments, we applied a t-test on the average IOD per pollen sample (average of the four subsamples), assuming unequal variance and applying a Welch modification. All statistical analyses were performed using R 3.3.1.³⁷⁾

Results

All 72 plants survived until after reproduction and showed no signs of feeding damage, but 12 of the 36 control plants from 6 different trays had already senesced at the time of the measurement and had dried out. All models of plant dry weight were regarded credible (Table 1), and the resulting average model (Table 2) revealed that none of the factors had a significant effect on plant dry weight. Overall there was large unexplained variance among the weights of individual plants (Fig. 1A), and treatment only explained 8.0% of all variation in the ANOVA. For both raceme length (Fig. 1B) and numbers of seeds (Fig. 1C), the null-model was best. Again most variation was found between individual plants and treatment only explained 1.3% and 1.2% in the corresponding ANOVAs, respectively. We found no significant difference in allergenicity between treatments (also not in a mixed model with treatment as fixed effect and subsample as random effect), even though the variation and mean seemed larger in the insecticide-treated samples (Fig. 2).

Table 1. The subset of credible models for plant dry weight with their degrees of freedom (df), ranked by their conditional Akaike Information Criterion (AICc), the difference with the AIC of the best model in this subset (Δ AICc) and their weight.

Fixed effects of models	df	AICc	ΔAICc	weight
treatment * log(seedling volume)	6	79.9	0.00	0.284
treatment + log(seedling volume)	5	80.2	0.24	0.252
treatment	4	80.3	0.33	0.241
log(seedling volume)	4	81.6	1.69	0.122
1	3	82.0	2.08	0.101

Table 2. The parameters of the average model for log-transformed plant dry weight, with their estimate, adjusted standard error, and 95% confidence interval of the estimates.

Parameter	Estimate	Adjusted SE	95% CI
(Intercept)	0.759	0.449	-0.121 - 1.639
log(seedling volume)	0.123	0.136	-0.143 - 0.389
treatment	0.405	0.488	-0.551 - 1.361
treatment : log(seedling volume)	-0.071	0.139	-0.342 - 0.201

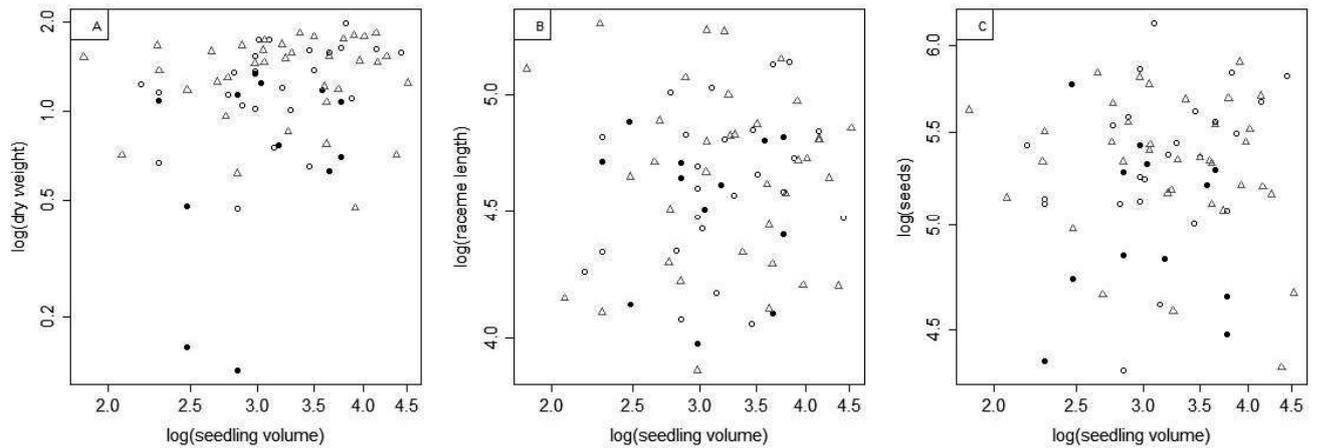


Figure 1. The effects of the insecticide treatment and seedling volume on the dry weight (A), raceme length (B), and number of seeds (C) of common ragweed, all at a log-log-scale. Circles represent plants of control treatments (open circles for vital plants, closed circles for those already senesced at the time of measurement), while triangles represent plants subjected to the insecticides treatment. No significant effects of treatment or seedling volume were found for any of the response variables.

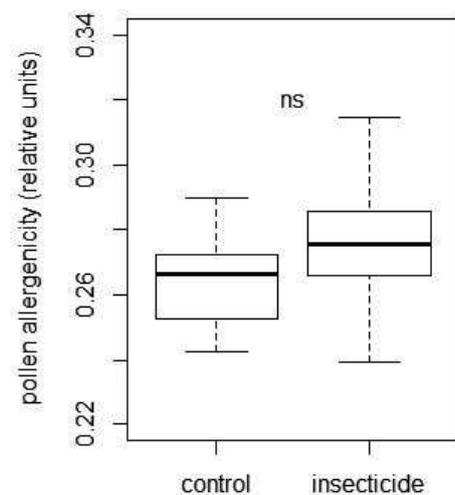


Figure 2. The effect of the insecticide treatment on common ragweed pollen allergenicity, assessed by a Slot-blot technique followed by image analysis of the optical density, relative to a standard. The “ns” indicates no significant difference between the means of the two treatments.

Discussion

Successful biological control of *A. artemisiifolia* reduces the production of seeds and pollen, without increasing allergenicity of the pollen. The application of our insecticide treatment, constituting the alternation of three different broad-spectrum compounds, resulted in negligible effects on plant dry weight, raceme length, seed production (Fig. 1), and pollen allergenicity (Fig. 2). This is due to the large amount of unexplained variation among individual plants. It seems typical of *A. artemisiifolia* that individual plant identity explains most of the variation in the traits of plants³⁸⁾ and pollen³⁹⁾ even under standardized conditions. This highlights the relevance of genetic, epigenetic, or maternal effects in determining the traits of plants and pollen in this species. *Ambrosia artemisiifolia* is known to have large intraspecific genetic variation, likely due to its reproductive system of wind-pollination and outcrossing¹⁵⁾, and the admixture of populations before their invasion.⁴⁰⁾ Under natural field conditions even more variation in phenotypic traits can be expected because of local environmental variation and the huge phenotypic plasticity of the species. It is, therefore, unlikely that insecticides will result in detectable direct effects in the investigated traits under natural conditions. In addition, effects of any successful biocontrol agent should be much larger. Therefore, our treatment can safely be applied to assess the impact of an insect agents when results are compared to controls not treated with insecticides.

One should take into consideration, however, that such broad-spectrum insecticides as used in our experiment eliminate the entire insect community and may therefore not reflect the effect of the biocontrol agent alone. In our case study this posed no problem since *O. communa* was always largely outnumbering the very few other insects sometimes found on the plants, and leaf-feeding damage resulting from other insects was never observed (Lommen, unpublished results).

We detected delayed plant senescence as a response to the insecticide treatment. This may be the result of slower or prolonged growth. The observation that the senesced plants tended to have smaller weights than still vital plants (Fig. 1A), renders the mechanism of prolonged growth more likely. These results imply that studies on plant phenology should utilize other methods of insect exclusion⁴⁾. For instance, when investigating if *O. communa* exerts a selection pressure on the timing of flowering of *A. artemisiifolia* in the wild, which has been found in the laboratory, the use of exclusion cages would be more appropriate.⁴¹⁾

Our study does not allow us to disentangle potential effects of the individual chemical compounds on *A. artemisiifolia*. In corn, acetamiprid has been reported to increase plant height in corn³²⁾, but deltamethrin had negative effects on this crop's growth.³⁴⁾ Unfortunately their joint effect on corn is unknown.

We acknowledge that our conclusions cannot be generalized to other study systems, and we advocate a case-by-case approach as long as general patterns regarding the direct effects of insecticides on plants remain unknown. The documentation of such case studies is important to standardize experimental applications across studies on the same system. This applies to investigating the efficacy of insects for weed biocontrol as well as to studying plant-insect interactions in other contexts⁴²⁾, allowing comparisons of effects found in different studies. The accumulation of such case studies, ideally replicated in time and space,

should aid in building up knowledge about the consistency of chemical effects across different molecules within classes of insecticides on different target plants.

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Supplemental material



Figure S1. The experimental design, with single seedlings in pots on trays. Experimental seedlings are in the 24 trays in the centre, while trays at the border serve as a buffer.



Figure S2. Collection of mature pollen (the yellow powder) from a raceme.

APPENDIX 6

Development and impact of *Ophraella communa* in Europe

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Contribution: Data collection, Analysis

Development and impact of *Ophraella communa* in Europe

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Abstract – The ragweed leaf beetle *Ophraella communa*, a potential biocontrol agent of invasive common ragweed *Ambrosia artemisiifolia*, that recently was discovered to have established in southern Switzerland and northern Italy. Combining field experiments with demographic modelling, we here provide the first evidence that this beetle has the potential to build up multiple generations with high densities on the Po plain, and to strongly reduce pollen and seed release of common ragweed in both the short and the long term in this European climate.

Key words: demographic model, field experiments, spatio-temporal variation, population growth

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Background

We present studies assessing the potential impact of the ragweed leaf beetle *Ophraella communa* on the invasive common ragweed, *Ambrosia artemisiifolia*, in Europe. The beetle is a potential biocontrol agent that was recently discovered to have established in southern Switzerland and northern Italy (Müller-Schärer et al., 2014). Although this beetle has good success in controlling ragweed in China (Huang et al., 2011, Zhou et al. 2014), prospective evaluation of its potential impact in Europe are lacking. Such studies are essential to decide upon the suitability of the candidate biocontrol agent for the target area (Morin et al., 2009). This accidental introduction provided the opportunity to test the beetle's development under European conditions in the field and its impacts on common ragweed.

Preliminary results

A cage experiment set up along an altitudinal gradient in northern Italy indicated that the multivoltine beetle can develop up to four generations in the growing season on the Po Plain, but less at higher altitudes with colder temperatures. At 1250 meters only a single new generation was completed. When monitoring 4 sites with natural ragweed and beetle populations on the Po Plain in 2016, we discovered that the leaf damage caused by the beetles at all sites dramatically increased within a few weeks in August, after 3-4 generations of the beetle have been produced. In this period, when the male flowers of common ragweed normally produce pollen, the beetle also inflicted damage to these structures, likely resulting in less pollen being released. Although the leaf damage was high at all sites, the mortality of the plants at seed set (before seed dispersal) varied a lot between sites. By killing plants at this stage, the beetle reduces the numbers of *Ambrosia* seeds being released. In the best case, *Ophraella* caused a 4-fold mortality rate compared to plants that had been kept free from the beetle. The maximum mortality rate found was over 95%. To assess the long-term impact of *Ophraella*, we constructed a deterministic demographic model of common ragweed and parameterised this with observations from 4 field sites in Ticino, Switzerland, and the Italian regions Piemonte and Lombardia. In two of these sites the beetle was experimentally excluded by applying insecticides on half of the study area. Data from 3 different years indicate strong year-to-year variation of the common ragweed dynamics and the impact of the beetle, resulting in highly different estimates of population growth rates. When projecting data from the most favourable year into the future, ragweed populations exposed to the beetle all showed a strong reduction of the population size. In the same year, in each of the two manipulated sites the beetle achieved a more than 10-fold decrease in population growth compared to the insecticide-treated parts (where populations were projected to grow). Altogether, this indicates that the beetle has varying success, but has the potential to build up multiple generations with high densities on the Po plain, and to strongly reduce pollen and seed release of common ragweed in both the short and the long term.

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APPENDIX 7

Taxonomic issues related to biological control prospects for the ragweed borer, *Epiblema strenuana* (Lepidoptera: Tortricidae)

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Contribution: Writing, ecological background and history of *E. strenuana* in biological control)



Taxonomic issues related to biological control prospects for the ragweed borer, *Epiblema strenuana* (Lepidoptera: Tortricidae)

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Abstract

The ragweed borer, *Epiblema strenuana* (Walker, 1863), has a long history of use as a biological control agent against important weed pests in the family Asteraceae. Recently, *E. strenuana* has been reported feeding on the invasive perennials *Ambrosia confertiflora* and *A. tenuifolia* in Israel. The geographic location of Israel has raised concern over the possibility that the moth may spread to areas such as Ethiopia where the oil-seed crop *Guizotia abyssinica* is cultivated, as this is a potential host for *E. strenuana*. However, the taxonomic status of *E. strenuana* and a current synonym, *E. minutana* (Kearfott, 1905) is unclear. These taxa have been treated as separate species in the past, and they potentially have different feeding habits and damage different parts of the plant. We analyzed DNA data and adult morphology and determined that *E. minutana*, **stat. rev.**, is a valid species which we raise from synonymy with *E. strenuana*. Wing coloration, the shape of the female sterigma, and COI DNA barcodes are consistently different between the two species. We also determined that the species previously identified as *E. strenuana* in Israel is actually *E. minutana*. While detailed host range tests have been conducted on the *E. strenuana* populations released in Australia and China, the host range of *E. minutana* remains to be clarified. We discuss the history of biological control using *E. strenuana* and the implications for finding *E. minutana* in Israel. We also provide species redescriptions for *E. strenuana* and *E. minutana* and illustrate diagnostic characters.

Introduction

The ragweed borer, *Epiblema strenuana* (Walker, 1863), has a long history of use as a biological control agent against important weed pests in the family Asteraceae. A native of North America, this species has been introduced to Australia to control *Parthenium hysterophorus* L. (McFayden 1985; McClay 1987) where it subsequently also attacked the invasive weed *Ambrosia artemisiifolia* L. (Dhileepan and McFayden 2012), and in China to control *A. artemisiifolia* (Wan *et al.* 1995; Zhou *et al.* 2014). The moth was also considered for field release as a biological control agent in India and South Africa, but it was rejected due to its ability to complete development on the oil-seed crop *Guizotia abyssinica* (L.f.) Cass. under laboratory conditions (Jayanth 1987; McConnachie 2015).

Recently, *E. strenuana* has been reported from Israel, possibly due to an accidental introduction with grain from the U.S.A. (Yaacoby and Seplyarsky 2011). In Israel, the moth has been found feeding on the invasive perennials *Ambrosia confertiflora* DC and *Ambrosia tenuifolia* Sprengel. The discovery of the moth in Israel has raised concern over the possibility that it may spread to areas where *G. abyssinica* is cultivated (e.g., Ethiopia). However, the taxonomic status of *E. strenuana* and a current synonym, *E. minutana* (Kearfott, 1905) is unclear. These taxa have been treated as separate species in the past (e.g., Brown 1973; Blanchard 1979), and they potentially have different feeding habits and damage different parts of the plant (Stegmaier 1971). Thus, the effectiveness of using *E. strenu-*

ana as a biological control agent and reducing the risk of non-host target feeding relies on the correct identification of this species and the taxonomic status of *E. minutana*.

Taxonomic history

The taxonomic history of *E. strenuana* and its synonyms, including *E. minutana*, is long and confusing. Walker (1863) described *Grapholita strenuana* and *G. exvagana* in the same publication from “North America.” Two years later, Clemens (1865) described *Steganoptycha flavocellana* from Virginia and probably Pennsylvania and noted differences in size and coloration between individuals. Zeller (1875) described *Grapholita subversana* from specimens collected by Boll in Texas and sent to him for identification from the Museum of Comparative Zoology. Fernald (1882) synonymized all of the aforementioned names under *Paedisca strenuana* in his catalogue of North American tortricids, and later (Fernald 1903) transferred *strenuana* to *Eucosma*. Heinrich (1923) was the first North American researcher to examine genitalia for nearly every tortricid species in his taxonomic revisions. Heinrich (1923) resurrected many of the genera synonymized by Walsingham (1914) under *Eucosma* and placed them in the subfamily Eucosminae. Included in this group of taxa was *Epiblema*, which Heinrich defined by the clasper on the valva in the male genitalia (Gilligan *et al.* 2014). Using this character, Heinrich (1923) assigned approximately 30 species, including *strenuana*, to *Epiblema*.

Eucosma minutana was described by Kearfott (1905) from “about forty specimens” from North Carolina, Ohio, Pennsylvania, Maryland, Illinois, Tennessee, and New Jersey. Kearfott (1905) stated that these specimens were mixed with *E. strenuana*, but were smaller and had differences in wing coloration and markings. He also noted that these species were likely not congeneric with *E. circulana*, the type species of *Eucosma*, and would eventually be placed in a different genus. Heinrich (1923) synonymized *E. minutana* with *E. strenuana*, stating that *E. strenuana* is one of the most variable species in the genus but that this variability could be found in the same series of reared specimens. He did note, however, that *E. minutana* specimens were the most distinct based on color. The two species remained synonyms until Blanchard (1979) compared a series of individuals he collected from North Padre Island, Texas with a series of Kearfott’s *E. minutana* types. Blanchard (1979) determined that *E. minutana* could be separated from *E. strenuana* by its smaller size and narrower wings, as well as differences in the female genitalia: shape of the sterigma, size of the signa, and shape of the corpus bursae. Based on this evidence, Blanchard (1979) elevated *E. minutana* to species status. He also noted that Brown (1973) had reached the same conclusions in his unpublished Master’s thesis several years prior. Brown (1973) cited the following differences as reasoning for separating the two species: the forewing color of *E. minutana* is lighter gray with brown scales absent, whereas *E. strenuana* is darker gray with lines of brown scales extending from the apical costal strigulae; the socii of the male genitalia are shorter in *E. minutana* than in *E. strenuana*; the female sterigma in *E. minutana* is laterally rounded with narrow lateral flanges and relatively shorter compared to that of *E. strenuana*, which has straight lateral margins, wide flanges that turn inwards, and is relatively longer; and the female corpus bursae of *E. strenuana* has two signa that are wide and laterally curved, whereas in *E. minutana* the signa are narrow and nearly straight. Brown (1973) also cited evidence from a rearing study conducted by Stegmaier (1971) on Lepidoptera, Diptera, and Hymenoptera associated with *Ambrosia artemisiifolia* in Florida. Stegmaier (1971) reared two tortricids, *E. strenuana* and “*Epiblema* sp. not *strenuana*,” and described differences in their feeding habits. *Epiblema strenuana* bored into the stem terminals, producing a fusiform swelling and infesting nearly every terminal, while the other species bored into the main stem and sometimes the lateral branches but did not infest the terminals or produce fusiform swelling (Stegmaier 1971). Specimens reared from the main stem were later identified as *E. minutana* by C. P. Kimball (Brown 1973).

Miller and Pogue (1984) attempted to resolve the taxonomic status of *E. minutana* using an allometric analysis. They conducted measurements of adult characters examined by previous authors (e.g., Blanchard 1979) and plotted these against insect size for 101 specimens collected from the eastern United States. Characters measured included: forewing length, forewing width, male valval neck width, male socius length, female signa base diameter, and the female sterigma length-width ratio (Miller and Pogue 1984). Forewing length was used to represent overall insect size. They concluded that all of the differences in measurements were effectively continuous and correlated with forewing length, and thus overall size (Miller and Pogue 1984). In addition, they hypothesized that the differences in feeding observed by Stegmaier (1971) could be explained by variance in host tissue nutritive value or chemical characteristics. Their final conclusion was that these findings may warrant returning *E. minutana* to synonymy with

E. strenuana (Miller and Pogue 1984). However, Powell (1983) had already synonymized the two species in the new North American checklist, although he provided no explanation for this change. The two species were treated as the *E. strenuana* “complex” by Miller (1987) and Gilligan *et al.* (2008), and listed as synonyms in subsequent catalogues (Brown 2005; Gilligan *et al.* 2018) and checklists (Pohl *et al.* 2018).

History of biological control

During field surveys in the native range of *Parthenium hysterophorus* in the late 1970s, *E. strenuana* was prioritized as a biological control candidate for this weed in Australia (McClay 1987). Based on the results of host range tests conducted in its native range and in Australia, the moth was approved for field release in 1982 and subsequently released in Queensland (McFadyen 1985; McClay 1987). It became established, spread over Queensland and New South Wales, and is now considered one of the most prominent biological control agents of *P. hysterophorus* in Australia (Dhileepan and McFayden 2012), thereby improving rangeland productivity (Dhileepan 2007). In its introduced range in Australia, *E. strenuana* also attacks the invasive weeds *A. artemisiifolia* L. and *Xanthium occidentale* Bertol. *Ambrosia artemisiifolia* is currently considered to be under good control in Queensland and New South Wales, with *E. strenuana* feeding cited as a major factor for this success (Gerber *et al.* 2011).

The Australian *E. strenuana* population served as source for introductions to China in 1990–1993 (Wan *et al.* 1995; Ma *et al.* 2008). Subsequent host range tests revealed that *E. strenuana* can complete its life cycle on a local variety of sunflower in no-choice conditions; however, the risk of economic damage to sunflowers was deemed to be low (Wan *et al.* 1995). The moth is now established in South, East, Central, and North China, without reports of sunflower yield losses due to *E. strenuana* (Ma *et al.* 2008). In addition to *A. artemisiifolia* and *P. hysterophorus*, *E. strenuana* also feeds on *Xanthium strumarium* L. and *Ambrosia trifida* L. in China. Efforts to control *A. artemisiifolia* in China by biological means is built on synergistic feeding of the leaf beetle *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) and *E. strenuana*, and mass-rearing programs for both species are now in place to increase their impact on this noxious weed (Zhou *et al.* 2014).

While *E. strenuana* is successfully used as a biological control agent on two different continents against two different target weeds, its relatively broad host range has led to the rejection of its release in India and South Africa. In both cases, the reason for concern was its capacity to feed on *G. abyssinica*. This plant, originating from the Ethiopian highlands, is grown as an oil crop in eastern Africa and India. Laboratory host range tests indicated that *E. strenuana* will attack this plant in the field, which led to the rejection of its release in India in 1987 (Jayanth 1987). In South Africa, *G. abyssinica* is not cultivated on a commercial scale; however, the possibility of the moth spreading to eastern Africa eventually led to concerns about its host range. This led to no-choice experiments of *E. strenuana* on various Ethiopian cultivars of *G. abyssinica*, and initial larval feeding severely damaged all tested cultivars. As a matter of responsibility, *E. strenuana* was deprioritized as a potential biological control agent of *P. hysterophorus* in South Africa (McConnachie, 2015).

Summary

Although Miller and Pogue (1984) provided a convincing case for a continuous gradient of morphological characters between *E. strenuana* and *E. minutana*, there do seem to be diagnosable differences (e.g., wing coloration) in the majority of specimens that may indicate these are different species. Molecular data, including DNA barcoding (Hebert *et al.* 2003), has been used successfully to resolve taxonomic issues where morphological characters are ambiguous and to determine which morphological characters are taxonomically informative (e.g., Brown *et al.* 2014, Gilligan *et al.* 2014, Gilligan *et al.* 2016). Here we use DNA barcoding combined with morphology and host preference to examine populations of the *E. strenuana* complex from North America, Australia, China, and Israel. We revise the taxonomy of this group and provide recommendations regarding the use of *E. strenuana* as a biological control agent.

Materials and Methods

We examined 123 adult specimens together with 43 associated genitalia preparations deposited in the following col-

lections: The Natural History Museum, London, United Kingdom (BMNH); Essig Museum of Entomology, University of California, Berkeley, California, U.S.A. (EME); Florida State Collection of Arthropods, Gainesville, Florida, U.S.A. (FSCA); Mississippi Entomological Museum, Mississippi State University, Starkville, Mississippi, U.S.A. (MEM); and National Museum of Natural History, Washington, D.C., U.S.A. (USNM). Specimens from populations in Australia, China, and Israel were obtained from Dr. Kunjithapatham Dhileepan (Australia), Dr. Zhongshi Zhou (China), and Nadav Nussbaum (Israel), and sent to US and TMG in ethanol.

Images of adults were taken with Canon 100 mm and MP-E 65 mm macro lenses attached to a Canon 7D digital SLR. Images of genitalia were taken with a Nikon DS-Fi2 digital microscope camera attached to a Nikon Eclipse 80i compound microscope. All images were edited using Photoshop CS6 or CC, and some are composite stacks of many individual images created with Zerene Stacker. Forewing length (FWL) is defined as the distance from the base to the apex including the fringe, reported to the nearest half millimeter. The forewing aspect ratio (AR) is defined as the forewing length divided by the medial forewing width. Measurements were made with a stereomicroscope equipped with an ocular micrometer or a compound microscope using a slide micrometer. The number of observations supporting a particular statistic is indicated by “n =.” Dissection methods follow those presented in Brown and Powell (1991), and morphological nomenclature follows Horak (2006) and Gilligan *et al.* (2008, 2014).

Sequences generated for this study were produced using the following methods: DNA was extracted from crushed legs or abdomens soaked overnight in lysis buffer and proteinase K using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, Calif.) following the manufacturer’s recommended protocol. PCR reactions were performed with TaKaRa Ex Taq HS polymerase (Takara Bio, Shiga, Japan) in total volumes of 50 μ l using the manufacturer’s recommended volumes of 10X Ex Taq buffer and dNTP mixture. The primers LepF1/LepR1 (Hebert *et al.* 2004) were used to amplify a 658bp segment of cytochrome c oxidase I (COI) on a Bio-Rad C1000 Touch (Bio-Rad Laboratories, Inc., Hercules, Calif.). PCR conditions included an initial denaturation step of 94°C (3min), 39 cycles of 94°C (20 sec)/ 50°C (20 sec)/ 72°C (30 sec), and an extension step of 72°C (5 min). Amplicons were purified using a Qiaquick PCR Purification Kit and eluted into 35 μ l of EB buffer. Sequencing was performed at the University of Chicago Cancer Research Center DNA Sequencing Facility using an Applied Biosystems 3730XL DNA sequencer (Applied Biosystems, Foster City, California). Individual forward and reverse contigs were assembled using Geneious Prime 2019 (Biomatters Ltd., Auckland, New Zealand), manually trimmed, and examined for errors.

An additional 55 publically available sequences for *Epiblema* that we could confidently assign to the *E. strenuana* complex were downloaded from the Barcode of Life Data System website (BOLD; Ratnasingham and Hebert 2007). These were combined with 24 newly generated sequences and two sequences of *E. foenella* from BOLD that were used to root the tree. All sequences (81 total) were aligned with MAFFT ver. 6 using the G-INS-i algorithm (Katoh *et al.* 2002). A maximum likelihood analysis was performed using Garli ver. 2.0 (Zwickl 2006) and the GTR + gamma nucleotide substitution model. Optimal likelihood trees were obtained using 1,000 independent searches.

Results and Discussion

The most optimal likelihood tree constructed with the DNA barcoding data is illustrated in Figure 1. Sequences from the *E. strenuana* complex fall into two primary clades (Group 1 and Group 2). Group 1 contains sequences from specimens originating from Australia, Canada (Ontario, Québec), China, Jamaica, Taiwan, and the United States (Alabama, Connecticut, Illinois, Maryland, Ohio). Group 2 contains sequences from specimens originating from Canada (Ontario, Québec), Israel, and the United States (California, Connecticut, Florida, Illinois, Kentucky, Mississippi, Ohio).

Adult specimens represented by sequences in Group 1 (Figs. 10–16) are primarily brown with a variably expressed interfascial spot that ranges in color from white to bronze. The paired costal strigulae on the distal one-half of the wing are whitish and usually inconspicuous (except for strigula 9) in most individuals. The associated gray striae extend toward the termen and are usually separated by lines of orange-brown scales. The male genitalia (Fig. 19) have socii that are long and fingerlike with lateral margins that are nearly parallel. In the female genitalia (Figs. 24–26), the sterigma is elongate and rectangular. Overall, the specimens in Group 1 are consistent with the type of *E. strenuana* (Fig. 10). Adult specimens represented by sequences in Group 2 (Figs. 2–9) are primarily gray to dark gray. The interfascial spot, when expressed, is pale gray. The white costal strigulae are usually well expressed and more prominent than in Group 1, and the orange-brown lines between the striae are absent. The male genitalia (Figs. 17–18) have socii that are generally shorter than in Group 1, and in some specimens the socii are nearly triangular.

In the females of Group 2 (Figs. 20–23), the sterigma is ovate and not as elongate as in Group 1. The specimens in Group 2 are consistent with the type of *E. minutana* (Fig. 2).

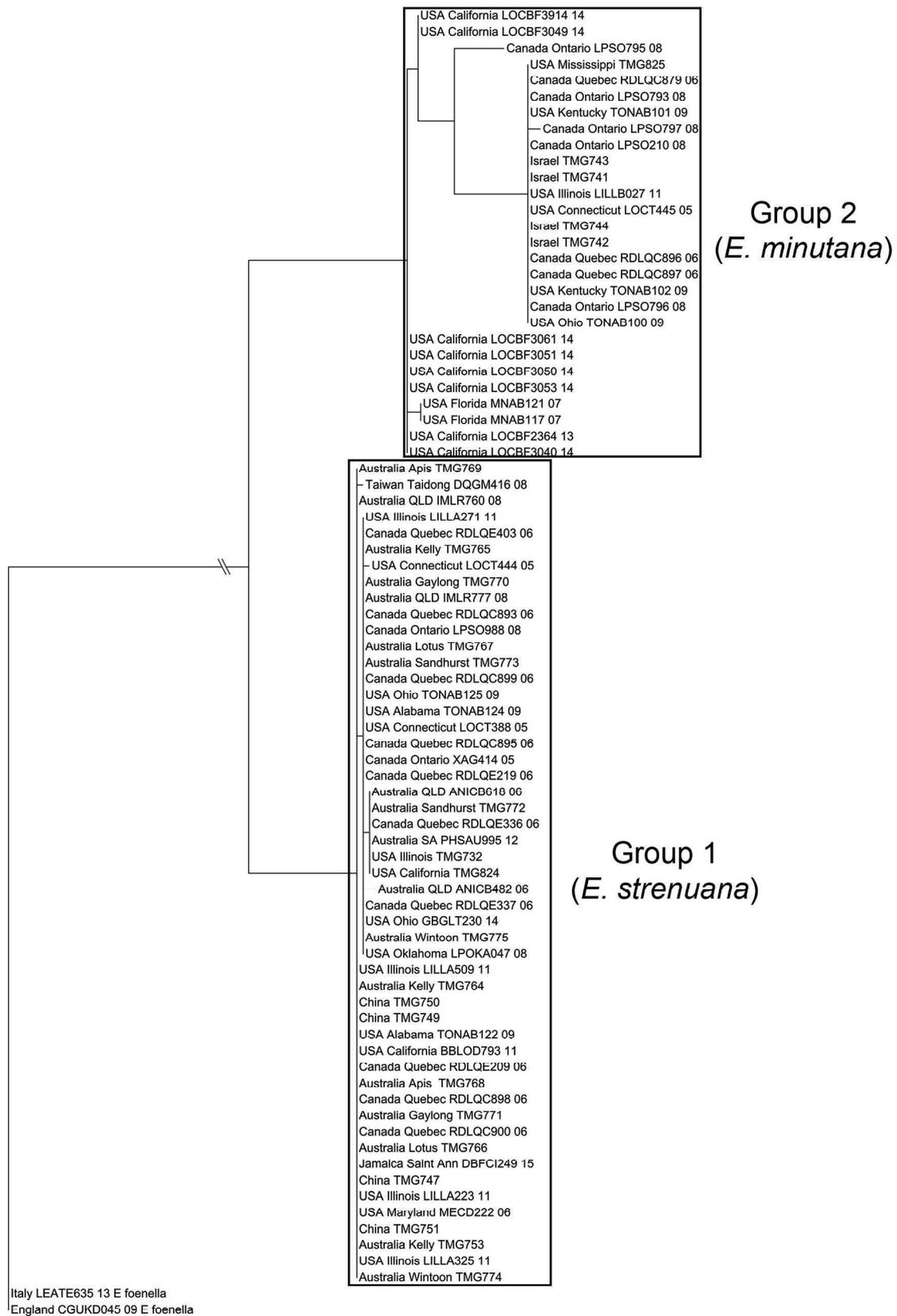


FIGURE 1. Maximum likelihood tree of DNA barcode data analyzed using the GTR + gamma nucleotide substitution model.

Based on the DNA data and differences in morphology, we believe that *E. strenuana* can be reliably separated from *E. minutana*. Wing coloration and the shape of the female sterigma are consistently different between the two species. *Epiblema strenuana* is brown with most costal strigulae inconspicuous and orange-brown scales near the apex of the forewing. *Epiblema minutana* is gray to dark gray with conspicuous white costal strigulae and a lack of orange-brown scales on the forewing. The female sterigma of *E. strenuana* is more elongate and rectangular than in *E. minutana*, where the sterigma is often ovate. There are some female specimens of *E. minutana* in which the sterigma is somewhat elongate, but the ostium is never twice as long as it is wide. In males of *E. strenuana*, the socii are long and fingerlike with parallel sides. In *E. minutana*, the socii can vary from fingerlike with parallel sides to short and triangular. In general, it appears that the male socii are less reliable than the female sterigma for separating the two species compared to the female sterigma. In all cases, genitalia characters should be combined with wing coloration to arrive at a final determination.

Other characters suggested by previous authors (size, female signa size and shape, etc.) are too variable to differentiate *E. strenuana* and *E. minutana*. Forewing length overlaps substantially between the two species (*E. strenuana* FWL: 4.0–9.0 mm; *E. minutana* FWL: 4.3–7.9 mm), although on average *E. strenuana* is slightly larger (mean FWL: 7.1 mm vs. *E. minutana* mean FWL: 6.0 mm). This size range is too close to separate any individual specimen, and we have observed large *E. minutana* collected in similar locations with small *E. strenuana*. Other characters, such as the female signa, also vary greatly in shape and size, and there is no consistent pattern to reliably separate the two species.

Although our analysis of DNA barcode data resulted in two clades representing two species, there is a substantial amount of variation in the *E. minutana* group. Sequences representing specimens from Florida and California clustered somewhat separately from the rest of the *E. minutana* sequences, hinting at the possibility that these sequences could represent a different taxon. We were not able to discern any consistent morphological differences in these specimens from other specimens of *E. minutana*, and thus assume these are simply population-level genetic differences. In the future, it is possible that more extensive DNA-based studies could reveal that the *E. minutana* clade represents a species complex; however, we have no current evidence to support this conclusion.

It is evident from this study that the species previously identified as *E. strenuana* in Israel (e.g., Yaacoby and Seplyarsky, 2011) is actually *E. minutana*. While detailed host range tests have been conducted with the *E. strenuana* populations released in Australia and China, the host range of *E. minutana* remains to be clarified. Interestingly, in his paper on the host specificity of *E. strenuana*, McClay (1987) stated that *E. minutana* attacks *A. confertiflora* in the native range in Mexico, but that “the inclusion or exclusion of *E. minutana* does not affect the recorded host range of *E. strenuana*.” Elucidating the host specificity of the population recently established in Israel is of particular relevance, both with regard to potential non-target risks and potential benefits. Israel’s native flora is rich in asteraceous species, and since the host range of *E. minutana* has not been studied, non-target attack of native species cannot be entirely excluded. In terms of the potential beneficial effects, several alien Asteraceae species are invasive in Israel, including *Xanthium* spp., *Verbesina enceloides* Cav., *P. hysterophorus* and several *Ambrosia* spp. (Danin, 2000). Until now, *E. minutana* in Israel has only been reported from *A. confertiflora* and *A. tenuifolia* (Yaacoby and Seplyarsky, 2011). It is of high economic and ecological interest whether *E. minutana* also attacks other asteraceous weeds, especially *P. hysterophorus* since this is a famously aggressive invasive weed (Adkins and Shabbir, 2014). In case *E. minutana* does not attack *P. hysterophorus* in Israel, it would be sensible to search for other biological control agents.

Species redescriptions

Epiblema strenuana (Walker, 1863)

(Figs. 10–16, 19, 24–26)

Grapholita strenuana Walker 1863:383.

Grapholita exvagana Walker 1863:383.

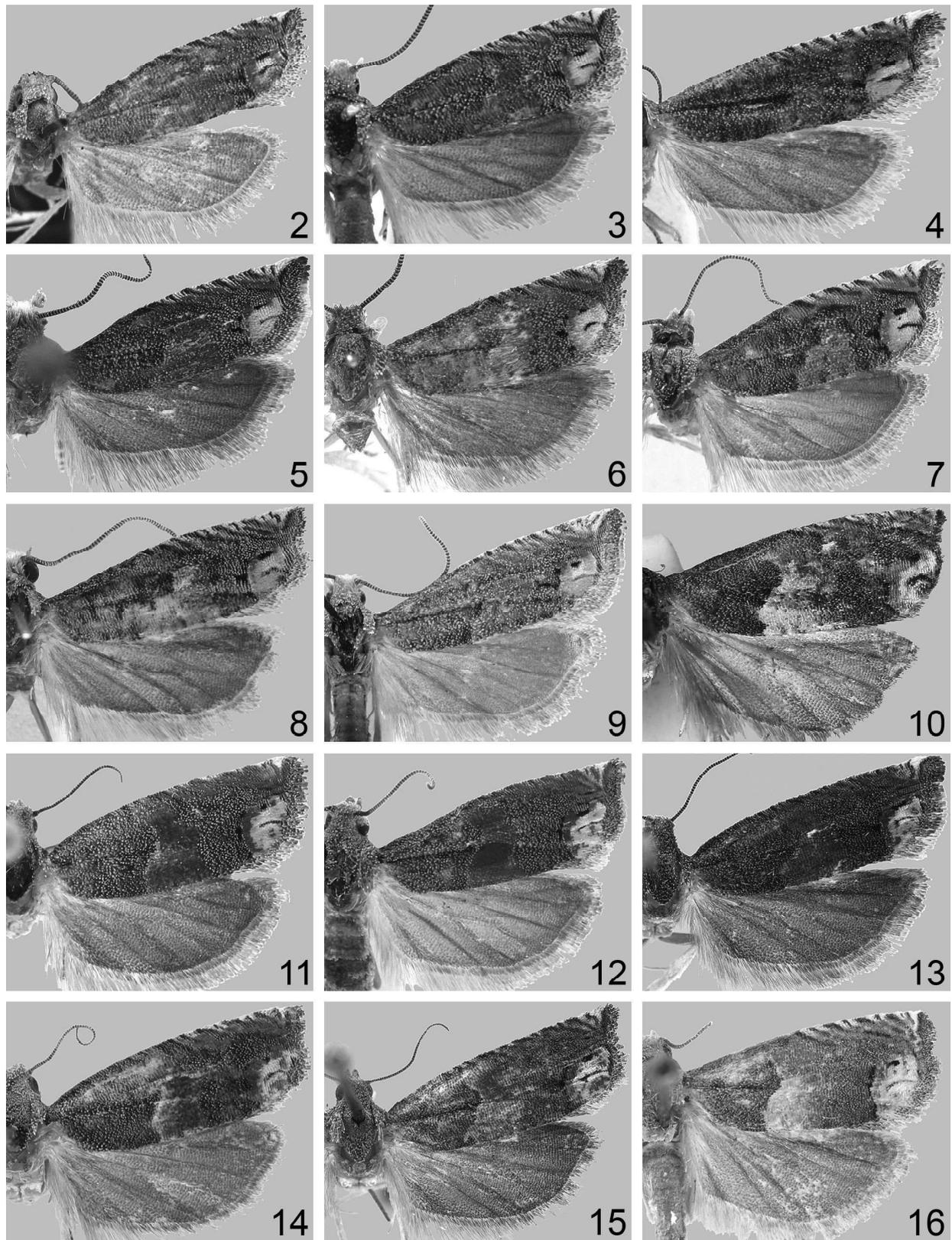
Steganoptycha flavocellana Clemens 1865:238.

Grapholita subversana Zeller 1875:318.

Paedisca strenuana: Walsingham 1879:52; Fernald 1882:40; Walsingham 1884:140.

Eucosma strenuana: Fernald 1903:458; Barnes and McDunnough 1917:170.

Epiblema strenuana: Heinrich 1923:140, figs. 257, 258; McDunnough 1939:48; Darlington 1947:95; Miller 1972:214; Powell 1983:35; Miller and Pogue 1984:227; Miller 1987:56; Brown 2005:286; Gilligan *et al.* 2008:121; Horak 2008:310; Powell and Opler 2009:135; Pohl *et al.* 2018:151.



FIGURES 2–16. Adults. 2–9, *E. minutana* (2, New Jersey, lectotype; 3, Mississippi, DJW8714; 4, California, DJW8655; 5, Kentucky, TONAB101-09; 6, Ohio, USNM152255; 7, Ohio, DJW8712; 8, Florida, DJW8739; 9, California, DJW8666). 10–16, *E. strenuana* (10, North America, lectotype; 11, Alabama, DJW8703; 12, Texas, DJW8667; 13, Ohio, USNM152288; 14, Alabama, DJW8704; 15, New Mexico, USNM152289; 16, California, DJW8683).

Types. *Grapholita strenuana*. Lectotype (designated by Miller and Pogue 1984) (Fig. 10). ♂, North America, Carter Collection, BMNH(E) 819923, slide 5737, BMNH. *Grapholita exvagana*. Lectotype (designated by Miller and Pogue 1984). North America, Carter Collection, BMNH(E) 819924, BMNH [hindwings and abdomen missing]. *Steganoptycha flavocellana*. Lectotype (designated by Darlington 1947). ♂, Type No. 7214, ANSP [abdomen missing]. *Grapholita subversana*. Syntypes? Texas, Boll, MCZ [Brown 2005 lists these types as lost].

Both *G. strenuana* and *G. exvagana* seem to have been described from specimens with a moderately well-expressed white interfascial spot; Miller and Pogue (1984) designated the same specimens that N. S. Obraztsov provisionally selected as lectotypes. The number of specimens supporting the description of *S. flavocellana* is unknown. Darlington (1947) attributed the lectotype designation to Heinrich (1923), but Heinrich did not provide enough information to designate a single specimen. Miller's (1973) image of *S. flavocellana* shows the interfascial spot to be obsolete. Zeller (1875) mentioned three or five syntypes in his description: "Texas (Boll). Massachusetts at Beverly (Burgess) where two ♀ on 27 June and 3 July were caught. One ♂, one ♀ in Museum Cambridge, one ♀ in my collection." Regardless of the exact number, Miller and Hodges (1990) did not report any types in the MCZ and Brown (2005) listed the types as lost. All of the above synonymies date to Fernald (1882).

Redescription. *Epiblema strenuana* is a brownish to grayish species of variable size (FWL: 4.0–9.0 mm, mean = 7.1) and average forewing geometry (AR = 2.80). The interfascial spot, which extends from the inner margin to the radius, varies from whitish (Figs. 10, 15–16) to shades of bronze or gray (Figs. 11–14), in the latter case being weakly distinguishable from the subbasal and median fasciae by its lack of white-tipped scales. Its proximal margin is often indicated by a thin line of pale scales (e.g., Fig. 14). The ocellus is white and conspicuous, with a central black longitudinal dash, a black mark on the costal margin, a narrow gray band on the proximal margin, and a black line along the basal edge of the gray band, the last often fragmented into two or three segments. The paired costal strigulae on the distal one-half of the wing are white to gray, usually inconspicuous (except for strigula 9), with associated gray striae extending toward the termen, the last usually separated by lines of orange-brown scales. The specimen in Fig. 16 is representative of a few specimens from southern California that have unusually well expressed costal strigulae.

The male genitalia (Fig. 19) are distinguished by long fingerlike socii, whose lateral margins are nearly parallel. In females, the sterigma (Figs. 24–26) is rectangular and elongate (length about 2 times ostium diameter), the posterior margin of sternum 7 is semicircular and diverges laterally from the sterigma, and the ductus bursae has a twist-like sclerotized contortion near the juncture with the ductus seminalis.

Remarks. Heinrich (1923) stated that the larva is a stem borer on *Ambrosia artimisiifolia* L. (annual ragweed) without providing the source of that information. Stegmaier (1971) reported rearing Florida specimens from larvae feeding in fusiform galls in the lateral branches of *A. artimisiifolia*. He also reported rearing a similar but unknown species of *Epiblema*, later determined by Miller and Pogue (1984) as *E. minutana*, from larvae boring in stems of *A. artimisiifolia*. Other larval hosts have been mentioned in the literature, including *Parthenium hysterophorus* L. (Santa Maria feverfew) (McClay 1987), *Xanthium* (cocklebur) (Miller 1987; Powell and Opler 2009), and *Chenopodium* (goosefoot) (Miller 1987), but these records need to be verified. *Epiblema strenuana* has been used as a biological control agent for a variety of invasive weeds; those species are listed elsewhere in this paper.

This species is broadly distributed over the North American continent. We suspect the distribution of the moth mimics that of its hosts, *Ambrosia* spp. *Xanthium* spp., and *P. hysterophorus* (Hilgendorf and Goeden 1983; McClay 1987). Of course, many of the literature records are uncertain due to the long-standing confusion regarding *E. strenuana* and *E. minutana*. We examined specimens from 14 states in the region extending from Minnesota to Texas, west to Colorado and New Mexico, east to Maryland and Florida, and several specimens from southern California. In the Midwest, *E. strenuana* has two primary flights, one in late June, and the other from mid-August to mid-September.

***Epiblema minutana* (Kearfott, 1905), revised status**

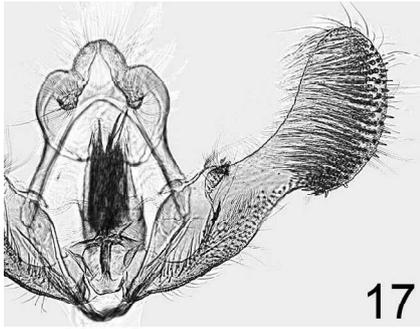
(Figs. 2–9, 17–18, 20–23)

Eucosma minutana Kearfott 1905:356; Barnes and McDunnough 1917:170.

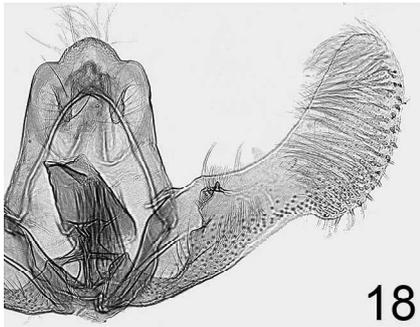
Epiblema minutana: Blanchard 1979:179; Miller and Pogue 1984:227.

Eucosma antaxia Meyrick 1920:344, unnecessary replacement name for *minutana*.

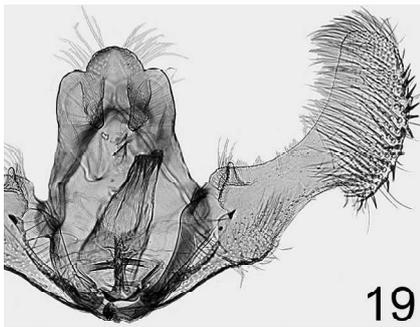
Epiblema strenuana (not Walker): Heinrich 1923:140; McDunnough 1939:48; Powell 1983:35; Miller 1987:56; Brown 2005:286; Gilligan *et al.* 2008:121; Pohl *et al.* 2018:151, senior synonym of *E. minutana*.



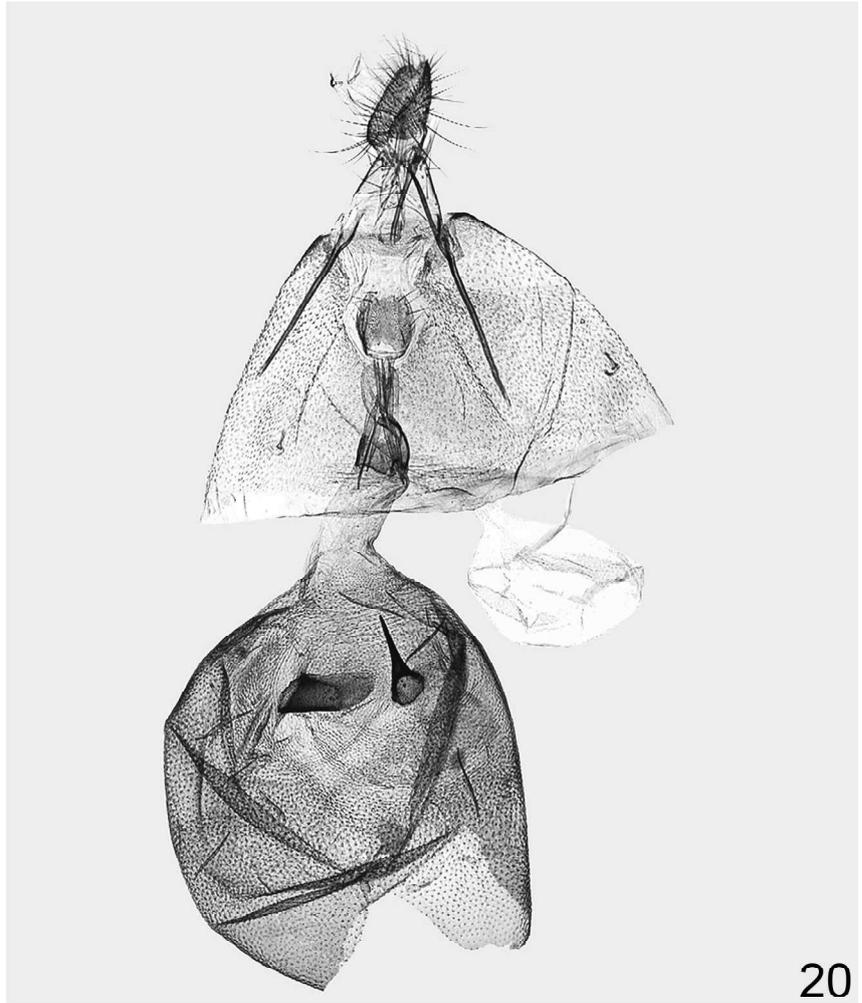
17



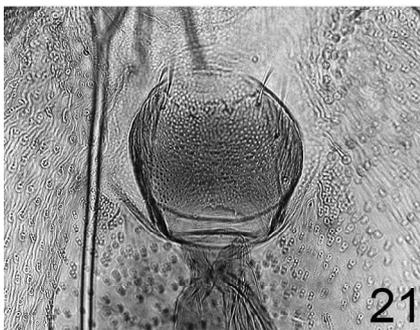
18



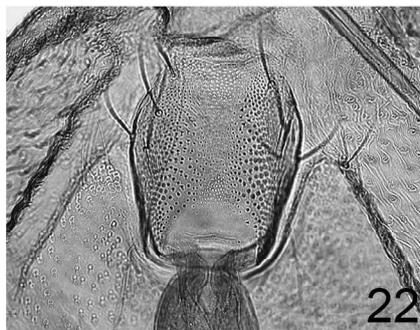
19



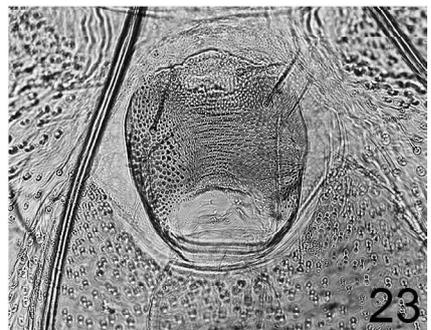
20



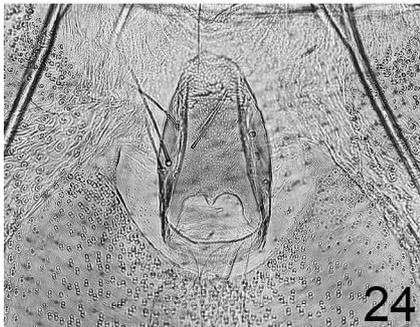
21



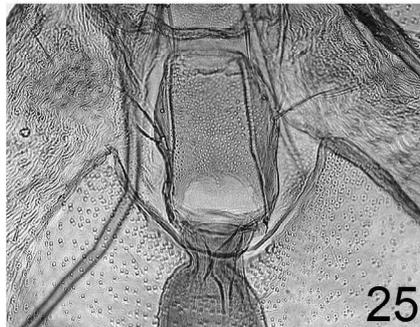
22



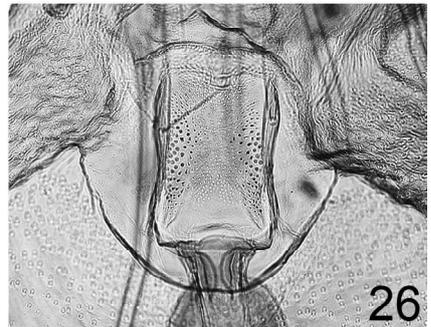
23



24



25



26

FIGURES 17–26. Genitalia. 17–18, *E. minutana* (17, Ohio, TMG782; 18, Ohio, USNM152256). 19, *E. strenuana* (Iowa, USNM152286). 20–23, *E. minutana* (20, Kentucky, TMG781; 21, New Mexico, USNM152260; 22, Kansas, USNM152258; 23, Israel, TMG784). 24–26, *E. strenuana* (24, TMG778; 25, Kansas, USNM152287; 26, Ohio, USNM152288).

Lectotype (designated by Blanchard 1979). ♂, New Jersey, Essex County, Montclair, W. D. Kearfott, July 1908, slide 24505, USNM.

Kearfott (1905) mentioned a series of about 40 specimens from Tryon, North Carolina; Cincinnati, Ohio; New Brighton, Pennsylvania; Plummers Island, Maryland; Belvidere, Illinois; Smith County, Tennessee; Anglesea, New Jersey; and Essex County Park, New Jersey. Klots (1942) stated that Heinrich (1923) had designated a lectotype from Essex County Park, New Jersey but disagreed with Heinrich's statement that the depository was the AMNH. Blanchard (1979) settled the matter by formally designating the lectotype listed above in the USNM.

Redescription. *Epiblema minutana* is a dark gray species that lacks any subcostal orange-brown coloration near the apex of the forewing. In size it varies (FWL: 4.3–7.9 mm) much like *E. strenuana* (FWL: 4.0–9.0 mm) but on average it is somewhat smaller than the latter species (mean FWL = 6.0 vs. 7.1 mm). Previous authors have noted, and we concur, that the forewing is slightly narrower in *E. minutana* than in *E. strenuana* (AR = 3.19 vs. 2.80). The interfascial spot is often present as a paler shade of gray (Figs. 5–8), but in some individuals it is barely discernable (Figs. 2–4). The ocellus resembles that of *E. strenuana*, but the white costal strigulae are usually more prominent than in the latter species.

Epiblema minutana is similar to *E. strenuana* in genitalia but differs from the latter species in the following respects: the socii (Figs. 17–18) are shorter and sometimes triangular (tapering from a broad base to a narrowly rounded apex vs. consistently fingerlike with parallel lateral margins), and the sterigma (Figs. 20–23) is ovate instead of rectangular, with length-ostium diameter = 1.56 vs. 1.95. Shape of the socii can vary and appear similar to those in *E. strenuana*. Genitalic characters should be used in combination with wing coloration to make a species-level identification.

Remarks. The typical phenotype of *E. minutana* (Figs. 2–8) is broadly distributed in eastern United States and is also found in central California. In the East, the larval host is presumed to be *Ambrosia artimisiifolia* L. (annual ragweed), but the adult determinations in literature reports of reared specimens need to be checked for accuracy vis-à-vis *E. strenuana*. *Epiblema minutana* has been reared in Mexico from field-collected larvae from *A. confertiflora* (McClay 1987) and in Contra Costa County, California, by J. A. Powell from *Ambrosia psilostachya* DC. (Cuman ragweed), a plant with a transcontinental distribution. The EME has numerous specimens from California with *minutana*-like genitalia and pale gray forewings (Fig. 9). They are somewhat larger than typical *E. minutana* (mean FWL = 6.9 vs. 6.0 mm) but are nearly identical in forewing geometry (AR = 3.18 vs. 3.19). This phenotype has been reared from *A. psilostachya* and from *Ambrosia chamissonis* (Less.) Greene (silver bur ragweed) (Powell and Opler 2009). The range of the latter plant extends along the Pacific coast from southern California to Alaska. Powell and Opler (2009) reported larvae causing deformities at nodes of lateral decumbent stems but not forming stem galls. Similar specimens are represented in the phylogenetic tree (Fig. 1) from San Diego County, California. These cluster with typical *E. minutana* but show some minor consistent differences in the sequences that could indicate they are a separate taxon. We can find no morphological differences in these California specimens, thus we tentatively refer them to *E. minutana* until a more comprehensive DNA analysis can be performed. We can confirm they are not *E. strenuana*, which is also present in California, and we have examined typical specimens of *E. strenuana* from the same location in San Diego County.

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What a ride it has been, people.

EHRENWÖRTLICHE ERKLÄRUNG

Hiermit bestätige ich,

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Benno Augustinus



CURRICULUM VITAE BENNO ANDREAS AUGUSTINUS

Summary

I am an enthusiastic, energetic, social, and determined person and good at thinking in possibilities. My academic education focusses on bio-interactions and biological control of insects and weeds. I am particularly interested in demographic modelling, and predicting density-dependent impact of insects on their hosts. My long-term goal is to include my Entomological background to aid improving sustainable agricultural practices.

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03/2015- current

Supervisors: Urs Schaffner, Head 'Ecosystem Management' section, CABI Europe-Switzerland, Professor Heinz Müller-Schärer, Department of Biology, University of Fribourg

BSc/MSc Biology, bio-interactions 9/2006-8/2014

Minor biological control of insects

Wageningen University and Research Centre, the Netherlands

Master thesis: '*Plant mediated effects of *Pieris rapae* egg deposition on performance and behavior of *Pieris brassicae* larvae*'

Performance and behavior studies with two different cabbage white species in greenhouse conditions. Gained deeper insights in bio-interactions, learned to adapt to a fluctuating experimental species population.

Bachelor thesis: '*Phloem mobility of the Nr-gene resistance factor of lettuce (*Lactuca sativa longifolia*) against the lettuce aphid, *Nasonovia ribisnigri**'

Performance and behavior studies using electrical penetration graph techniques. Learned to use complicated techniques to investigate resistance mechanisms

Abitur Biology, English, Health, 07/2006
IGSM Rockenhausen, Germany

Professional Experience

Internship Koppert España 11/2013-05/2014

'Aphid control with generalist predators in sweet pepper'

Laboratory and Semi-field experiments to estimate fitness of predators and their effect on prey populations. Gained hands-on experience in IPM, handled big datasets and worked in an international environment.

Production Assistant pharmacy Spits Tiel

06/2010-08/2010, 06/2009-11/2009, 11/2014-01/2015

Warehouse employee DPD Veenendaal 12/2012-11/2013

Sales representative bike shop 2Wielercentrum 01/2011-07/2012

Approved Research Projects and Grants

Jean and Blurette Nordmann Foundation scholarship 03/2019-08/2019

'Exploring demography and host range of *Epiblema strenuana*, a potential biological control agent of *Ambrosia artemisiifolia* in Europe'

6 month grant for Research stay at the Hebrew University of Jerusalem

Congress Grant NCCR 11-14/11/2018

To finance the visit to the 2018 ESA, ESC and ESBC Joint Annual meeting in Vancouver, BC, Canada

Travel Grant NCCR 13/07-03/08/2018

Reimbursement to visit and establish collaboration with Bar Ilan University, IL and the Hebrew University of Jerusalem, IL

Short Term Scientific Mission EU COST-Action 'Smarter' 15-27/04/2015

Research Visit at the Radboud University Nijmegen

Short Term Scientific Mission EU COST-Action 'Smarter' 30/9-15/10/2016

Field work on host range and occurrence of *Ophraella communa* in Northern Italy

Supervised students

Anne-Marthe Tournet: Research internship for the BSc at the Applied University 'HAS hogeschool Venlo', NL, at the University of Fribourg, 03-07/2017

Carine Beuchet: BSc thesis spring semester 2017

Personal Skills

Languages

-German: native
-Dutch: native
-English: C2
-French: B1
-Italian: A2
-Spanish: A1

Skills

R, Microsoft Office, Endnote
electrical penetration graph, life cycle parameter estimation, semi-field work, field work, qPCR, PCR drivers' license
motivator, networker, determined, dedicated

Interests

Sports: cycle-tourism, mountainbiking, hiking, trekking
Music: intensive education on the violoncello, chamber music, orchestra's, solo pieces
Hobbys: beer brewing

Career Break

Cycletour along the Andes (11/2009-08/2010)

I stopped my studies for one year to go cycling in South-America. Learned basic Spanish, increased my appreciation for cultural differences and pushed my personal boundaries.

Major Scientific Achievements

Publications

First author:

Augustinus B, Y Sun, C Beuchat, U Schaffner, H Müller-Schärer, 2019. Predicting impact of a biocontrol agent: Integrating distribution modeling with climate-dependent vital rates. *Ecological Applications* e02003

Augustinus BA, MF Guarino, F Colombo, S Citterio, U Schaffner, H Müller-Schärer, R Gentili. 2015. Nuove segnalazioni di *Ambrosia artemisiifolia* e *Ophraella communa* in valtellina (Alpi Centrali, Lombardia). *Natura Bresciana* 39: 45-48.

Other author:

Gilligan TM, DJ Wright, RL Brown, **BA Augustinus**, U. Schaffner, 2020. Taxonomic issues related to biological control prospects for the ragweed borer, *Epiblema strenuana* (Lepidoptera: Tortricidae). *Zootaxa* 4729(3); 347-358

Lommen STE, S Fogliatto, F Vidotto, S Citterio, **BA Augustinus**, and H Müller-Schärer. 2018. Direct effects of insecticides on common ragweed - implications for natural enemy exclusion trials. *Journal of Pesticide Science*: D17-048.

Mouttet R, **BA Augustinus**, M Bonini, B Chauvel, T Gachet, T Le Bourgeois, H Müller-Schärer, M Thibaudon, U Schaffner, 2018. Estimating economic benefits of expected biological control of an allergenic weed: a case study for *Ophraella communa* against *Ambrosia artemisiifolia* in southeastern France. *Basic and Applied Ecology* 33: 14-24

Lommen STE, **BA Augustinus**, U Schaffner, H Müller-Schärer and the COST-SMARTER Task Force Ophraella, the COST-SMARTER Task Force Population Dynamics 2017. Development and impact of *Ophraella communa* in Europe. *Notiziario della Società Botanica Italiana*, 1(1): 1-10

In press:

Augustinus BA, STE Lommen, S Fogliatto, F Vidotto, T Smith, D Horvath, M Bonini, RF Gentili, S Citterio, H Müller-Schärer, U Schaffner (2020). In-season leaf damage by a biocontrol agent explains reproductive output of an invasive plant species. *NeoBiota*, 54

Schaffner U, S Steinbach, Y Sun, C Skjøth, LA de Weger, STE Lommen, **BA Augustinus**, M Bonini, G Karrer, B Šikoparija, M Thibaudon, H Müller-Schärer, 2020. Biological weed control to relieve millions of allergy sufferers in Europe. *Nature Communications*

In review:

Augustinus BA, RF Gentili, D Horvath, R Naderi, Y Sun, ATE Tournet, U Schaffner, H Müller-Schärer. Assessing the risks of non-target feeding by the accidentally introduced ragweed leaf beetle on native European plant species. Submitted to *Biological Control*

Not peer-reviewed

Augustinus BA, U Schaffner, H Müller-Schärer. Occurrence monitoring and non-target survey of *Ophraella communa* in Ticino and Northern-Italy

Presentations

Biology'16, 11-12/02 2016

EMAPI - International Conference on Ecology and Management of Alien Plant Invasions 4-8/9/2017

ISBCW15, International Symposium on Biological Control of Weeds, 26-31/8/2018

Entomology 2018, ESA, ESC, and ESBC Joint Annual Meeting, 11-14/11/2018

Ragweed management and the potential benefit and risk of *Ophraella communa* in Northern Italy: Researchers meet their Stakeholders, 21/10/2016