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Assessing the risk of potential biological control agent of *Ambrosia artemisiifolia* to the closely related species.

Master Thesis
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Abstract

Biological control is one of the most effective methods to regulate and eradicate noxious invasive species, and a permanent, environmentally friendly and cost-effective management tool. To find an effective biocontrol agent represents a challenge, because the elaborate and time-consuming work for risk assessments, e.g. evaluate host specificity and also difficulties to select an agent that will suppress enough control effectiveness on the host population. In the quest of controlling the highly allergenic common ragweed (*Ambrosia artemisiifolia),* the accidental introduction of a natural enemy (*Ophraella communa*) could represent a potential successful scenario for biological control. Three studies were conducted during May-November 2015 in the proximities of the Malpensa Airport of Milano, Italy, to test the preference of oviposition and feeding behavior of *O. communa* in closely related plant accessions to common ragweed.

Firstly a latin-square design open field choice experiment was set in 4 localities (Magnago, Magenta, Abbiategrasso in Italy and Rovio in Switzerland), consisting of 7 plant accessions in 7 rows, including common ragweed as control, arranged in a way that none of the accessions was repeated in a same row or column. The experiment was repeated three times (May to July = Cohort 1, July to September = Cohort 2 and September to November = Cohort 3) on each locality, imitating the growing season of early, mid and late varieties of sunflower. During the 9 weeks each cohort lasted, plants were monitored 6 times (first when planted, one week after, two weeks after, 6 weeks after, 9 weeks after and final assessment 12 weeks after); the number of egg-batches, larvae (L1-L2 and L3 instar), pupae, adults and percentage of damage was recorded on each assessment. The results show a high preference of *O. communa* towards common ragweed for oviposition, as well as for the distribution of all larvae instars, pupae and adults. Damage caused by *O. communa* was higher on common ragweed, but also found on all the plants in different percentages. Damage in the third cohort was significantly higher among all plant accessions.

A second non-choice cage experiment was conducted in Corbetta, Italy, during 6 weeks, where 72 recently hutched larvae were transferred to 18 common ragweed plants and 18 sunflower var. PR64H42 plants arranged in groups of 12 individuals (6 common ragweed + 6 sunflower) inside the three cages of 2x1x1 meters. Two larvae per plant were enclosed inside clip-on cages (one onto a high leaf and the other onto a low leaf). The larvae used for the experiment were reared from 12 egg-batches to ensure the larvae were half-sibs. Adults were caught on-field (1 female and 2 males), taken from different localities. Clip-on cages were monitored daily to check for survival and the stage the individuals of *O. communa*. After 28 days, the experiment stopped and the weight of those beetles that became adults was recorded at the University of Fribourg. The results showed that *O. communa* performed better on common ragweed, as larval and pupae survival was significantly higher on common ragweed, but there was no significant difference in developmental time between survived *O. communa* larvae developing on ragweed or sunflower. We found significant differences of interactions between some insect families (i.e. from 12 mother adults) and the plant hosts that may suggest genetic variation in host preference.

Finally, a non-target survey was realized on a selected group of closely related plant species to common ragweed (Family: Asteraceae, Tribe: Helianthae), including both native and non-native, according to the centrifugal phylogenetic risk evaluation method. The survey was realized in 28 localities (25 in Italy 3 in and Switzerland) where the occurrence of both common ragweed and *O. communa* was confirmed, covering 15 non-target plant species. The number of egg-batches, larvae (L1-L2 and L3 instar), pupae, adults and percentage of damage was registered on the survey, as well as the demographic characteristics (population size, density, abundance, phaenology) of the plant population, predators of *O. communa* and potential host plants. We found that *O. communa* can complete its life-cycle on three other non-native plant species in the same tribe as common ragweed (*A. trifida, Xanthium strumarium* and *Helianthus tuberosus*), but also *O. communa* can cause punctual feeding damage on sunflower and three native plant species in the Asteraceae family (*Centaurea nigrescens, Buphthalmum silicifolium)*, including endemic *Xerolekia speciosissima*.

All the results suggest that there is a risk of attack on non-target plant species by *O. communa* in the newly introduced range in northern Italy and southern Switzerland. Nevertheless, the punctual damage found on native species was unlikely to have major repercussions, same for the damage found in early varieties of sunflower. The fact that the plant species where *O.* communa completes its cycle are also invasive, suggests that a simultaneous control could have beneficial implications. Only the negative effect of *O. communa* on late varieties of sunflower remain unsolved to predict and more research is needed in this subject. The apparent benefits that the potential biocontrol agent *O. communa* may bring, surpass the punctual risks found on the field, suggested that *O. communa* is a suitable control agent for *A. artemisiifolia*.

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1. Introduction

1.1. Invasive plant species

Plant invasion is one of the most problematic threats to biodiversity (Vila & Weiner, 2004). In certain areas, native vegetation succumb to the effect of vigorous alien plants (Cronk, 1995), and also the structure of natural habitats becomes disturbed (Huey *et al.*, 2005). Non-native species can also impact human health by introducing new diseases (Davies, Svejcar, & Bates, 2009) and affect local economies by displacing crop plants (Briese, 2003).

Not all alien plants are considered invasive, as invasive plants must be capable of establishing self-sustaining populations in areas of natural vegetation (Davies et al., 2009). Aggressiveness of spread is one important feature for invasive species, increasing the possibility to change rapidly and irreversibly the landscape (Cronk, 1995). Agriculture is related historically to spread of invasive plants, associated ruderal plants and weeds that were accidentally introduced together with the domesticated crops (Cronk, 1995). Despite the negative ecological effects of plant invasion, invasion offers an opportunity to study rapid evolution, as many species experience a severe bottleneck and spread can be accelerated by adaptation (Huey *et al.*, 2005).

1.2. Biological control

Biological control is essentially the use of some form of life to overcome another form causing an economic loss to men and/or ecologic loss to the environment (Ordish, 1967), usually done by introducing and augmenting natural enemies, to interrupt spread and impact on non-invasive species (Davies et al., 2009). Biological control is considered the best control method regarding the minimizing of the impact on the native flora and fauna (Cronk & Fuller, 1995). For invasive plants, herbivorous insects and pathogens are common biological control agents, but in most cases the agents are non-native themselves (Davies et al., 2009) and while most of these invertebrate are highly host specific, some can have impact on native species (Briese, 2003). Biological control programs must take in account adverse effects on non-target fauna and flora (Cronk, 1995), leading to conduct many trials of the potential biological control agent to ensure that host range expansion would not occur (Davies et al., 2009). An ideal agent must come from a climate similar to the region targeted, host specific and capable of significantly damaging the target plant, but also not feeding in the same way on native plants and be taxonomically distinct from the native plants (Lawton, 1985).

The failure rate of biological control is mainly due to the impossibility of establishment of the control agent or the low densities of which it establishes, but the damage on native plants seems rarely to happen (Cronk, 1995).

1.3. Study system

Common ragweed *(Ambrosia artemisiifolia)* is an annual weed from the Asteraceae family native to North America, known as an invader with a high potential for colonizing new areas (Müller-Schärer et al., 2014), it is spread in Europe and some Asian countries (Bosio, Massobrio, Chersi, Scavarda, & Clark, 2014) and its major concerns with this species are crop yield loss due to competition and human health problems regarding its highly allergenic pollen (Qin, DiTommaso, Wu, & Huang, 2014). In the native range, the weed is attacked reportedly by 200 species of arthropods and about 25 fungal pathogens (Bosio et al., 2014). In other continents, classical biological control of common ragweed has been implemented, specially using the stem-galling moth *Epiblema stenuata* and *Ophraella communa* (Kiss, 2001). The latter is regarded in China as the most successful weed biological control agent, as all three larval stages and adults feed on it and can devour plants over large areas before seed set (Gerber et al., 2011). In southern China beetles reached up to six generations per year (Müller-Schärer et al., 2014), contrasting to the 3 generations of their native range (Bosio et al., 2014) and four to five in Japan (Watanabe & Hirai, 2004).

O. communa is an olliphagous chrysomellid insect, reportedly accepting several hosts on various plants of the tribe Helianthae (Cao, Wang, Meng, & Li, 2011), but mainly feeding on common ragweed and giant ragweed (*A. trifida*), they may attack other plants early in autumn (Watanabe, 2004).

1.4. Study aim and hypothesis

In the summer of 2013, *O. communa* was discovered in southern Switzerland and Northern Italy resulting from an accidental introduction, presented a unique opportunity for the recently started EU-COST Action 'Sustainable management of *Ambrosia artemisiifolia* in Europe (SMARTER)' to assess this potential biological control agent to either spread it or create actions against its spread (Müller-Shärer *et al.*, 2014).

The aim of this work is to continue in the efforts of investigating the effects of potential biological control agent *O. communa* on non-target plant species, following the previous studies by Gaelle Kadima and Stefanie von Bergen in 2014. In this study we tried to answer the following questions: 1) Which plant species is preferred for oviposition and feeding behavior by *O. communa?* 2) Does *O. communa* have a better larval performance when fed exclusively on ragweed than fed only on Sunflower? 3) Does *O. communa* lay eggs or feed naturally on non-target plant species?

We hypothesize, that *O. communa* has a preference for common ragweed for oviposition and feeding behaviors in larval and adult stages. In second place that the growth performance of *O. communa* is better on common ragweed than on sunflower and finally, that there will be less feeding damage and less oviposition on previously recorded non-target plant species, also that no plant species will be recorded with damage or oviposition.

2. Preference experiment

2.1. Introduction

Host preference is defined in this study according to the behavioral approach, as the likelihood of acceptance of a particular host based on the ability of the potential biological control agent to detect the plant (Sheppard, Van Klinken, & Heard, 2005). Nevertheless, we also considered the ecological approach for monitoring, which takes in account the difference in distribution of consumption and/or number of eggs over different plant species in proportion to their relative abundance (Schaffner, 2001).

Host preference tests help to predict the ecological impact of potential biological control agents on native species that are secondary hosts (Louda, Arnett, Rand, & Russell, 2003). The evaluation of host specificity and preference is one of the primary criteria used to rank the risks that potential biological control agent pose for non-target organisms (Blossey, 1995). Different tests can be applied for host specificity evaluation, but there are mainly three types: non-choice tests, choice tests and field tests (Sheppard *et al.*, 2005). Preference is usually evaluated with choice tests, where the response of the potential biological control agent is measured in the presence of both the targeted invasive species and the alternative hosts (Louda *et al.,* 2003).

Choice tests are commonly conducted to evaluate host suitability and ranking of preference, and they are more valuable for highly mobile arthropods in their different life stages, allowing previous experience and learning affect preference (Sheppard *et al.*, 2005). Open field choice tests show more accurately the acceptances of test plants in natural conditions (Schaffner, 2001), because under field conditions, the trials are considered ideals as they are the closest to nature (van Klinken, 1999). Nevertheless previous laboratory tests are also necessary (Sheppard *et al.*, 2004). The design for open field tests must be suited to address the relationship between preference behavior and mobility of the potential biocontrol agent, but also to evaluate ecological parameters such as host plant density and spatial distribution (Schaffner, 2001). Open field choice experiments are more valuable when tests are synchronized with plant phenology (Sheppard *et al.,* 2005).

2.2. Materials and methods

The open-field choice experiment of preference was realized in order to imitate the situation of sewing different varieties of sunflower with diverse purposes, during the colonisation of *O. communa* throughout one season. We used a latin-square design of 3 m × 3 m, with seven rows and seven columns in which we sowed seven plant accessions from the three species (Table 1). None of the seven plant accession is repeated in the same line. This distribution copes with the effect of host plant density and distribution, as well as the mobility of the tested insect (Schaffner, 2001). In the setting we compared the oviposition preference and feeding behaviour of *O. communa* on closely related crop species *Guizotia abyssinica* and *Helianthus annus*, the latter with three varieties contrasted (Table 1). Common ragweed (*Ambrosia artemisiifolia*) was included in the design as control. In the same setting, it was also evaluated the effect of *O. communa* on three different phenotypic stages (hereafter phenostages) of sunflower variety PR64H42 (Girasole): seedling (with cotyledons), medium (with 4 leaves) and large (with 6-8 leaves). The latin-square design was repeated three times on each site (except Rovio where it was repeated twice) replicating the growing season of early sunflower varieties (Cohort 1= May to July), mid-late varieties (Cohort 2 = July to September) and late varieties (Cohort 3 = September to November).

Table 1. Description of the different species and varieties used for the latin-square experiment, describing the time they are sown and the use. Note that for the experiment, each phenostage (seedling, medium and large) of Girasole were treated as a different accession.

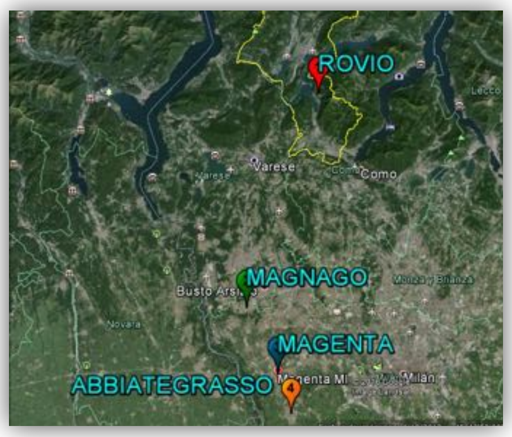
SPECIES	**VARIETY**	**SOW TIME**	**USE**
Helianthus annuus	PR64H42 (Girasole)	Late spring	Oil production
Helianthus annuus	Sunrich (Sunrich Orange F1)	Summer	Ornamental
Helianthus annuus	Iregui	Late summer	Green manure
Guizotia abbyssinica	---	Late summer	Green manure
Ambrosia artemisiifolia	---	Naturally appearing in spring	No use, target species

2.2.1. Growth conditions of the study species

Seeds from all sunflower varieties and *G. abbyssinica* were acquired in UFA Samen, Switzerland and common ragweed plants came from a single mother plant coded SASBAN (SMARTER Ambrosia Seed Bank Accession Number) number 66.10 from a population in Busto Arsizio, Italy. All seeds were sown in soil type Proter + Type 4, Bern, Switzerland mixed with 1/3 of sand. Plants were grown in the greenhouse of the University of Fribourg two months before the experiment started, for each of the 3 cohorts (for more info Annex 2), except for the seedling and medium phenostages of sunflower variety Girasole, which were sown respectively 2 weeks and 1 month before the experiment began.

2.2.2. Study sites

The experiment was performed in four localities (Fig. 1), around the Malpensa airport zone where the occurrence of *O.communa* was confirmed. All sites were barren lands where common ragweed grew naturally and were mowed before experiments begin.



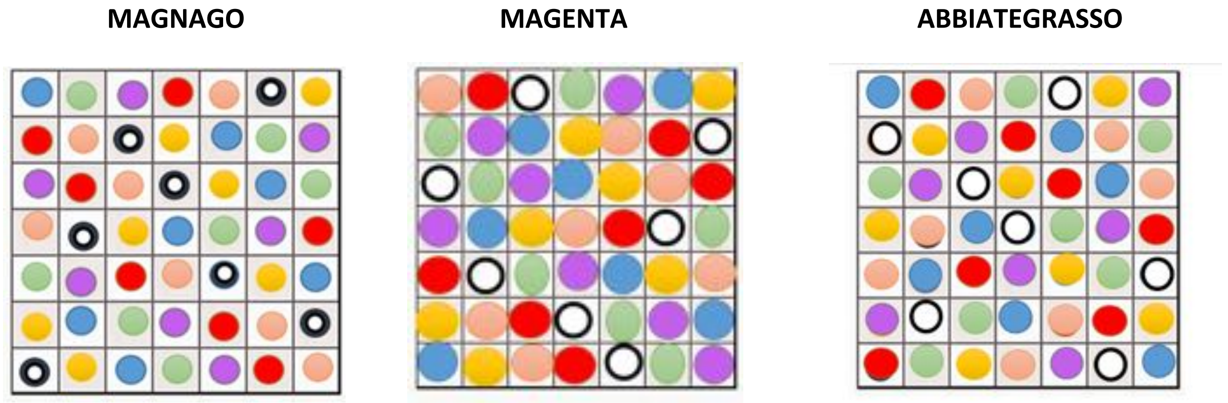
COUNTRY	LOCATION	COORDINATES
Switzerland	Rovio	45.93087 N 9.98377 W
Italy	Magnago	46.57073 N 8.78546 W
Italy	Magenta	45.45953 N 9.97472 W
Italy	Abbiategrasso	45.383185 N 8.928022 W

Figure 1. Map of the 4 sites where the latin-square experiments were performed, with the coordinate's information on the right table.

2.2.3. Experimental setup

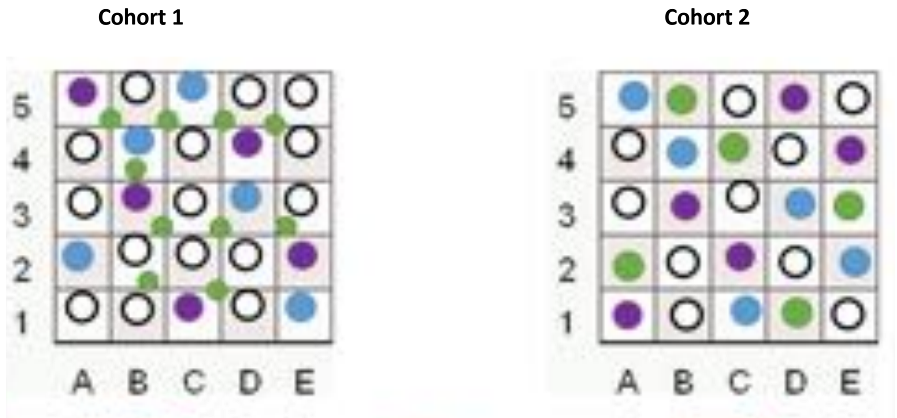
Each of the seven accessions was present with the same abundance in the latin-square design (Fig. 2) with one individual per accession on each row and each column, and located 50 cm away from the next plant. The setup was realized in three cohorts (early May, mid-July and early September) and each monitored five times (week zero, after one week, after two weeks, after six weeks and after nine weeks) during the nine week that a cohort lasted (for extra information see Annex 1), plus an additional final monitoring where the fresh weight of the plants was registered. For each cohort, the latin-square was implemented in 3 locations in Italy (Fig. 2) and additionally one reduced latin-square 5x5 in Rovio in Switzerland in the first and second cohort (Fig. 3)

To set the latin-squares, all plants were transported to the study sites. In Rovio, naturally growing common ragweed plants were used for the experiment exceptionally, as requested by local authorities. The 3m × 3m latin-squares were set according to a protocol (see Annex 3), centred inside a 7m × 7m square using trigonometry principles, the latter square was mowed to avoid influence of natural growing weeds in the experiment, also spontaneous ragweed were constantly hand-removed throughout the three cohorts. Individuals were randomized for the location and position, and then transplanted according to the design of Fig. 2 (and Fig. 3 for Rovio). After transplanting, all plants were labelled with the same coded colours as in Fig. 2 and then attached to a bamboo stick as support and to facilitate locating the plants in further assessments.



SYMBOL	PLANT ACCESSIONS AND DESCRIPTION	Stage (SIZE)
○	Helianthus annuus PR64H42 (CULTIVATED)	2-4 leaves
🔴	Helianthus annuus PR64H42 (CULTIVATED)	6-8 leaves
🟠	Helianthus annuus PR64H42 (CULTIVATED)	>8 leaves
🟡	Helianthus annuus Sunrich (ORNAMENTAL)	>8 leaves
🔵	Helianthus annuus Iregui (MANURE)	>8 leaves
🟣	Guizotia abyssinica Niger (MANURE)	>8 leaves
🟢	Ambrosia artemisiifolia Ragweed (CONTROL)	>8 leaves

Figure 2: Latin square designs randomized for the three locations in Italy in which the 3 cohorts were implemented. The two circles in the table show the two groups that are being compared within the design. The blue circle encloses the same variety (Sunflower PR64H42) in three different stages at the beginning of the experiment. The red circle encloses 5 different species /varieties at the same stage.



COLOR	ACCESSIONS	CARACTERISTICS
🔵 BLUE	*Helianthus annuus* IREGUI	40 cm high, used for Green manure
🟣 PURPLE	*Guizotia abbyssinica* NYGER	20 cm high, used for Green manure
○ WHITE	*Helianthus annuus* GIRASOLE	5 cm high, used for oil production
🟢 GREEN	*Ambrosia artemisiifolia* RAGWEED	Control

Figure 3: Reduced latin-square designs randomized for Rovio, Switzerland where 2 cohorts were implemented. Local naturally growing common ragweed were used for the experiments.

2.2.4. Monitoring

For each monitoring, the abundance of *O.* communa (number of egg-batches, mean number of eggs per batch, number of first and second instar larvae, number of third instar larvae, number of pupae and number of adults) was recorded on forms (Annex 6); as well as plant phenology and a visual estimation of the level of damage caused by *O. communa* following a damage guideline (Annex 7). Additionally height from the base of the plant until the apical leaves and the maximum width of the leaves were recorded, to measure the volume of the plant as an alternative to the plant biomass. Fresh biomass was weighted in the last assessment (12 weeks later for the first cohort, 12 weeks later for the second cohort and 9 weeks later for the third cohort).

2.2.5. Statistical analysis

To analyse oviposition preference of *O. communa*, we used the distribution index to indicate the relative attractiveness of the beetle to a host plant. The distribution index was calculated by dividing the absolute number of egg-batches found on each plant accession by the total number of egg-batches found on the experiment (Gould & Sweet, 2000). Similarly we calculated the distribution index of first instar larvae (second instar included here to minimize risk of counting twice), third instar larvae and pupae. In the case of adults, we calculated the adult load by dividing the cumulative number of adults recorded by plant accession, divided by the number of assessments in which adults were present. For damage we registered the maximum damage found at the final assessment.

All statistical analyses were performed using JMP® Pro 11.1.1, SAS Institute Inc. Cary, (NC 1989-2013), and the graphs created in Microsoft® Excel 2013 and R (Version 3.1.2. R foundation for Statistical Computing 2014). Analyses included general linear models and analysis of variance (ANOVA), in which significant treatment differences were indicated by a significant F-test with $P \leq 0.05$. A post hoc test of Tukey HSD was used to compare the means between treatments. ANOVAs including the main effects of plant accession, cohort and site were performed for the mean distribution of egg-batches, first instar larvae, third instar larvae, pupae, adults and maximum damage per plant accession to determine if this factor differed systematically. Each site was analyzed separately and cohorts were compared among each other.

2.3. Results

To ease the reading, all results are summarized on Table 2 (Page 25).

2.3.1. Oviposition preference of *Ophraella communa*

Oviposition diminished throughout the growing season after having a peak at the end of the first cohort (Annex 5). The number of egg-batches of *O. communa* differ significantly from site to site ($F_{3,471}=6.4$, $P<0.006$), most of the egg-batches were found in Magnago (N=528) then Magenta (N=186), Rovio (N=117) and Abbiategrasso (N=103) (Fig. 4).

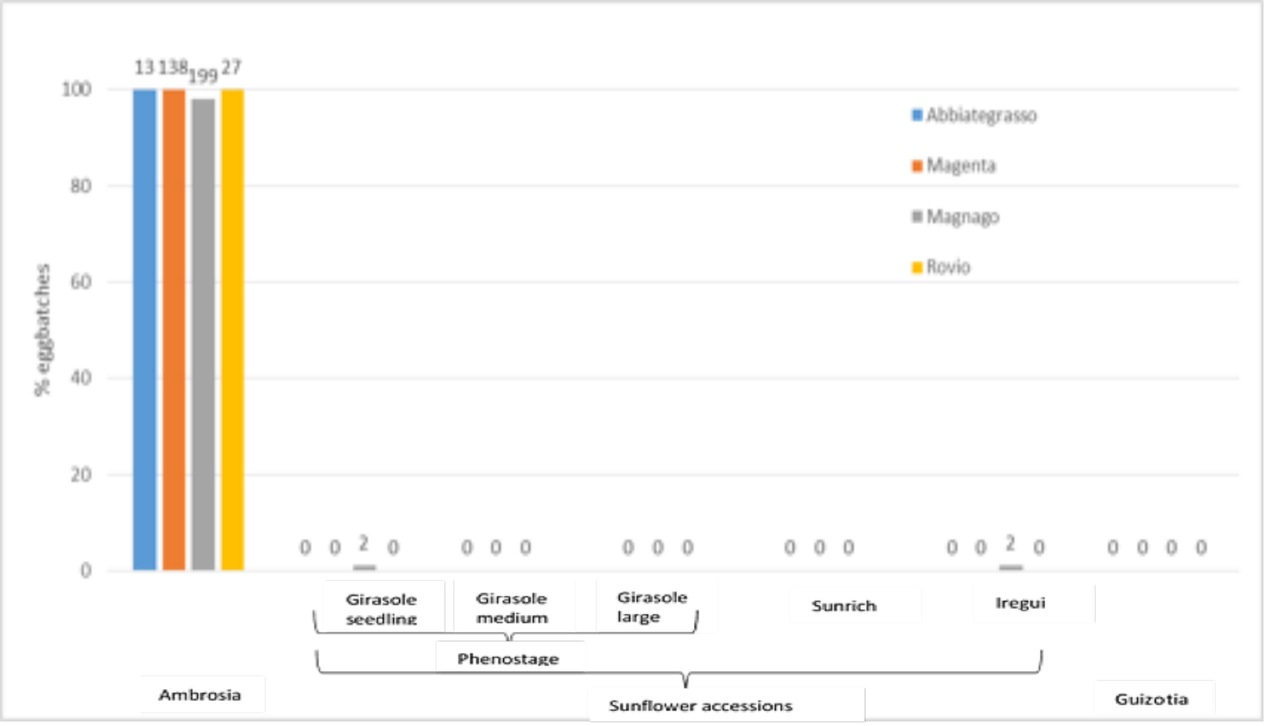


Figure 4. Distribution of egg-batches among plant accession within a site for cohort 1 (May-July). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

In all Italian sites we found a significant effect of plant accession for the distribution of egg-batches of *O. communa* ($F_{6,126} > 9.67$, $P<0.0001$), as well as cohort ($F_{2,126}>3.25$, $P<0.042$) and the interaction between cohort and plant accession ($F_{12,126}>2.59$, $P<0.004$). A comparison using the post hoc test of Tukey HSD indicated that the distribution of egg-batches *on* common ragweedis significantly different from the distribution on other plant accessions (Tukey's HSD; $P<0.0001$). The test also showed that the interaction between common ragweed and the first two cohorts (Tukey's HSD; $P<0.001$) was significantly different from the rest of the interactions, including the one between common ragweed and third cohort. Lastly the third cohort was significantly different from the first two cohorts in Abbiategrasso and Magenta according to the Tukey test (Tukey's HDS; $P=0.001$).

In Rovio, only plant accession had a significant effect on the distribution of egg-batches ($F_{3,48}$=17.53, P<0.001). The post hoc test showed that common ragweed was significantly different from the rest of the plant accessions (Tukey's HSD; P<0.001) (Fig. 5).

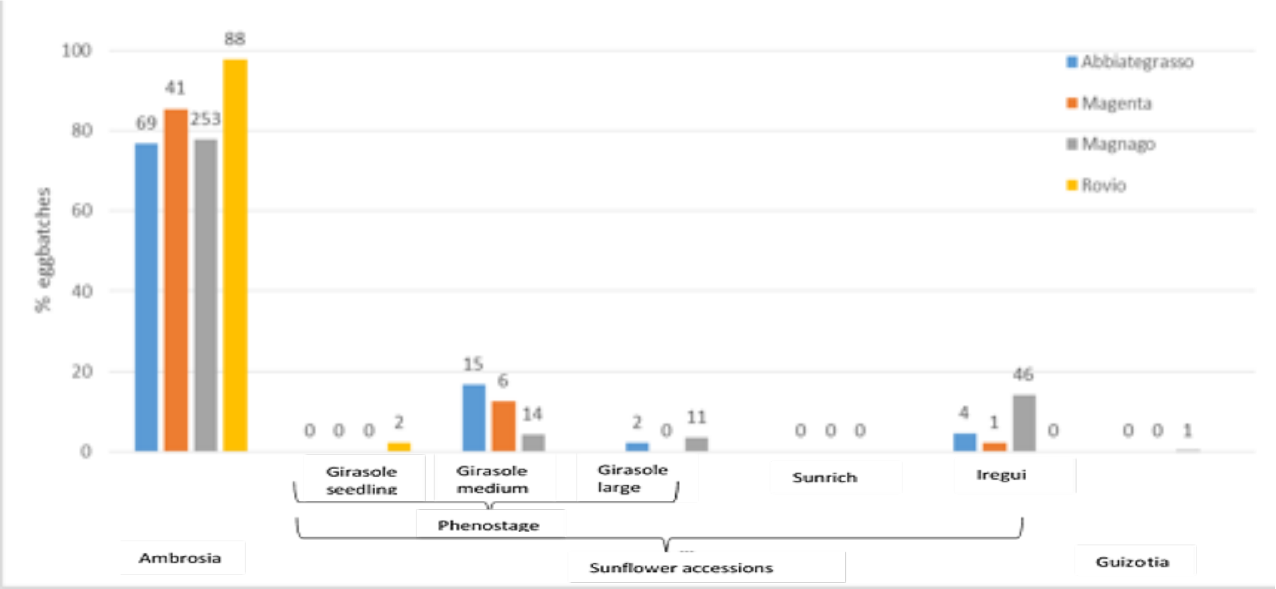


Figure 5: Distribution of eggbatches among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accession, arranged in a latin-square (see text for details).

2.3.2. Larvae and pupae abundance of *Ophraella communa*

Most of the first instar larvae were found in Magnago (N= 357), followed by Rovio (N=157), Abbiategrasso (N=146) and Magenta (N=76) (See Annex 5 for more information). Site had a significant effect for the number of first instar larvae ($F_{3,471}$=8.16, P<0.001).

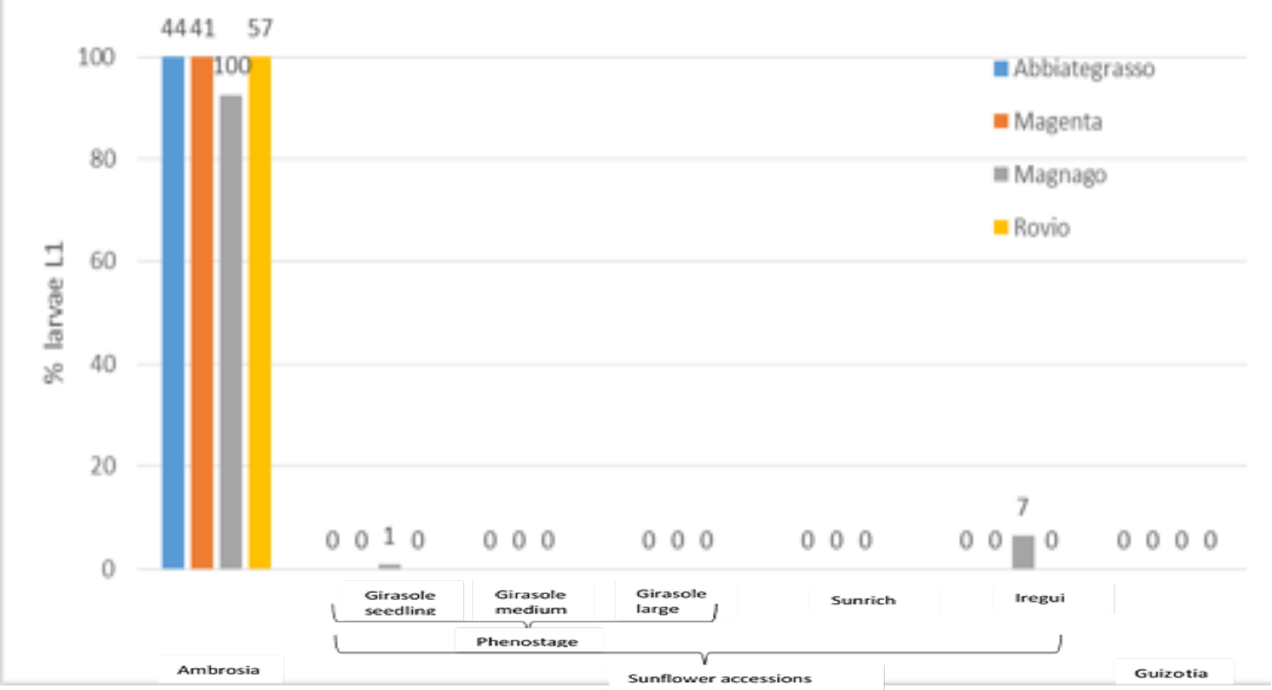


Figure 6: Distribution of L1 larvae among plant accessions within a site for cohort 1 (May-July). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

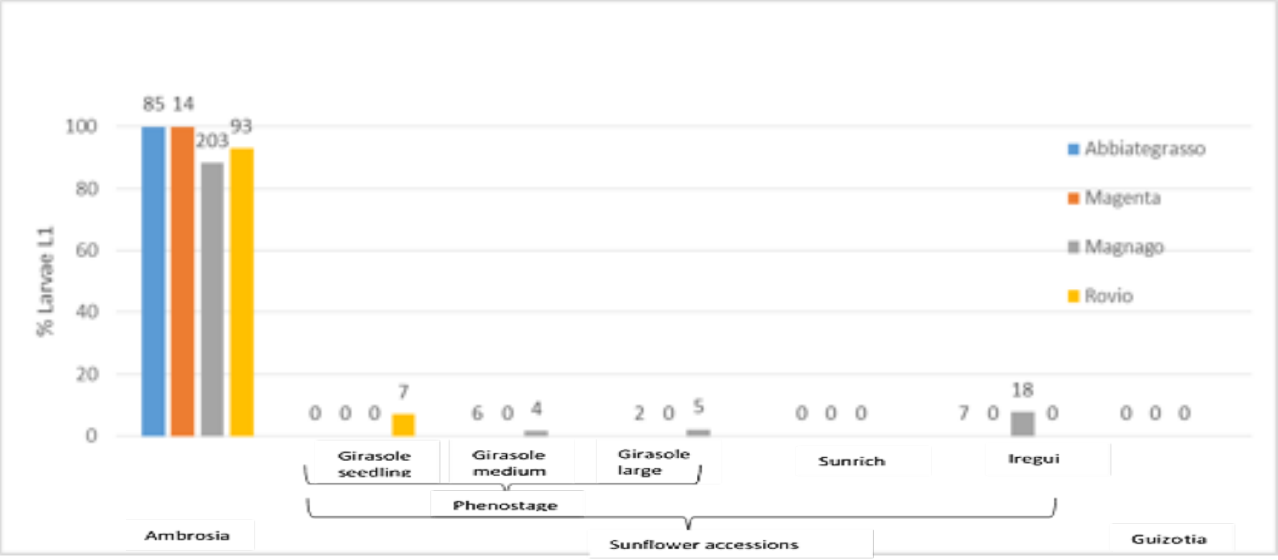


Figure 7: Distribution L1 larvae among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accessions arranged in a latin-square (see text for details).

For all sites, only the effect of plant accession was found significantly determinant for the distribution of first instar larvae ($F_{6,126}$>13.39, p<0.001), but neither from cohort ($F_{2,126}$<1.6, P>0.077), nor their interaction ($F_{12,126}$<1.6, P>0.077). A post hoc test of Tukey was done to evaluate the difference between plant species, the target species was significantly different (Tukey's HSD; P<0.001) from the rest of the plant accessions (Tukey's HSD; P>0.87).

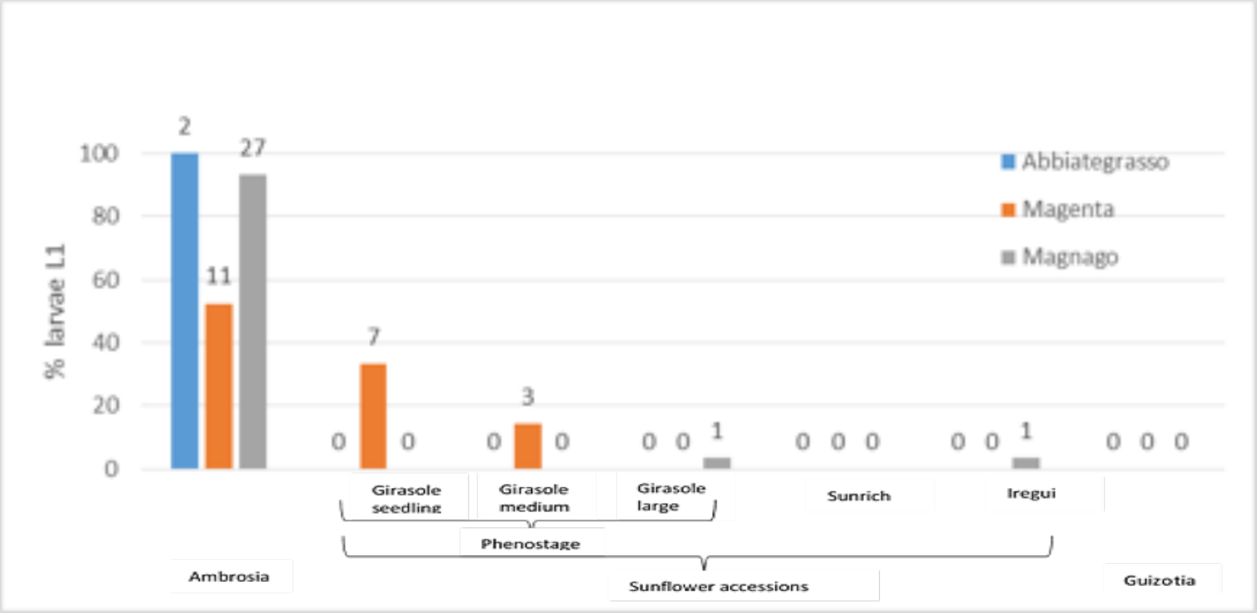


Figure 8: Distribution of L1 larvae among plant accessions within a site for cohort 3 (September-November). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

Most of the third instar larvae were found on Magnago (N=248), then Abbiategrasso (N=58), then Magenta (N=27) and finally Rovio (N=15). Site was significant (F=17.59, P<0.001).

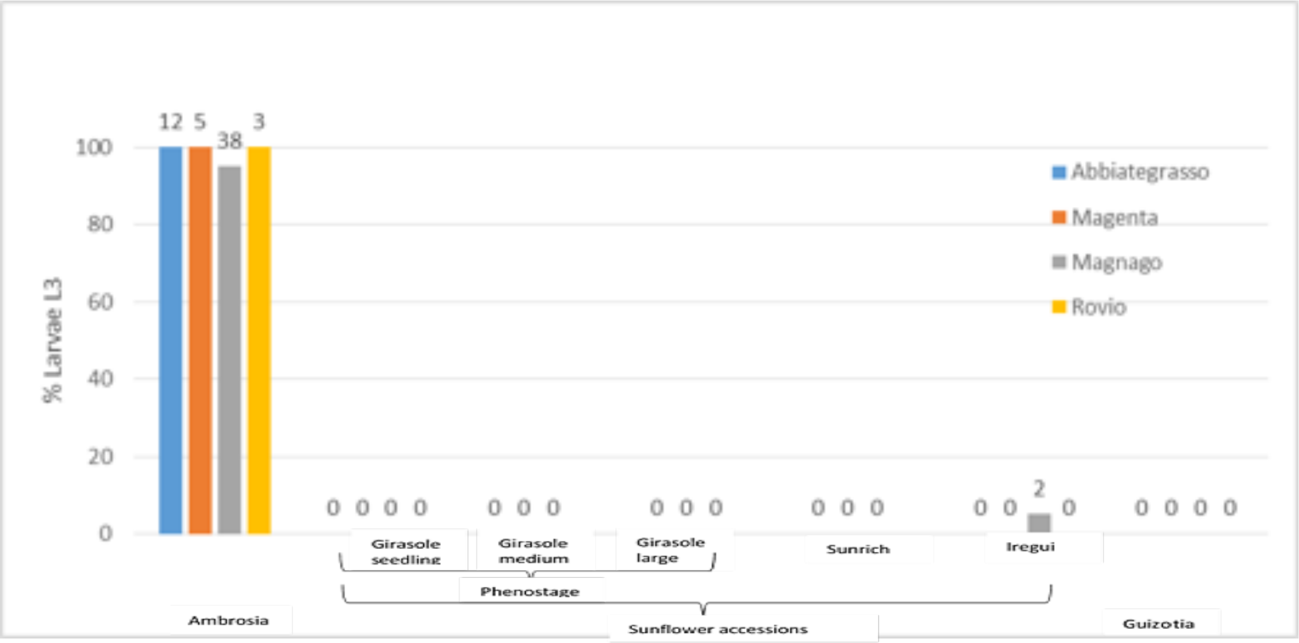


Figure 9: Distribution of L3 larvae among plant accessions within a site for cohort 1 (May-July). Data are based on 7 plant individuals per plant accession, arranged in a latin-square (see text for details).

The distribution of third instar larvae of *O.* communa in Abbiategrasso and Magenta was affected significantly by plant accession ($F_{6,126}$>10.35, P<0.001) and by the interaction between plant accession and cohort ($F_{12,126}$>2.59, P<0.002), but not by cohort alone ($F_{2,126}$<2.99, P>0.06). A Post hoc evaluation of the differences between plant species showed that the distribution of third instar larvae was higher on common ragweed (Tukey's HSD; P<0.001), and also the interaction between the first two cohorts and *A. artermisiifolia* there was a significant interaction (Tukey's HSD; P<0.001).

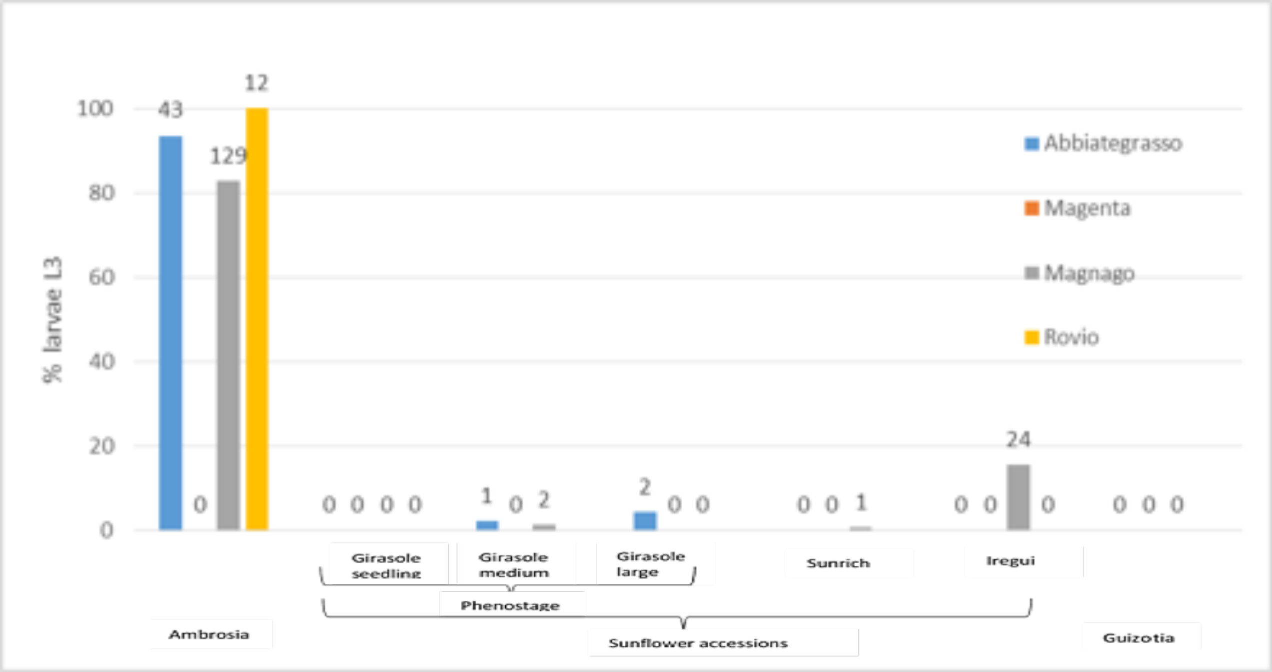


Figure 10: Distribution of L3 larvae among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

In Magnago ($F_{6,126}>7.86$, $P<0.001$) and Rovio ($F_{3,48}>7.86$, $P<0.001$). only plant accession had a significant effect on the distribution of third instar larvae. A post hoc test of Tukey indicated that *A.* artemisiifolia had a significantly higher distribution of third instar larvae (Tukey's HSD; $P<0.02$).

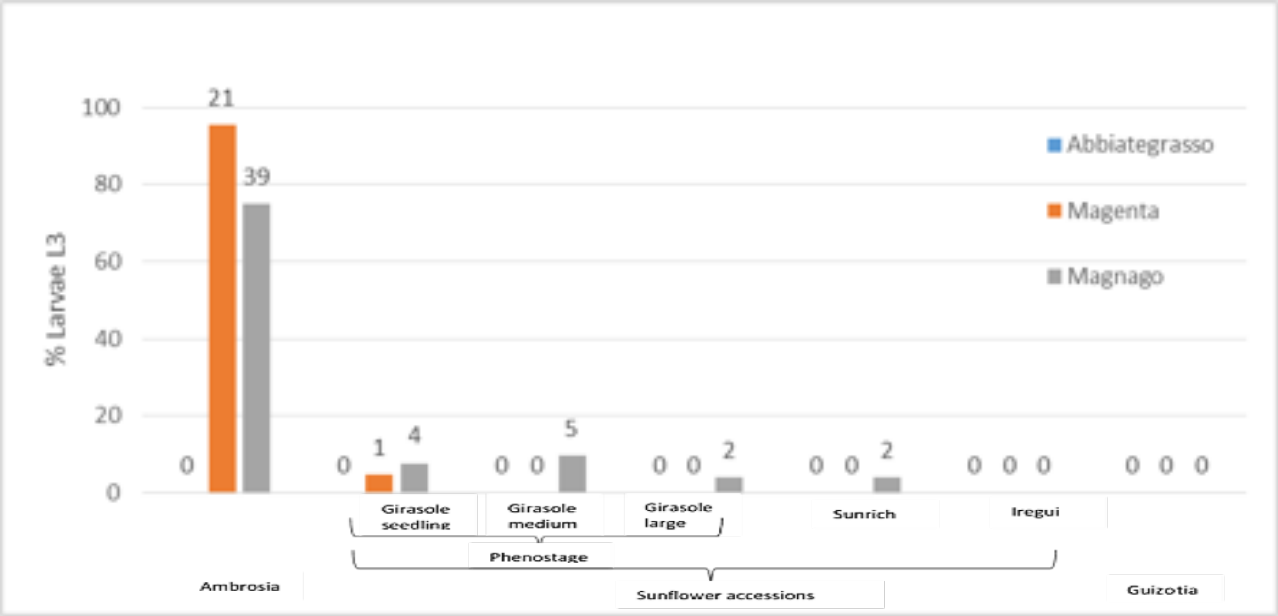


Figure 11: Distribution of L3 larvae among plant accession within a site for cohort 3 (September-November). Data are based on 7 plant individuals per plant accession arranged in a latin-square.

Pupae were found mostly in Magnago (N=248), followed by Abbiategrasso (N=51), then Rovio (N=7) and lastly Magenta (N=2). There was a significant effect of site (F=9.54, P<0.001) and the interaction between site and plant accession (F=6.07, P<0.001).

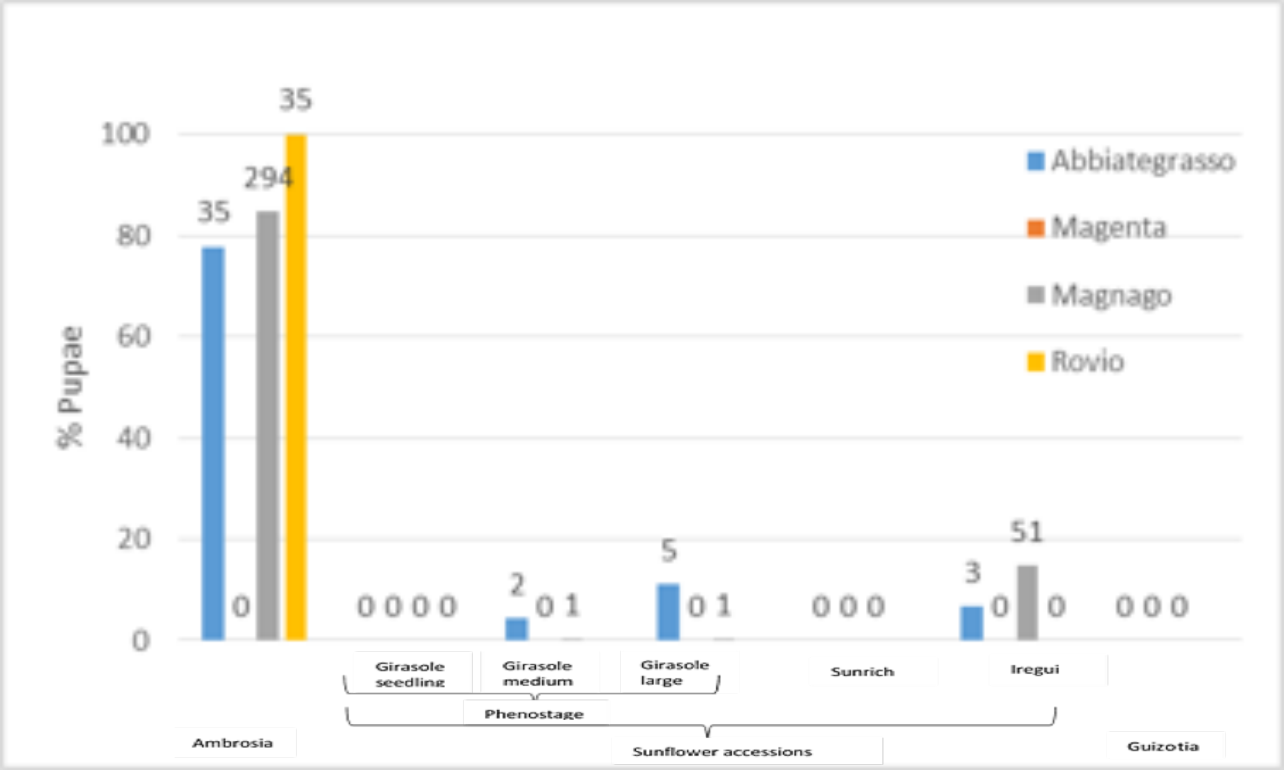


Figure 12: Distribution of pupae among plant accessions within a site for cohort 1 (May-July). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

Neither in Abbiategrasso, Rovio or in Magenta there was any significant effect of plant accession ($F_{6,126}$<1.19, P>0.05), nor cohort ($F_{2,126}$<1.08, P>0.07), but in Magnago there was a significant difference in the distribution of pupae for plant accession ($F_{6,126}$=2.78, P=0.014), but the post hoc test of Tukey did not found a significant difference between plant accessions (Tukey's HSD; P<0.07).

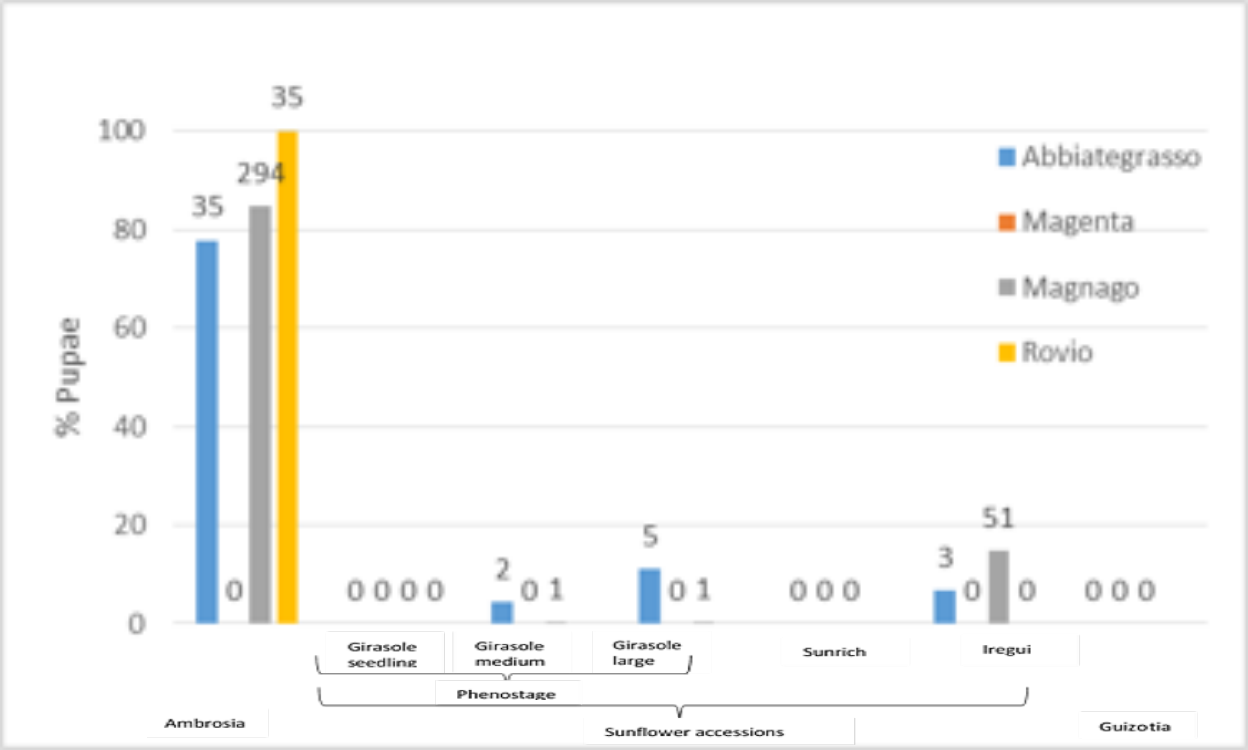


Figure 13: Distribution of pupae among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

2.3.3. Adult load of *Ophraella communa*

The load of adults was significantly different among sites ($F_{3,471}$=5.32, P=0.005).The highest load of adults was found in Magnago with N=1105, then Magenta (N=663), Abbiategrasso (N=376) and Rovio (N=30).

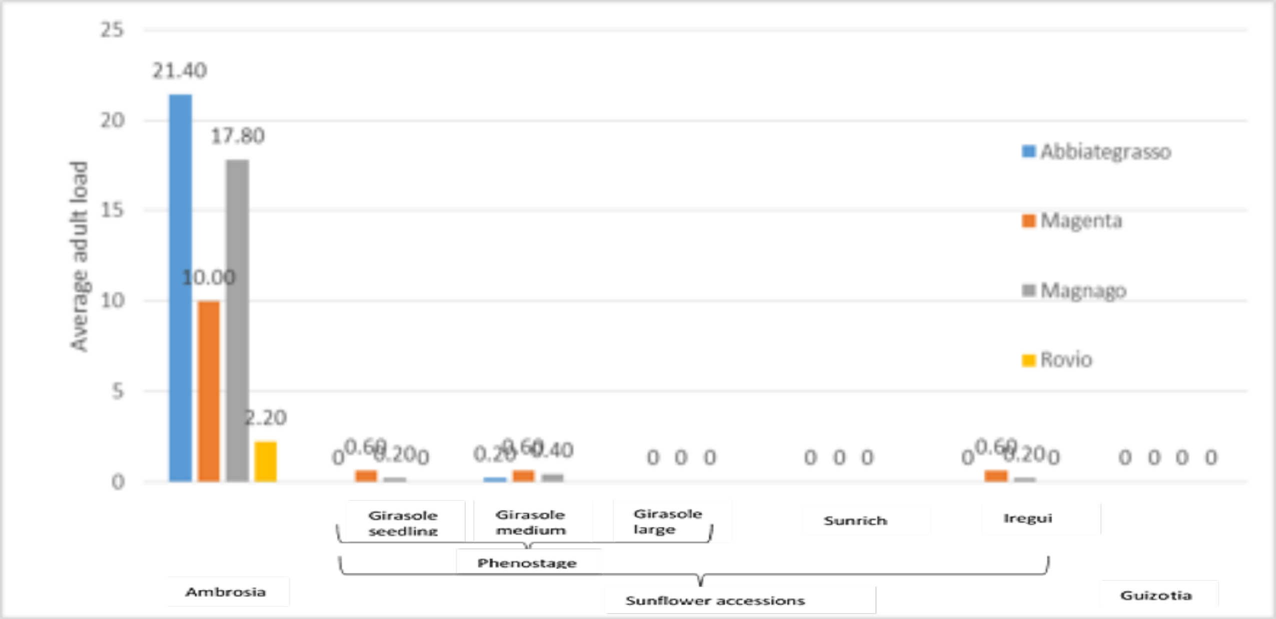


Figure 14: Distribution of average adult load among plant accessions within a site for cohort 1 (May- July). Data are based on 7 plant individuals per plant accession arranged in a latin square.

In Abbiategrasso only the effects of plant accession was found significant for the distribution of beetle adult load ($F_{6,126}$=21.08, p<0.001). A post hoc test done after showed that *A. artemisiifolia* is significantly different from the other plant accessions (Tukey's HSD; P<0.001).

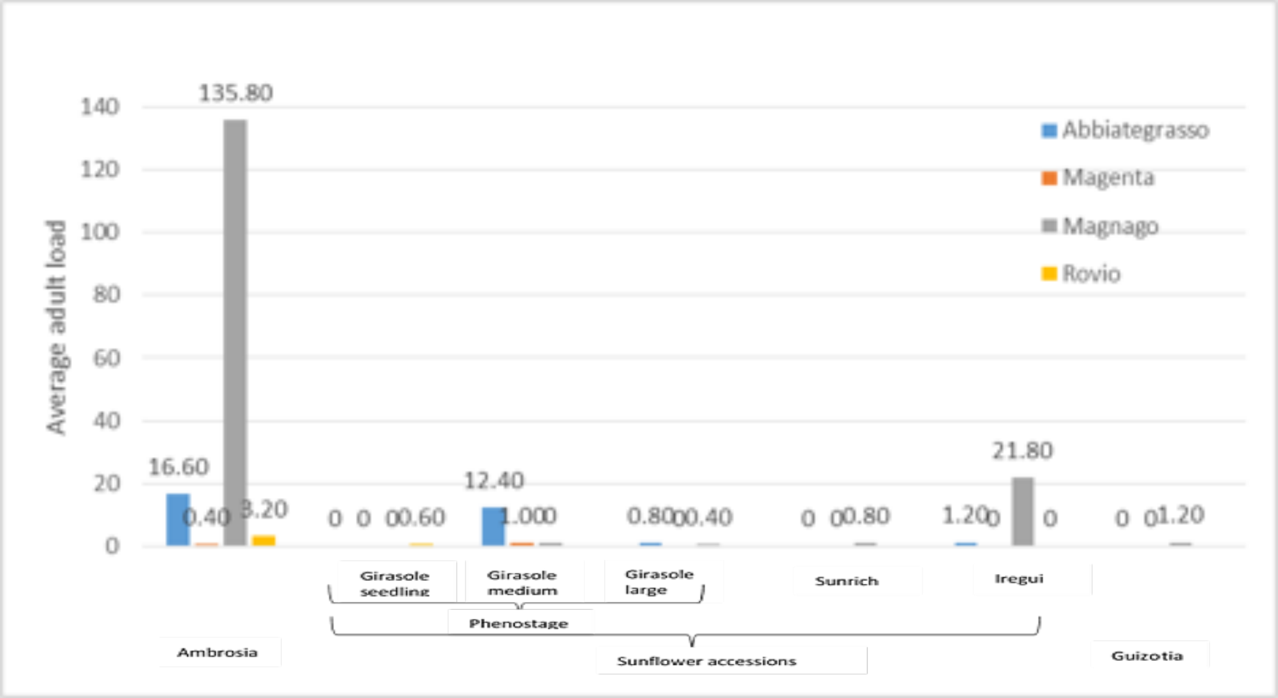


Figure 15: Distribution of average adult load among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

In Magenta and Magnago the cohort ($F_{2,126}$>7.97, P<0.001), plant accession ($F_{6,126}$>48.422, P<0.001) and the interaction between them ($F_{12,126}$>29.549, P<0.001) affected significantly the load of *O. communa* adults throughout the experiment. The post hoc test of Tukey indicated that common ragweed in cohort three (Tukey's HSD; P<0.001) was significantly different to other interactions between plant accession and cohort (Tukey's HSD; P>0.05). Cohort three (Tukey's HSD; P<0.001) was significantly different from the first two cohorts (Tukey's HSD; P>0.37) and also common ragweed was different according to the same test (Tukey's HSD; P<0.001) to the rest of the plant accessions (Tukey's HSD; P>0.78).

In Rovio the accession ($F_{3,48}$=20.17, P<0.001) and the interaction between cohort and plant accession ($F_{3,48}$=4.37, P=0.018). A Tukey test indicated that common ragweed is significantly different to the rest of the accession (Tukey's HSD; P<0.001), Also the interaction of the second cohort and common ragweed was significantly different (Tukey's HSD; P<0.001).

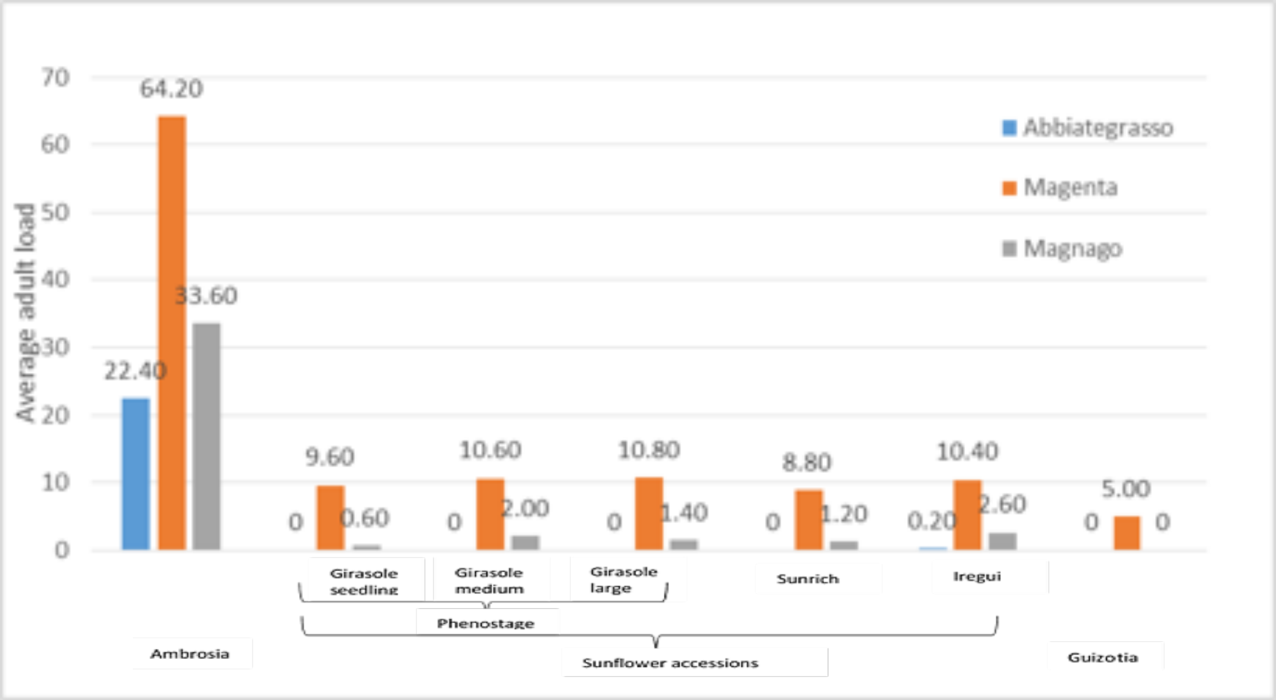


Figure 16: Distribution of average adult load among plant accessions within a site for cohort 3 (September-November). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

2.3.4. Damage to test plants

There was no significant difference between sites in the distribution of maximum damage on the seven plant accessions ($F_{3,471}$=0.41, P=0.67). In Abbiategrasso, feeding damage caused by *O. communa* to the 7 plant accessions vary significantly among cohorts, plant accessions and the interaction between cohort and plant accession ($F_{12,126}$>19, P<0.001). A post hoc test of HSD Tukey indicated that there are four groups for the maximum damage found throughout the experiment. The first one with common ragweed in the last 2 cohorts (Tukey's HSD; P<0.001); secondly common ragweed in the first cohort (Tukey's HSD; P<0.001), thirdly 3 accessions of sunflower from the second cohort (Girasole large, Girasole medium and Sunrich (Tukey's HSD; P<0.001). the last group puts the rest of the plants together (Tukey's HSD; P=1).

Similarly to Abbiategrasso, in Magenta the plant accession, cohort and their interaction had a significant effect on the distribution of the maximum damage caused by *O. communa.* (F>11.58, P<0.001). The effect of the third cohort showed in a Tukey test that was the highest for damage (Tukey's HSD; P>0.001), followed by the first cohort (Tukey's HSD; M=6.796, P<0.001) and the second (Tukey's HSD; P=0.043).

Following the test for comparing the plant accessions, we found that common ragweed was significantly different (Tukey's HSD; P<0.001) from the rest of the plant accessions regarding the maximum damage found (Tukey's HSD; P>0.06). It was also found significant the difference in maximum damage found in the variety Sunrich and the seedlings of the variety Girasole compared to *G. abyssinica* (Tukey's HSD, P<0.02).

The same test of Tukey HSD showed that the effect of the interaction between common ragweed and the third cohort is significantly different to the rest of the interactions (Tukey's HSD; P<0.001), but also that the effect of the interaction of the sunflower accessions in the third cohort (Tukey's HSD; P<0.05) plus common ragweed with the first cohort were different to the distribution of maximum damage on *G. abyssinica* and the rest of the interactions (Tukey's HSD; P>0.5).

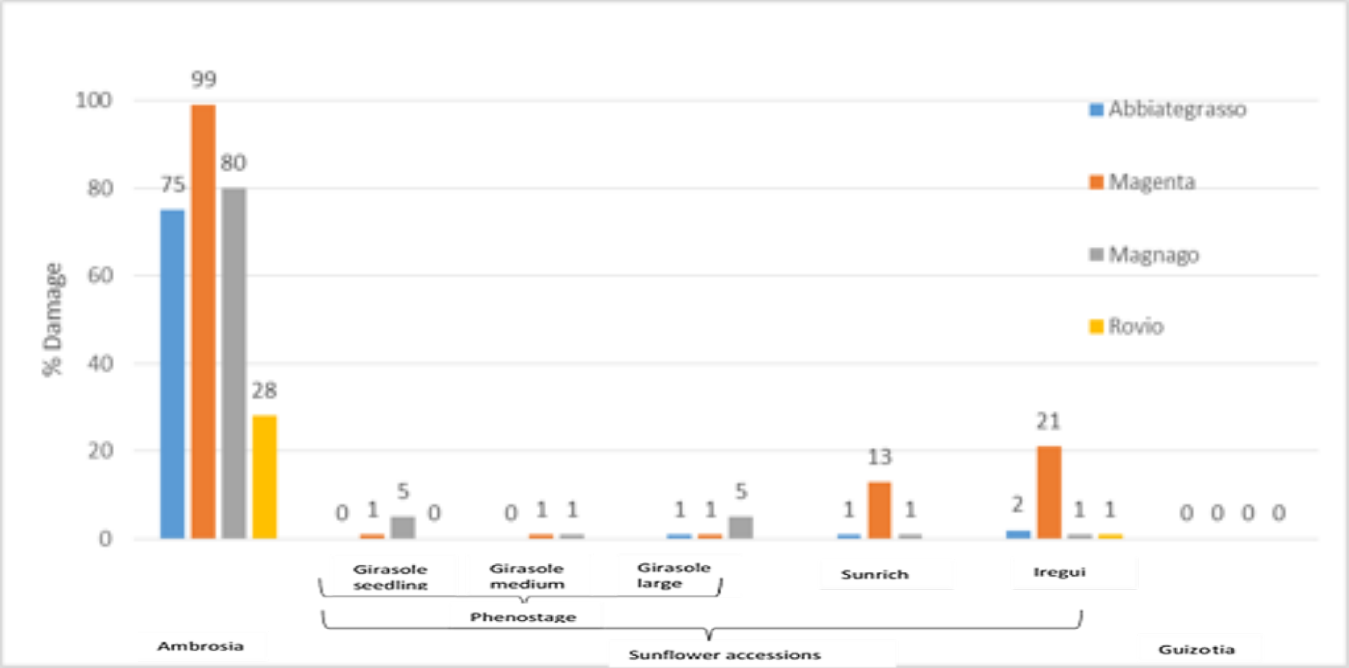


Figure 17: Distribution of the maximum damage among plant accessions within a site for cohort 1 (May-July). Data are based on 7 plant individuals per plant accession arranged in a latin-square.

In Magnago, cohort, plant accession and their interaction were significant (F>2.34, P<0.009). The Tukey test indicated that the last two cohorts differ significantly from the first one (Tukey's HSD; P<0.001). Common ragweed was significantly different (Tukey's HSD; P<0.001) to the rest of the plant accessions according to this post hoc test. Regarding the interaction between cohort and plant accession, the test indicated that common ragweed in the three cohorts (Tukey's HSD; P<0.02) was significantly different from the rest of the interactions in the distribution of the maximum damage.

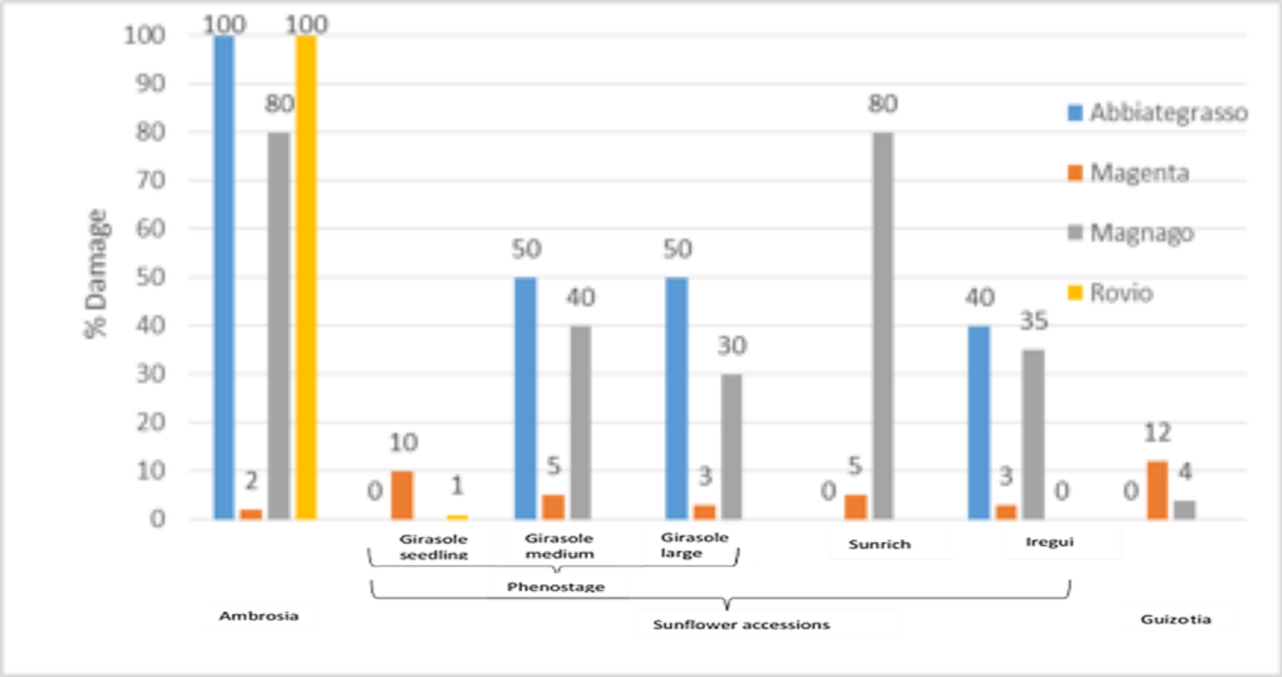


Figure 18: Distribution of damage among plant accessions within a site for cohort 2 (July-September). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

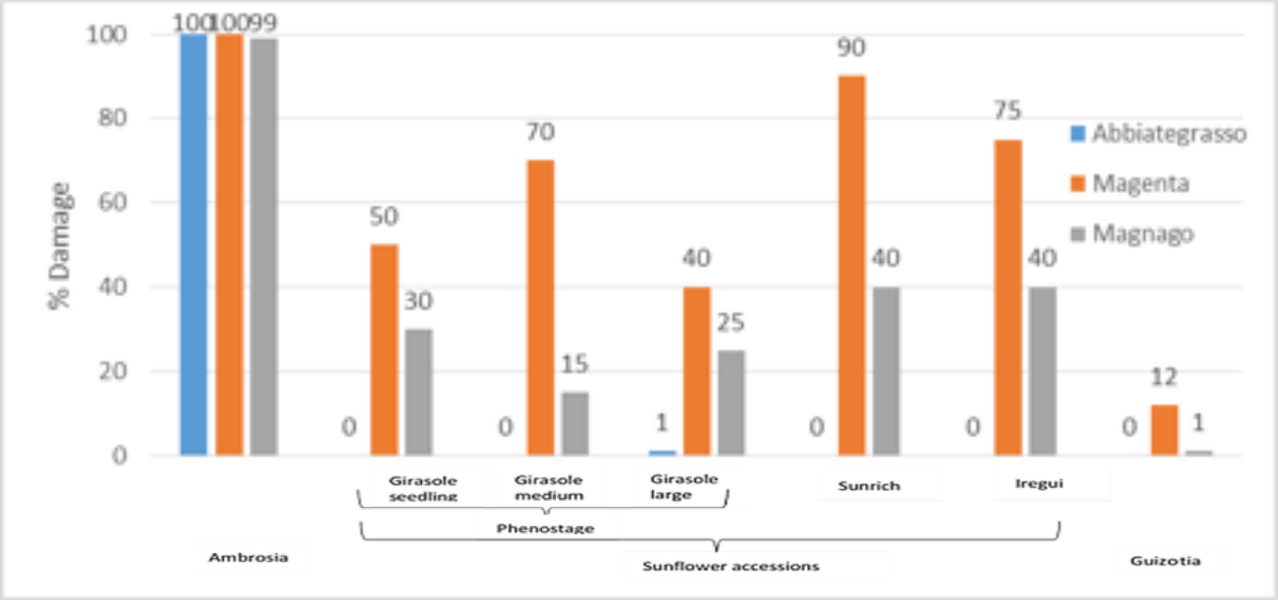


Figure 19: Distribution of damage among plant accessions within a site for cohort 3 (September-November). Data are based on 7 plant individuals per plant accession arranged in a latin-square (see text for details).

In Rovio, the effect of plant accession on damage caused by *O. communa* among plant accessions was significant ($F_{3,48}$=24.6, P<0.001) as well as the interaction between cohort and plant accession ($F_{3,48}$=6.69, P=0.003). A post hoc Tukey test indicated that common ragweed was significantly different from the rest of the accessions (Tukey's HSD; P<0.001), as well as the interaction between the second cohort and common ragweed (Tukey's HSD; P<0.001) was different from the rest of the interactions.

We also analyzed the influence of the average number of *O. communa* in the 4 different life stages on the maximum damage found on common ragweed. On the first cohort (Fig. 20) the average number of L1 larvae ($F_{1,176}$=7.09, P=0.01), pupae ($F_{1,176}$=10.98, P=0.001) and adult load ($F_{1,176}$=21.01, P<0.001) had an effect on the damage percentage. On the second cohort, presence of L3 larvae ($F_{1,161}$=29.62, P<0.001) and pupae ($F_{1,161}$=7.15, P=0.008) were significant for the maximum damage found on common ragweed (Fig. 21). Only adult load ($F_{1,142}$131.3, P<0.001) was found significant on the third cohort (Fig. 22)

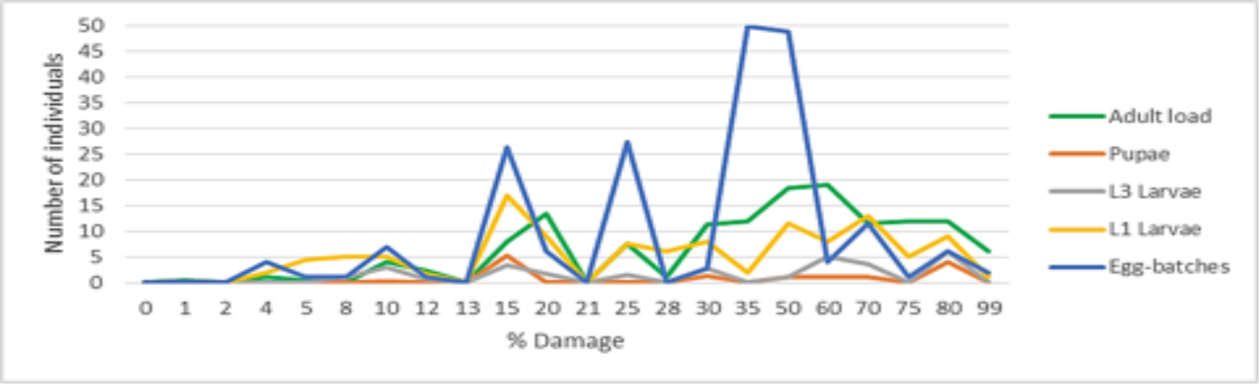


Figure 20: Number of *O. communa* individuals of the four life stages (egg-batches, L1 larvae, L3 larvae, pupae and adults) found on different percentages of feeding damage on *A. artemisiifolia* on cohort 1 (May-July).

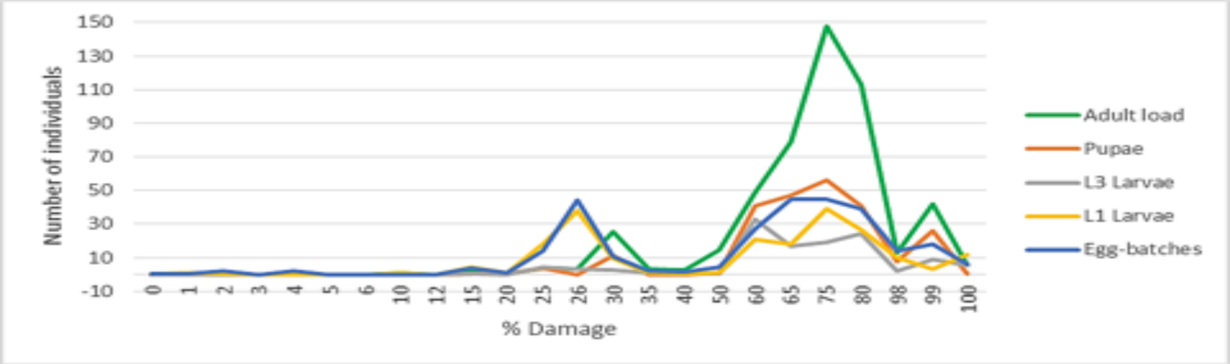


Figure 21: Number of *O. communa* individuals of the four life stages (egg-batches, L1 larvae, L3 larvae, pupae and adults) found on different percentages of feeding damage on *A. artemisiifolia* on cohort 2 (July - September).

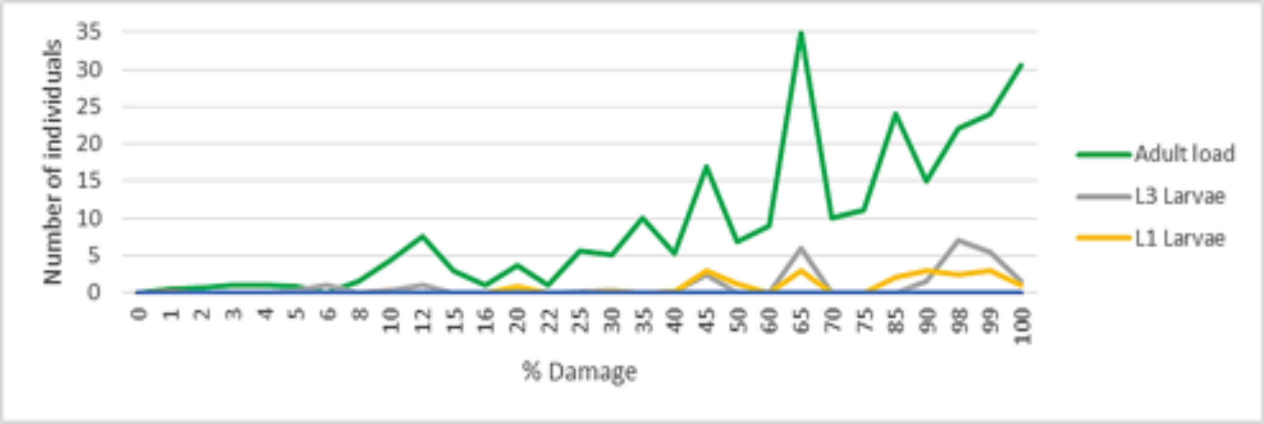


Figure 22: Number of *O. communa* individuals of the four life stages (egg-batches, L1 larvae, L3 larvae, pupae and adults) found on different percentages of feeding damage on *A. artemisiifolia* on cohort 3 (September - November).

Table 2: Summary of the distribution of the 4 stages of *O. communa* on the 7 plant accessions in the 4 sites.

***Ophraella* stage**	**Site**	**Cohort**	**TOTAL**	**Girasole seedling**	**Girasole medium**	**Girasole large**	**Sunrich**	**Iregui**	**Guizotia**	**Ragweed**
Egg batch	Abbiategrasso	1	**13**	0	0	0	0	0	0	100%
		2	**90**	0	16.67%	2.22%	4.44%	0	0	76.01%
		3	**0**	0	0	0	0	0	0	0
	Magenta	1	**138**	0	0	0	0	0	0	100%
		2	**48**	0	12.5%	0	2.08%	0	0	85.42%
		3	**0**	0	0	0	0	0	0	0
	Magnago	1	**103**	0.98%	0	0	0	0	0	98.03%
		2	**325**	0	0	0	0.98%	0	0	90.15%
		3	**0**	0	4.31%	3.69%	1.84%	0	0	0
	Rovio	1	**27**	0	-	-	-	0	0	0
		2	**90**	2.22%	-	-	-	0	-	97.77%
Larva L1	Abbiategrasso	1	**13**	0	0	0	0	0	0	100%
		2	**163**	0	6%	2.53%	7%	0	0	85%
		3	**100**	0	0	0	0	0	0	100%
	Magenta	1	**138**	0	0	0	0	0	0	100%
		2	**14**	0	0	0	0	0	0	100%
		3	**21**	33%	14.28%	0	0	0	0	52.38%
	Magnago	1	**203**	0.92%	0	0	6.48%	0	0	92.59%
		2	**230**	0	1.74%	2.17%	0.87%	0	0	95.21%
		3	**29**	0	0	3.44%	3.44%	0	0	93.10%
	Rovio	1	**27**	0	0	0	0	0	0	100%
		2	**100**	7%	-	-	-	0	-	93%
Larvae L3	Abbiategrasso	1	**12**	0	0	0	0	0	0	100%
		2	**46**	0	2.17%	2%	0	0	0	94.47%
		3	**0**	0	0	0	0	0	0	0
	Magenta	1	**5**	0	0	0	0	0	0	100%
		2	**0**	0	0	0	0	0	0	0
		3	**22**	4.53%	0	0	0	0	0	95.45%
	Magnago	1	**40**	0	0	0	5%	0	0	95%
		2	**156**	0	0.64%	0.64%	0	0.64%	0	98.07%
		3	**52**	7.69%	9.61%	3.85%	0	3.84%	0	0.75%
	Rovio	1	**3**	0	0	0	0	0	0	100%
		2	**12**	0	-	-	-	0	-	100%
Pupae	Abbiategrasso	1	**5**	0	0	0	0	0	0	100%
		2	**45**	0	4.44%	4.34%	6.67%	0	0	77.78%
		3	**1**	0	0	100%	0	0	0	100%
	Magenta	1	**1**	0	0	0	0	0	0	100%
		2	**0**	0	0	0	0	0	0	0
		3	**1**	0	0	0	0	100	0	0
	Magnago	1	**24**	0	0	0	0	0	0	100%
		2	**347**	0	0.28%	0.29%	1.15%	0	0	98.27%
		3	**6**	0	0	0	100	0	0	0
	Rovio	1	**0**	0	0	0	0	0	0	0
		2	**7**	0	-	-	-	0	-	100%
Adult Load	Abbiategrasso	1	**108**	0	2	0	1	0	0	50
		2	**163**	0	62	4	6	0	0	90
		3	**122**	0	0	1	0	0	0	121
	Magenta	1	**59**	3	3	0	3	0	0	50
		2	**7**	0	5	0	0	0	0	2
		3	**626**	48	53	54	52	44	25	321
	Magnago	1	**93**	1	2	0	1	0	0	89
		2	**865**	0	11	2	1	4	0	787
		3	**245**	3	10	7	13	6	0	168
	Rovio	1	**11**	0	0	0	0	0	0	11
		2	**19**	0	-	-	-	3	-	16
Maximum Damage	Abbiategrasso	1	-	0	0	1%	2%	1%	0	75%
		2	-	0	50%	50%	50%	0	0	100%
		3	-	0	0	1%	0	0	0	100%
	Magenta	1	-	1%	1%	1%	21%	13%	0	99%
		2	-	0	12%	0	1%	0	0	10%
		3	-	50%	70%	40%	75%	90%	12%	100%
	Magnago	1	-	5%	1%	5%	1%	1%	0	80%
		2	-	0	40%	40%	50%	35%	0	80%
		3	-	30%	15%	25%	40%	40%	1%	99%
	Rovio	1	-	0	-	-	-	1%	0	28%
		2	-	1%	-	-	-	0	-	100%

2.3.5. Analysis without target plant

When common ragweed was excluded from the analysis of number of egg-batches, the effect of plant accession was not significant ($F_{5,400}$=39.91, P=0.09) but the interaction between cohort and plant accession was ($F_{10,400}$=1.86, P=0.05). A post hoc test of Tukey shown that the combined effect of cohort 2 with sunflower Sunrich variety (Tukey's HSD; P<0.02). Regarding the distribution of first instar larvae, the effect of plant accession ($F_{5,400}$=2.52, P=0.03) was significant, but no effect was found for third instar larvae or pupae (F<1.19, P>0.3). The post hoc test of Tukey showed that sunflower Sunrich variety is significantly different from the rest of the plant accessions (Tukey's HSD; P<0.04). When common ragweed was excluded from the analysis of the load of O. *communa* adults of the plant accessions, no significant effect was found ($F_{5,400}$=1.61, P=0.16).

We found a significant effect of plant accession ($F_{5,400}$=4.63, P=0.001), cohort ($F_{2,400}$=3.9, P<0.001) and their interaction ($F_{10,400}$=42.25, P<0.001) for the distribution of maximum damage. The post hoc test of Tukey indicated that *G. abyssinica* (Tukey's HSD; P<0.04) was significantly less damaged than the other accessions. Also that the three cohorts were significantly differentiated from one another (Tukey's HSD; P<0.001). Finally that the interaction between cohort number three and three sunflower varieties (Sunrich, Iregui and seedlings from sunflower Girasole) were significantly different from the rest of the interactions (Tukey's HSD; P<0.008).

2.3.6. Comparison between phenostages of sunflower Girasole variety.

Comparing the distribution of egg-batches on the three phenostages of sunflower Girasole variety (seedling, medium and large), we found a significant effect of cohort ($F_{2,205}$=23.12, P<0.001), phenostage ($F_{2,205}$=8.04, P=0.004), and also of their interaction ($F_{4,205}$=9.16, P<0.001). The Tukey posthoc test showed that the medium phenostage (Tukey's HSD; P=0.003) is significantly different from the large phenostage and the seedling, and that medium phenostage in the second cohort had a higher effect on the distribution of egg-batches compared to the rest of the interactions of cohort and phenostages. The analysis for L1 larvae, L3 larvae and pupae showed no significant effect of phenostage ($F_{2,205}$<2.88, P>0.52) nor the interaction between cohort and phenostage ($F_{4,205}$<0.97, P>0.42) were significant.

For maximum damage, the effect of the interaction of cohort and Girasole variety phenostage was significant ($F_{4,205}$=5.45, P<0.001). A post hoc test of Tukey indicated that the interaction between the third cohort with the three phenostages were significantly different from the rest of the interactions (Tukey's HSD; P<0.03).

2.4. Discussion

There was a clear preference of *O. communa* in all sites for oviposition on common ragweed, especially in the first cohort between June and July when there is an overlap of the first two generations (Bosio *et al.*, 2014), as almost 100 % of the egg-batches were found on common ragweed. Temperature and humidity were found to have a strong influence on oviposition behavior of *O. communa* (Zhou, Guo, Chen, & Wan, 2010), temperature differences between seasons may explain the peak we found in July at the end of the first cohort (Annex 5). Moreover no more egg-batches were found on any plant accession in September, which was already observed in the region of Piedmont (Bosio *et al.* 2014). *O. communa* enters in diapause before overwintering (Watanabe & Hirai, 2005), and stops all oviposition behaviour. This could be regarded to be a beneficial life-history trait when assessing the risk of *O. communa* attack to non-target plant species, because early sunflower varieties are harvested (OHS sementi e consigli, 2014) by the time *O.communa* increases oviposition on sunflower.

Oviposition behavior apparently is more plastic than larval host range (Schaffner, 2001) and adults accidentally laying eggs on non-host plants may occur eventually. Even if there was a significant high number of egg-batches on sunflower varieties on the second cohort, larvae may have left after hutching in search of their preferred host (Welch, 1978). According to Müller *et al.* (2014) oviposition on sunflower under choice conditions is low, and most of the recently hutched larvae move away searching for common ragweed, their preferred host plant. High mobility was observed in previous laboratory and field experiments (Welch, 1978; Tanaka & Yamanaka, 2009; Hu & Meng, 2007). A clear gap between the number of eggs on the assessed plants and the larvae suggests high levels of mortality of young larvae and eggs, thus mortality assessments for eggs and first instar larvae by predation or climatic variables are still needed to be further investigated.

The number of larvae and pupae found in the first two cohorts, increased as expected throughout the seasons, for the population growth of *O. communa* between mid-May and August (Zhou, 2010). Larvae completing a full-cycle on non-target plants could signify an ultimate challenge in the prediction of risks to non-target species, as it suggests that a physiological adaptation to the new host, could have a tendency to rapid evolvability (Schaffner, 2001). Egg-batches and larvae of *O. communa* were found in all varieties of sunflower, except for Sunrich variety, which paradoxically registered the highest damage caused by adults late in the growing season. Sunflower Sunrich is an ornamental variety, thus feeding damage can imply unfavorable consequences for its production.

Punctual damage on non-target plants increases when the preferred host has a decrease on its abundance (Sheppard *et al.*, 2005).Damage on all plant accessions was the highest in the third cohort, coinciding with the increase of adult beetles at the end of august, when different generations co-occur and the population of *O. communa* builds up quickly (Fukano & Yahara, 2012), common ragweed was heavily attacked and destroyed already by the third assessment (Annex 5, Fig. A6, A12 & A17). In many risk assessments, non-target species recorded an increase of feeding damage when the abundance of the preferred host decreased (Watanabe & Hirai, 2004), when insects become sufficiently deprived to accept lower ranked hosts (Van Klinken, 1999). This might explain the increase of feeding damage on non-target plants after the third assessment, even *G. abyssinica* that remained untouched for the three cohorts got adults feeding on it when common ragweed was no longer available. Damage on common ragweed throughout the growing season was apparently related with the abundance of the different life stages of *O. communa.* Damage over 70% in the first cohort was found on plants with the highest number of egg-batches, larvae and adults found. For the second cohort, the highest load of adult beetles corresponded to plants with over 70% of damage; lastly for the third cohort, the damage levels depended almost entirely on the load of *O. communa* adults. Damage caused by adult feeding on common ragweed was found to be the most determinant late in the season (Fukano, 2014), but there is not much evidence of the influence of the abundance egg-batches and larvae on damage levels. Impact on non-target plants is not proportional to level of damage caused by the potential control agent, thus predicting impact on non-target plants is better explained with the ratio of agent attack rate (Sheppard *et al.*, 2005). In the experiment we assessed ratio of damage and percentage to be able to predict impact. Levels of damage was used to compare between cohorts, as one of objectives of the study was to evaluate incidence of attack throughout the growing season.

Oviposition and feeding behavior of biocontrol agents was in several studies correlated to plant phenology and therefore assessing them on different phenotypic stages is important for understanding the impact of the biological control (Collins & Müller-Schärer, 2012). In the case of Girasole variety, we found that medium stages were preferred for oviposition, especially in the second cohort. As Girasole is an early variety, damage in the first cohort would have been more likely to put in risk crops, but the survival of the plants may be dictated mainly by the earliest phenostages (Collins & Müller-Schärer, 2012). Delaying the planting date of sunflower was effective measure to reduce populations of a damaging Chrysomelide beetle *Zigogramma exclamationis* (Charlet & Knodel, 2003), thus a similar solution could be found for *O. communa* in the case of sunflower Girasole variety being attacked in the region.

Data from the second cohort in Magenta and the third in Abbiategrasso may not have been representative enough, given that the last three assessments in both cases suffered from plant loss, as a drought in the fields in Magenta dried out most of the plants and small mammals consumed the majority of plants from the experiment. The site of Rovio in Switzerland had special requirements for the use of common ragweed plants for the experiments. The difference in the design could explain the relatively lower abundance of *O. communa*, but population dynamic studies are being carried out in the same sites (Lommen: unpublished data, 2014) therefore we expect to find the real difference of population sizes between these sites.

3. PERFORMANCE EXPERIMENT

3.1. Introduction

Growth performance is referred commonly to a series of parameters related to weight gain, height or length increase in rearing assessments (Workagegn, 2012). The measurements of growth performance differ depending on the taxa that is being studied (Roder, Rahier, & Naisbit, 2008). In insects, the parameters includes survivorship and developmental time to produce adults with different fecundities and fertilities (Schaffner, 2001) but also performance is referred to the rate of mass gained in one of its development states (Roder et al., 2008). Performance is usually evaluated with no-choice tests, in which only one of the alternative hosts is available (van Klinken, 1999). As a part of a host specificity testing, non-choice tests confine life stages of the potential biocontrol agent onto one species at a time, especially for evaluating relative suitability of hosts for development. (Sheppard *et al.*, 2005). Larval performance on alternative hosts is compared by using growth parameters and development time (Louda *et al.*, 2005).

Ophraella communa forms part of the chrysomelidae family, most commonly known as leaf beetles, which is one of the richest in number of species worldwide with around 50000 species distributed in 19 subfamilies (LeSage, 1986) as well as in central Europe, with around 539 species (Harde & Severa, 2000). All known adults are phytophagous, feeding from all sorts of plant tissue, and mainly prefer deciduous trees, bushes and weeds as host plants (LeSage, 1986).

Many Chrysomelidae feed only on a single plant species or on a few closely related plants, meaning that most of them are host-specific (Clark *et al*, 2004). Plants that are subject to consumption by small herbivores, such as by insects, are known as host plants (Ricklefs & Miller, 2000). One of the most accepted hypothesis about the specialization on host specificity posits that a given insect species cannot simultaneously maximize performance on host plants with different defensive compounds due to the cost of maintaining a greater range of detoxification enzymes, making generalists less efficient than specialists (Keese, 1998).

This close relation between beetles and host plants has positioned some Chrysomelidae species as major pests, due to their feeding habits on important cultivated plants, with over 25 species included in the Midwest list of Vegetable Insect pests (Foster & Flood, 1995), other beetle species are considered beneficial due to their use as biocontrol organisms against invasive weeds and shrubs, because of their feeding specificity on these plants (Martinkova & Honek, 2004).

3.2. Materials and methods

To evaluate the difference in performance of the specialist herbivore *Ophraella communa* when fed on two host plants: *Ambrosia artemisiifolia* (common ragweed) and *Helianthus annuus* var. PR64H42 (sunflower), Five performance variables were measured (larval survival, larval developmental time, pupae survival, pupae developmental time and dry weight of adult *O. communa* after eclosion) of 72 larvae of *O. communa* from 12 different genetic families. A family was defined as half-sib larvae hutching from the same egg-batch and was taken in consideration to assess the influence of genetic variation among populations of *O. communa* on the performance.

3.2.1. Growth conditions of the study plant species

40 ragweed plants and 40 sunflower plants var. PR64H42 (Girasole) were grown in the greenhouse at University of Fribourg, about two months before the experiment began, with a day/night regime of 16/8 hours and a temperature of 28/20 ºC, planted in narrow plastic pots of dimensions 20 cm ×5 cm Ø using 2/3 of the soil Proter + Type 4, Bern, Switzerland mixed with 1/3 of sand. The seeds of sunflower came from UFA Samen, Winterthur, Switzerland and the seeds of common ragweed came from a single mother plant coded SASBAN (SMARTER Ambrosia Seed Bank Accession Number) 66.10 from a population in Busto Arsizio, Italy.

3.2.2. Rearing conditions of *Ophraella communa* larvae

21 rearing jars were prepared in Corbetta, Italy, to obtain the half-sibs egg-batches of *O. communa* by planting a single ragweed plant in a closed cylindrical plastic jar of dimensions 15.5 cm ×10 cm Ø with neutral soil from OBI, Magenta, Italy. To allow gas exchange, the lid of the jar was perforated with a hole of 7cm Ø area which was covered with 20 cm × 20 cm mesh tissue by placing it underneath the lid. The plants used for the rearing jars were grown in a greenhouse at University of Fribourg, Switzerland, in the same condition as the plants used in the experiment.

Adults of 8 different populations were collected and sexed in the area of the Malpensa airport, Italy and in Rovio, Switzerland (Fig. 1). Inside each rearing jar, three adults of *O. communa* (two males and one female) were locked together to ensure that the produced egg-batches on the rearing boxes were at least half-sibs. I checked daily for egg-batches and watered the plants every two days, making sure the adults wouldn't flee. Ten days after, 72 recently hutched larvae were selected from 12 egg-batches (defining 1 egg-batch as 1 insect family) from different rearing jars (1-3 families were used to represent each population). The chosen egg-batches had six or more larvae hutched in the last two days. All of the 8 populations were represented at least by one insect family.

Figure 23. Map of the Malpensa area. The stars show the eight different populations of *Ophraella communa* where they were collected.

3.2.3. Experimental setup

Both ragweed and sunflower plants with an average height of 40-60 cm were transported to Corbetta, Italy, (45.47089 N, 8.98683 E) where the experiment took place. An abandoned field site between crops that was arranged by the local authorities was used for the experiment, where both common ragweed and *O. communa* occur.

18 ragweed plants and 18 sunflower plants were transplanted into plastic pots and were grown in the neutral soil from OBI. Three metal cages of dimensions 2 m × 1 m × 1 m that covered with tissue mesh cloth were set up. Each cage contained 12 plants (6 sunflower plants and 6 ragweed plants) that positioned randomly with 20 cm of distance between them (Fig.2).

Each of the 36 plants got 2 larvae of *O. communa* out of the 12 recently hutched egg-batches from different insect families available. One of the larvae was placed on an upper leaf and one on a lower leaf. To isolate the larvae of *O. communa* from each other, a single larva was placed inside a clip-on cage (i.e. a limited feeding chamber), where it can freely move in a circle of 2.5 cm diameter. Once the larva has eaten all available leaf surface, the entire clip-on cage with the larva inside the feeding chamber is displaced to a new leaf, so the larva can continue feeding on a new leaf surface. The clip-on cages were sustained by attaching them to bamboo sticks (Fig. 2).

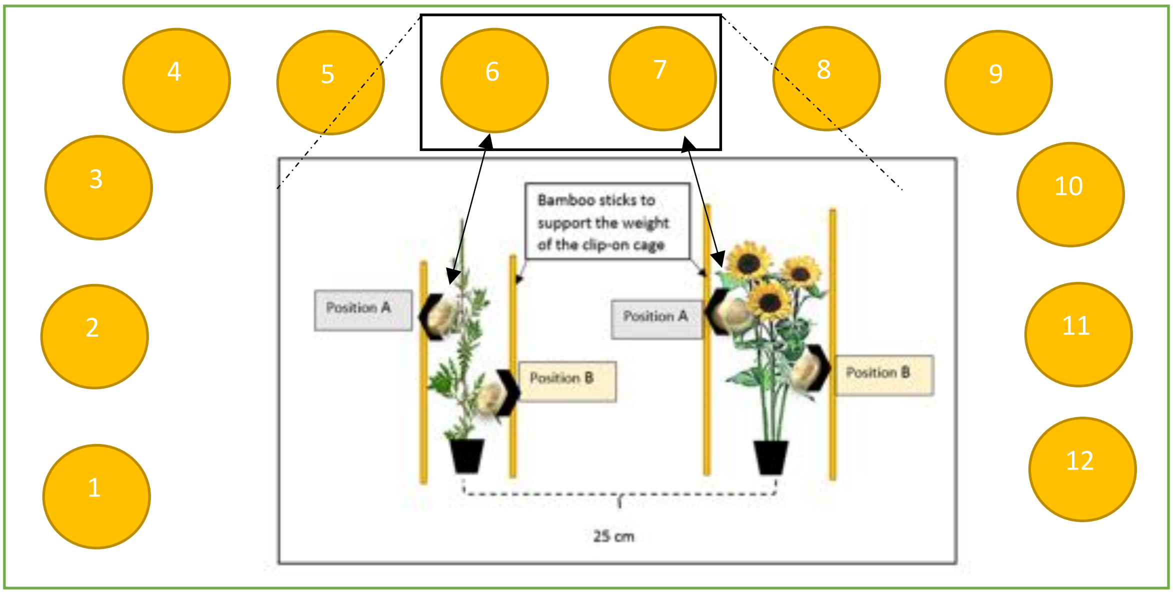


Figure 24: Position of the pots containing the 2 plant species (*Ambrosia artemisiifolia* and *Helianthus annuus* var. PR64H42 inside the cage. For each cage the position of the 6 *A. artemisiifolia* plants and 6 *H. annuus* were randomized. An upper position (Position A) and a lower position (Position B) were chosen to set up the larvae inside clip-on cages. Clip-on cages were then supported by attaching them to bamboo sticks, allowing also readjustment of the clip-on cage once the leaf area covered was eaten.

The clip-on cages were self-produced at the University of Fribourg, Switzerland, following a modified design proposed by Thomas Mowry (1993) used originally to confine aphids on leaves, but we adequate the design adapting to the dimensions of *O. communa* beetles (Annex 3). This design consists mainly in two perforated aluminum blades attached by a binder clip that gives the clipping quality; and a feeding chamber in the perforated part of the blades, with walls made of foam resistant to heat and humidity and covered in mesh tissue to avoid insects from escaping, but allowing gas exchange at the same time. Cable binders and aluminum wires were used to attach the blades to the binder clip, and the feeding chamber was fixed using hot glue.

The distribution of the 72 (6×12 insect families) larvae was randomized (Table 1) so that 3 larvae were placed in ragweed and 3 on sunflower, avoiding repeating leaf position or cage.

Table 3: Randomization of the larvae obtained from 12 families inside the clip-on cages on top and low positions, distributed in 3 cages as blocks. White indicates to feed the beetle on a sunflower plant and grey means to feed the beetle on a ragweed.

Position	CAGE 1											
UP	F1	F11	F3	F7	F4	F2	F8	F10	F5	F9	F12	F6
DOWN	F7	F5	F4	F2	F9	F10	F11	F9	F3	F6	F8	F12
Position	CAGE 2											
UP	F10	F8	F9	F11	F6	F2	F12	F3	F7	F1	F4	F5
DOWN	F9	F5	F11	F6	F10	F1	F5	F12	F4	F7	F2	F8
Position	CAGE 3											
UP	F3	F12	F6	F2	F10	F1	F3	F8	F9	F7	F4	F11
DOWN	F6	F5	F10	F8	F2	F9	F7	F4	F3	F1	F11	F12

3.2.4. Monitoring

Clip-on cages were checked daily for survival, recording the state at which the individuals were found during the 28 days the experiment took place, coding L= larvae; P= pupae and A= adults. Every two or three days, when the food provision started to run out, the clip-on cage was moved to other leaves for fresh green tissue, taking care not to disturb the larvae. Once an individual switched to pupa, the clip-on cage was no longer re-accommodated. Adults that hatched were killed by freezing and transported back to the University of Fribourg to be sexed and weighted in a high sensitive balance.

3.2.5. Statistical analysis

All analyses were performed in R (Version 3.1.2, the R foundation for Statistical Computing 2014). (Generalized) linear mixed models (GLMMs/LMMs) were fit using the glmer/lmer functions obtained from the R package "lme4", with maximum likelihood to estimate the model parameters, to explore the effect of plant species and family on the performance of *O. communa*. We first assessed the number of days *O.* communa survived during the course of the experiment (maximum 28 days for the duration of the experiment) regardless of the final stage they achieved (larva, pupae or adult), using the number of days alive as a Poisson response variable with GLMMs. We then analyzed the performance of each stage of *O. communa.* Larval/pupae survival were analyzed as a binomial response variable (a binary response of survival or death of each larva or pupa) with GLMMs. Larval developmental time (considering the number of days a larva was alive before developing into pupae) was analyzed as a Poisson response variable with GLMMs. Pupae developmental time (the number of days a pupa was alive, considering only the pupae that developed into adults) was analyzed as a Poisson response variable with GLMMs. The biomass of adults (dry weight of each individual adult) after log-transformation was then used as the response variable in LMMs with a Gaussian distribution. In all these models, the plant species, insect family and/or sex (adults after eclosion) were included as fixed factors and cage and position were treated as random factors.

3.3. Results

Considering the days survived of the individuals of *O. communa* throughout the 28 days of the experiment, the effect of plant species (χ^2=16.101, P<0.001), the effect of the interaction of insect family and plant species (χ^2=31.854, P<0.001) and the effect of insect family (χ^2=23.111, P=0.017) were all highly significant. We also analyzed each stage of *O. communa* regarding the survival and developmental time of larvae and pupae, as well as the dry weight of adults after eclosion, with longer survival on ragweed (Fig. 3).

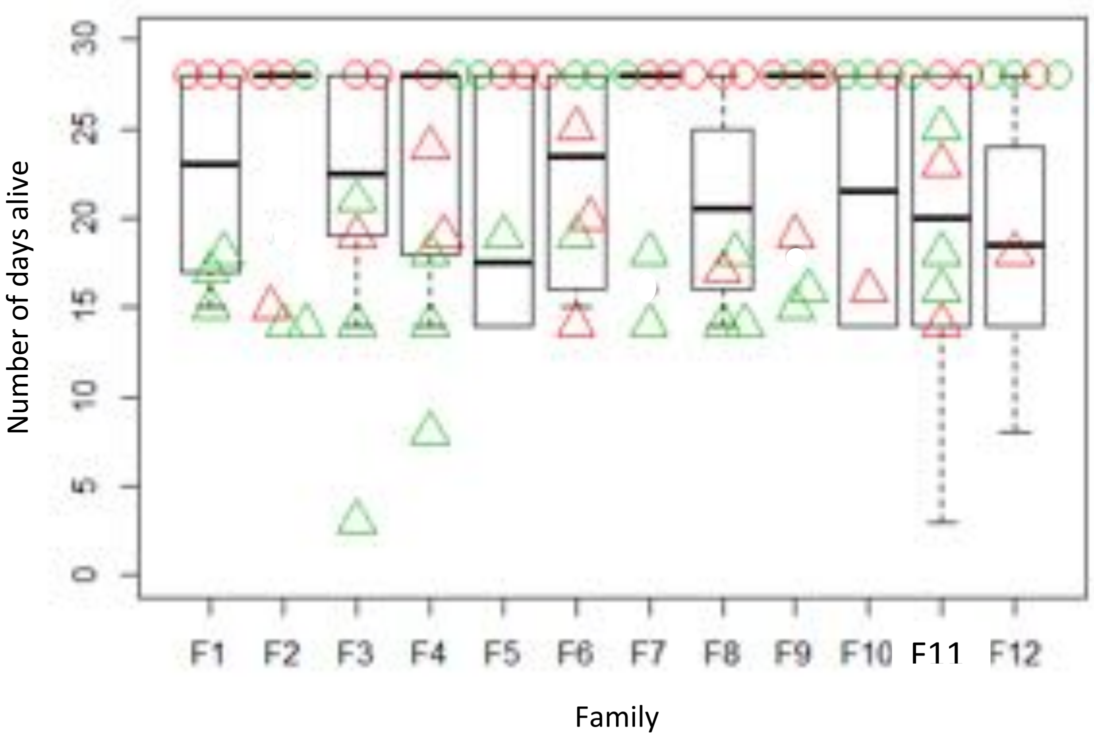


Figure 25: Survival of the 12 families of *O. communa* during the experiment. Red figures represent the three beetles per family that fed on ragweed and green figures show the three beetles that fed on Sunflower. Triangles indicate death and circles indicate surviving at the end of the experiment.

3.3.1. Larval performance of *Ophraella communa*

The larval survival of *O. communa* (yes/no) was higher when fed on ragweed than sunflower (χ^2=5.668; P=0.017), 66.7% (N=36) of the larvae developed into pupae on ragweed while only 38.9% (N=36) on sunflower. For larvae that developed into pupae, there was no significant difference between larval developing days on ragweed and on sunflower (χ^2=0.067; P=0.795) (Fig. 4).

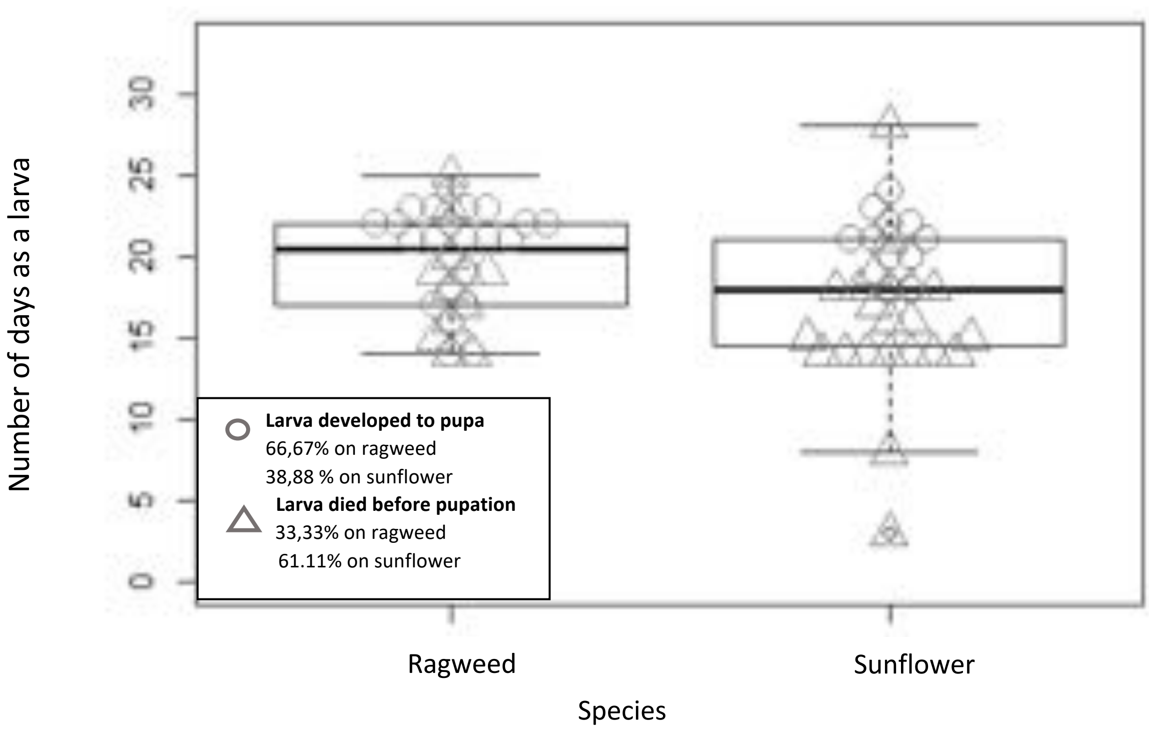


Figure 26: Larval performance on ragweed and sunflower. The boxplots show the number of days that *O. communa* survived as larvae. The larval survival is also represented in the figure. Triangles indicate larvae that perished and circles indicate surviving larvae.

The effect of the insect family was not significant (χ^2=18.953, P=0.062) for the larval survival (yes/no), but the interaction of plant species and insect family (χ^2=24.258, P=0.012) was significant (Fig. 5).

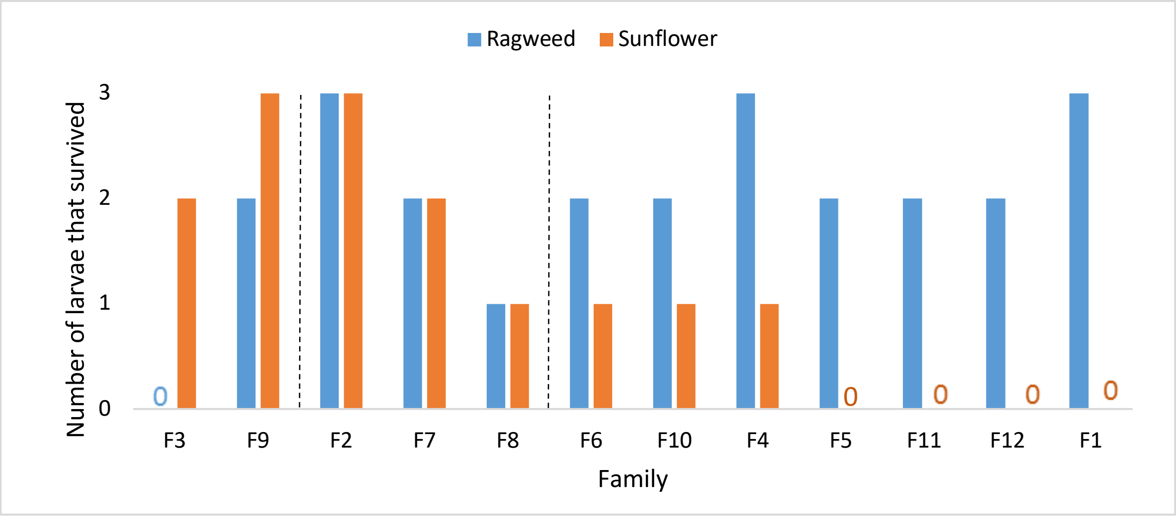


Figure 27: Number of surviving larvae of the 12 families (F1-F12). Bars in red show the number of larvae surviving on sunflower and the ones in blue on ragweed. The families are separated in 3 groups, with families performing better on sunflower (left); families performing equally on the two plant species (middle) and families performing better on ragweed (right).

3.3.2. Performance of *Ophraella communa* pupae

Similarly to larval survival, plant species also affected pupae survival (χ^2=8.191, P=0.042), with 95.83% (N=24) of the pupae surviving on ragweed until the experiment was over or developing into adults, and 78.5% (n=14) of the pupae surviving on sunflower (Fig. 6). The interaction of plant species and the *O. communa* family (Fig. 7) had a strong effect on survival (χ^2=24.602, P=0.010), but not on developmental time (χ^2=6.253, P=0.396) and there was no significant effect from insect family or plant species (χ^2<8, P>0.7).

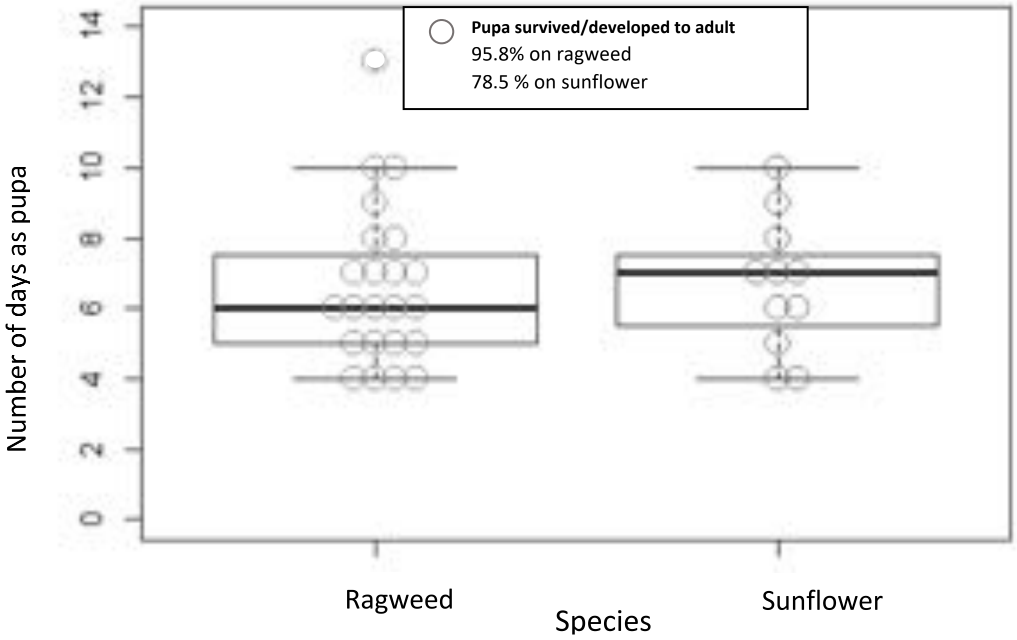


Figure 28: Pupae performance on ragweed and on sunflower. The boxplots show the distribution of the days of pupa spent developing to adults (or to the end of the experiment). The circles represent each pupa, taking in account only those that survived (the 4 pupae that perished were excluded).

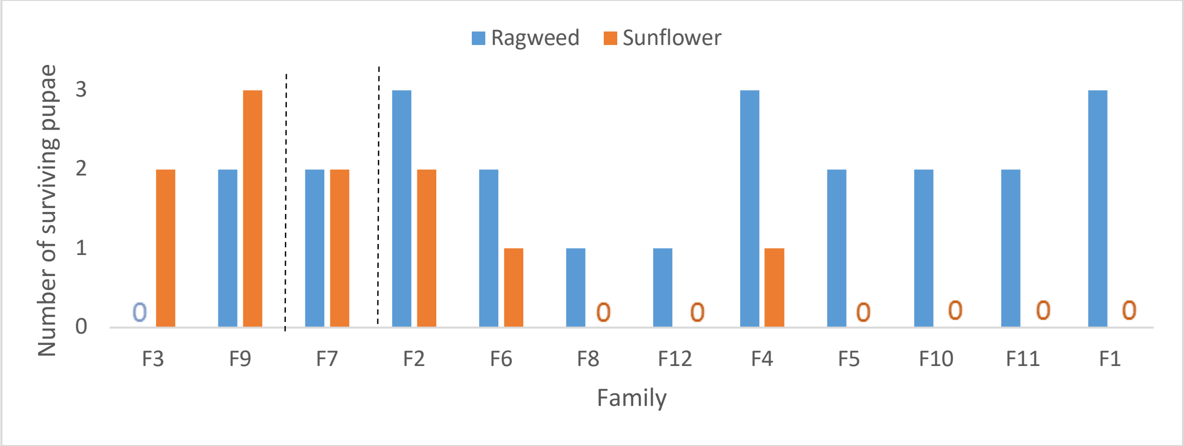


Figure 29: Number of surviving pupae of the 12 families (F1-F12). Bars in red show the number of pupae surviving on sunflower and blue on ragweed. The families are separated in 3 groups, with families performing better on sunflower (left); families performing equally on the two plant species (middle) and families performing better on ragweed (right).

3.3.3. Weight after eclosion of *Ophraella communa* adults

Regarding the final dry weight of adults after eclosion (20.8% (N=72) of adults developing into adults during the course of the experiment), no effect of family, plant species nor their interactions was significant (χ^2<7.800, P>0.400), but the effect of sex of the adults was highly significant (χ^2=24.525, P<0.001) with females being heavier. In addition, also the interaction of sex of the adults and plant species (χ^2=28.910, P<0.001) was significant, but only four females (three on ragweed and one on sunflower) and 11 males (nine on ragweed and three on sunflower) eclosed.

3.4. Discussion

The performance of phytophagous insects often depends on the quality of the plant host as a source of food, due to the strong specialization from most of these insects to a restricted group of hosts. (Roder et al., 2008). Regarding survival, there was a significantly higher mortality of larvae enclosed onto sunflower than on common ragweed. Lower survival rate of *O. communa* on sunflower was already observed (Ya-peng et al., 2007), but it is considered to be part of its fundamental host range (Funk, Futuyma, Orti, & Meyer, 1995).

Suitability of plant host comparison can be done by insect growth parameters and proportion of neonates that develop to adults for plant host suitability (van Klinken, 1999). In the experiment we did not find difference in growth parameters (survival, developmental time and adult weight after eclosion) of *O. communa* when fed on sunflower comparing to feed on common ragweed. Not surprisingly there was a significantly higher proportion of neonates developing to adults on common ragweed, suggesting that common ragweed is more suitable for *O. communa*. Completion of larval development for insects is an essential step in their life cycle (van Klinken, 1999), in the study a proportion of larvae completed their life cycle on both plant species. The use of *O. communa* as a biological control for ragweed was rejected in Australia due to similar results (Müller-Schärer *et al.,* 2014), although this fact should not be disregarded when assessing risks on non-target species, Non-choice tests on larval development have been repeatedly debated because results can lead to elimination of biological control agents that would be safe to release (Schaffner, 2001), by generating “false negatives” when the insects are forced to face a choice that would not be relevant in the field (van Klinken, 1999). Although the objective of our study is to predict potential risks on non-target species by olliphagous leaf beetle *O. communa*, the final conclusion for spread is based in all the experiments done. It is already known that sunflower is a host species to *O. communa* (Funk et al., 1995) and that it is naturally attacked by *O. communa* in Italy (Bosio, *et al.,* 2014). our experiment could allow us to explore the effect of half sibs on performance and the possibility of sufficient genetic variation in host specificity among populations.

Common ragweed is accepted as the most suitable host plant of *O. communa* (Bosio *et al.*, 2014; Watanabe, 2003; Kiss, 2001). Agent performance in different life stages is not always correlated with host plant preference (Sheppard *et al.*, 2005). We found that developmental time of *O. comunna* larvae on sunflower and on common ragweed were not significantly different, but this should not be taken as a fact for defying the host specificity of *O. communa*, which are a two-dimensional concept, involving host range breadth and relative acceptability of host (Van Klinken, 1999).

Insect family was found to have an effect on performance of the larvae when fed on both plant species. We found that the insect families 3 and 9 (Fig. 27), had lower mortality and longer survival on sunflower than on common ragweed. Experiments on different *Ophraella* species exchanging plant hosts showed no evidence of sufficient genetic variation to adapt to a new plant host (Futuyma, Walsh, Morton, Funk, & Keese, 1994), due to constrains that avoid beetles to accept other plant hosts than their preferred one (Futuyma et al., 1994). Genotypes could vary dramatically on the use and acceptance of diverse non-target host plants (van Klinken, 1999). There is a possibility that genetic variation between the assessed insect families of *O. communa* can explain the difference in performance among them, but the data set used in the experiment is too small to allow us come to conclusions yet. Evaluation of genetic variation between populations of *O. communa* is being carried on by the SMARTER project (Sustainable management of *Ambrosia artemisiifolia* in Europe), in which populations from all the insect families were included, and will hopefully provide a better understanding of the difference on performance of *O. communa*.

4. NON-TARGET SURVEY

4.1. Introduction

Fundamental host range represent the absolute limits of the host range of an insect (van Klinken, 1999) and includes all plant species where an agent can complete its life cycle (Schaffner, 2001). The realized host range is the expression of the fundamental host range under different conditions (van Klinken, 1999) such as environmental settings and genetic variation in host acceptability of insects (Sheppard *et al.*, 2005). The list of plants considered in the realized host range is influenced by the sort of species that co-occur with the herbivore, and whether it can be recognized by it (Schaffner, 2001). Host specificity tests in the field help predicting risk analysis previous to release of a control agent (Sheppard, 2005).

The plant species for host specificity tests are selected by the centrifugal phylogenetic evaluation of risk procedure, which prioritize a sequence of potential hosts, from the most closely related to the more distant included in the fundamental host range (Schaffner, 2001). This method is based in two components, the first (Component A) includes test plants in order of taxonomic relatedness beginning with other forms of the same species, then other species form the same genus, and continues until order. The second component (Component B) includes safeguard criteria, such as cultivated plants related to target weed, crop plants or any other plants on which the candidate has been previously recorded (Briese, 2003). In the case of field surveys, not only the phylogenetic relatedness needs to be considered, but also an overlap of habitats and a similar life-history and form (Briese, 2003), or in other cases plant architecture, phenology or similar chemistry may need to be considered (van Klinken, 1999).

Surveys regarding attack by potential biological control agents on plant species occurring sympatrically with the targeted plant in the study range, provide important data on the range of plant species that actually are accepted by the insect in natural condition (Schaffner, 2001). Relative availability and suitability of target and non-target plant species must be considered in a heterogeneous environment (van Klinken, 1999).

Ophraella communa is an oliphagous beetle, it was previously recorded feeding on various species from the genus *Ambrosia, Xanthium* and *Helianthus*, as well as *Iva axilaris, Dittrichia graveolens, Parthenium hysterophorus, Ratibida pinnata, Artemisia annua* and *Bidens pilosa* (Annonymous, 2015). In the region of Lomabardia and Piedmont after it was introduced, it was observed feeding on *Xanthium strumarium* and *Ambrosia trifida,* but also some feeding damage found on *Helianthus tuberosus* (Bosio *et al.*, 2014) and on *Inula graveolens* (Müller-Schärer *et al.*, 2014).

4.2. Materials and methods

The non-target survey of *O. communa* evaluates presence, feeding damage and oviposition of the beetle on non-host plants, relaying on the centrifugal Phylogenetic scheme to select plant species to monitory. This survey focus on finding populations of the selected non-target plant species where there's also an occurrence of common ragweed to test the presence of eggs, larvae, pupae, adults and a level of damage in percentage, following a damage guideline (Annex 5).

4.2.1. Study species and study sites

The species to be evaluated (Table 4) were selected from a list of potential hosts (Annex 10), which determined according to the centrifugal phylogenetic method. The first list was shortened (Table 4) when considering only plant species which share the area of occurrence of *O. communa* in the area.

Table 4: Selected species for the non-target survey. The species are ordered according to the phylogenetic distance to *Ambrosia artemisiifolia.*

Species	Tribe	Subtribe	Family	Category
Ambrosia artemisiifolia	Heliantheae	Ambrosiinae	Asteraceae	**Exotic**
Ambrosia trifida	Heliantheae	Ambrosieae	Asteraceae	**Exotic**
Xanthium strumarium	Heliantheae	Ambrosinae (Xanthiae)	Asteraceae	**Exotic**
Helianthus tuberosus	Heliantheae	Helianthinae	Asteraceae	**Exotic**
Helianthus annuus	Heliantheae	Helianthinae	Asteraceae	**Cultivated**
Bidens tripartitus	Coreopsidae	Bidentinae	Asteraceae	**Native**
Inula salicina	Inuleae	Inulineae	Asteraceae	**Native**
Inula sp	Inuleae	Inulineae	Asteraceae	**Native**
Pulicaria dysenterica	Inuleae	Inulineae	Asteraceae	**Native**
Buphthalmum salicifolium	Inuleae	Inulineae	Asteraceae	**Native**
Xerolekia speciosissima	Inuleae	Inulineae	Asteraceae	**Native** **Endemic**
Conyza canadensis	Astereae	Erigeroneae	Asteraceae	**Exotic**
Artemisia vulgaris	Anthemideae	Artemisineae	Asteraceae	**Native**
Picris hieracioides	Cichorieae	Picrideae	Asteraceae	**Native**
Centaurea nigrescens	Cardueae	Centaureeae	Asteraceae	**Native**
Persicaria maculosa			Polygonaceae	**Native**

The map in Fig. 30, shows the location of the sites and the nature of the flora that was evaluated, recognizing cultivars, exotic plants, mixed of native and exotic or, in the case common ragweed was not present, only native plants were monitored.

Figure 30: Map with the Localities where the Non-Target survey was carried out between early August and early September.

4.2.2. Measurements and data processing

The survey was designed as a two-page form to be filled out (Annex 10), contemplating information about the selected area, the characteristics of the population of the selected plant and a similar form for common ragweed, plus the evaluation of 50 plants per species and the search for new host plants in the same area. The number of replicates varies from one species to another, depending on the quantity of surveys being made, counting on having at least three of all species evaluated. All registered data was then evaluated using Microsoft® Excel 2013 and R (Version 3.1.2).

4.3. Results

We carried out 65 surveys on 28 sites (3 in Switzerland and 25 in Italy) on the 16 selected non-target species (see Annex 12 for further info). The results are summarized on Table 6, rows indicating sites and columns indicating plant species ordered from more closely related to common ragweed to more distant. Abundance of common ragweed and *O. communa* per site are coded by the figures shown in the column of common ragweed. If egg-batches (E), pupae (P) or larvae (L) were found, we included in the table, together with the number of adults per survey and the incidence of attack rate expressed as percentage.

Table 6: Summary of the number of adults and incidence of attack rate in percentage found in the non-target surveys per plant and location. Abundance of beetles and common ragweed per site are shown in the column of Target. Plants are ordered from left to right regarding the relatedness to common ragweed.

Site	**Target**	**Exotics**				**Natives**					Endemic	**Exotic**	**Natives**			
	Ambrosia arrtemisiifolia	*Ambrosia trifida*	*Xanthium strumarium*	*Helianthus tuberosus*	*Helianthus annuus*	*Bidens tripartitus*	*Inula graveolens*	*Inula hirta*	*Pulicaria dysenterica*	*Buphtalmum salicifolium*	*Xerolekia speciossissima*	*Conyza canadensis*	*Artemisia vulgaris*	*Picris hieracioides*	*Centaurea nigrescens*	*Persicaria maculosa*
Balerna CH							0/0%									
Contone CH	0/0				0/0%											
Rovio CH	🌿🌿🌿 🪲🪲🪲												0/0%			
Alba IT	🌿🌿 🪲		2/10%									0/0%	0/0%			
Abbiategrasso IT	🌿🌿🌿 🪲🪲🪲		15/20 %	E,L,P 12/ 30%								0/0%	0/0%			
Baraggio IT													0/0%			
Bastida Pancarana IT	🌿 🪲🪲		E,P,L 12/ 17%	E,P,L 7/17%												
Cassine IT	(0/ 🪲)		15/ 15%													
Carvico IT	🌿 🪲		19/ 50%													
Corbetta IT	🌿🌿 🪲🪲		6/5%	2/20%									0/0%			
Liscate IT	🌿 🪲		0/0%													0/0%
Lomato IT	🌿 🪲🪲		7/50%													
Magenta IT	🌿🌿🌿 🪲🪲🪲												0/0%			
Magnago IT	🌿🌿🌿 🪲🪲🪲												2/0%			
Mezzana corti IT	🌿🌿🌿 🪲🪲🪲	E,L,P 285/ 90%	E,L,P 97/ 100%	E,L 16/ 30%		0/0%						0/0%	0/0%			
Monte Barro medow IT	(0/🪲)										12/ 30%					
Monte Barro IT	(0/🪲🪲)							39/0%		180/ 60%			0/0%	13/0%	58/ 20%	
Palazzolo IT	(0/🪲🪲🪲)		5/10%										3/0%			
Palosco IT	0/0												0/0%			
Parco delle cave IT	(0/🪲🪲)				75/ 40%											
P. curone IT	🌿 🪲		74/ 50%										0/0%			
Ponte della becca IT	🌿🌿🌿 🪲🪲🪲		E,L,P 63/ 80%			0/0%										
Ponte San Pietro IT	🌿 🪲			E,L,P 12/ 37%				0/0%				1/0%	1/ 0,2%		0/0%	0/0%
San Steffano IT	🌿🌿 🪲🪲											0/0%	0/0%			
Sirmione IT	🌿 🪲								0/0%							
Shilpario IT	0/0							0/0%								
Vigevano IT	🌿🌿🌿 🪲🪲🪲		E,L,P 95/ 70%									E,P 19/0%	E,P 16/0%			0/0%

Ambrosia abundance	*Ophraella* damage
🌿=1-100	🪲=1-10%
🌿🌿=101-1000	🪲🪲=11-50%
🌿🌿🌿>1000	🪲🪲🪲=51-100%

E= egg-batchs; L= larvae; P=pupae;
Number of adults/Infestation %

4.3. Discussion

The surveys show that three out of the 15 non-target species can be included in the realized host range of *O. communa*, which is defined by all the plants that are accepted for oviposition and initiation of feeding (van Klinken, 1999). Under this definition, sunflower was excluded as host, as it was only found lightly attacked on Italian crops, but no eggs nor larvae were found. In the native range of *O.* communa, the insect was never reported to be a pest on sunflower and previous tests showed that *O. communa* population cannot increase on sunflower (Kiss, 2007). Throughout this thesis, non-choice and choice experiments were conducted to evaluate impact of *O. communa* on sunflower, and both indicated that sunflower, at least some varieties, are suitable hosts for the beetle. Differences on results from realized host range testing are more common than expected and may be explain by differences in population, such as genetic variability in host specificity (Sheppard *et al.*, 2005). In Australia small non-choice tests indicated that *O. communa* can complete a life cycle on sunflower, and was therefore discarded as a control agent against common ragweed (Müller-Schärer *et al.*, 2014), but as we did not find any larvae or eggs on the field survey, a careful interpretation of the results is needed to predict damage on the field, including results of non-choice, choice and field tests (Sheppard *et al,* 2005). The undeniable influence of environmental factors to the behavior of insects affect greatly field surveys (van Klinken, 1999). Careful planning needs to be done in order to cope with differences among areas, and also seasons and within different years.

Although only on rare opportunities the release of beetles as biological control agents had side effects on non-target species, such as the release of *Galerucella calmiensis* adults of feeding on non-target plants (Briese, 2003) and *Zygogramma bicolorata* to control *Parthenium hysterophorus* that caused considerable damage on sunflower crops (Jayanth et al., 2010). In both cases, the assessments disregarded the reversible effects of agents punctually feeding on non-target species, even though they could not complete a life cycle on those species (Briese, 2003). We found 4 plants with adult feeding damage (sunflower and three native Asteraceae), including *Xerolekia speciosissima*, endemic to subalpine meadows in Lombardy, that was included in the red book of Flora Lombarda (I.U.C.N., 2004) in the category of least concert (LC). Least concern is the lowest category ranked for endangered taxa, meaning that risk of extinction is unlikely and abundance is high (UICN, 2012). When non-target damage is sufficiently “minor”, a potential biological control agent can still be used (van Klinken, 1999). In the case of *X. speciosissima* damage was considerably low, but as it is included in the Red list of Flora, a closer follow-up and re-evaluation of the category may be prudent to be done, as biocontrol agents can reduce diversity in the introduce community (Louda, 2003).

5. Conclusion and outlook

Louda *et al.* (2005) stated that the use of a host-specificity evaluation was sufficient to predict the ecological risk of an insect in a new environment, by testing the adult preference and larval performance on the field, accompanied with the knowledge of the plant species used by the potential biological control agent in its native region. The three studies carried out in this thesis cover accurately these three points, also evaluating new potential plant hosts for oviposition and feeding behavior of *O. communa* on the new range. Taking in account the results of the two experiments (preference and performance) together with the non-target survey, my prediction would be that, even though some feeding damage can occur on non-target species, including commercially important species and native plants, the presence and spread of *O. communa* will have more benefits than costs, as the impact on non-target species is considerably lower than on common ragweed; and only substantial damage to non-target species can be found later on the season, when sunflower is already harvested and oviposition declines.

For the effectiveness of *O. communa* as a biological control agent of common ragweed in the evaluated region, a different range of studies still need to be done. But as long as my studies covered, *O. communa* seems to have a great impact on common ragweed throughout the season, especially late in summer, thus a biological control program that includes *O. communa* as a biocontrol agent will have a good chance of being successful for controlling common ragweed.

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Firstly I would like to express my gratitude to Prof. Heinz Müller-Schärer, for all his guidance and support throughout the Master work, but also for the trust put on me to continue the efforts in this project. Secondly to the whole HMS-group (Suzanne Lommen for the vision and planning, Benno Augustinus for the enthusiasm and company, Yan Sun for the help in statistics and advices, Gaelle Kadima and Stephanie von Bergen for the practicalities and a special thanks to Joelle Romanens for all the patience and help).

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Annex 1: Experiment sites

Table A1: Information on the coordinates of the field sites and dates of the field experiments.

Experiment	Sites	Cohorts	Date (2015)	Coordinates	Remarks
Preference	Abbiategrasso	1 cohort	01.05-29.07	45.383185 N 8.928022 W	3 cohort with 6 assessments
		2 cohort	07.07-16.09		
		3 cohort	10.09-08.11		
	Magenta	1 cohort	01.05-29.07	45.45953 N 8.87472 W	3 cohort with 6 assessments
		2 cohort	07.07-16.09		
		3 cohort	10.09-08.11		
	Magnago	1 cohort	01.05-29.07	45.57073 N 8.78546 W	3 cohort with 5 assessments
		2 cohort	07.07-16.09		
		3 cohort	10.09-08.11		
	Rovio	1 cohort	05.05-28.07	45.93087 N 8.98377 W	2 cohort with 4 assessments
		2 cohort	11.07-07.08		
Performance	Corbetta	1 cohort	21.07-16.08	45.47089 N 8.93683 W	

Annex 2: Planting schedule

Table A2: Planting schedule for the 7 plant accession for the experimental setting for the first cohort in May for the choice experiment in a latin square (Chapter 2).

Code name	**Plant Species**	**Stage/ Size**	**Number of Seeds planted**	**Number of plants required**	**Number of plants taken**	**Planting date**
Girasol	*Helianthus annuus*	2-4 leaves	50	21 (7 X 3 sites)	30	20.04.2015
Girasol	*Helianthus annuus*	6-8 leaves	50	21 (7 X 3 sites)	30	01.04.2015
Girasol	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites)	30	10.03.2015
Sunrich	*Helianthus annuus*	More than 20 leaves	35	21 (7 X 3 sites)	30	10.03.2015
Iregui	*Helianthus annuus*	More than 20 leaves	36	21 (7 X 3 sites)	30	16.03.2015
Nyger	*Guezotia abbyssinica*	More than 20 leaves/ 20 cm	50	21 (7 X 3 sites)	30	10.03.2015
Ragweed	*Ambrosia artemisiifolia*	More than 20 leaves/ 20 cm	60	21 (7 X 3 sites)	30	23.03.2015
TOTAL			290	147	210	01.05.2015

Table A3: Planting schedule for the 7 plant accession for the experimental setting for the second cohort in May for the choice experiment in a latin-square (Chapter 2).

Code name	**Plant Species**	**Stage/ Size**	**Number of Seeds planted**	**Number of plants required**	**Number of plants taken**	**Planting date**
Girasol	*Helianthus annuus*	2-4 leaves	100	21 (7 X 3 sites) + 15 Rovio	70	20.06.2015
Girasol	*Helianthus annuus*	6-8 leaves	50	21 (7 X 3 sites)	30	01.06.2015
Girasol	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites) + 5 Rovio	40	13.05.2015
Sunrich	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites + 5 Rovio	40	13.05.2015
Iregui	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites + 5 Rovio	40	13.05.2015
Nyger	*Guezotia abbyssinica*	More than 20 leaves/ 20 cm	60	21 (7 X 3 sites + 5 Rovio	40	13.05.2015
Ragweed	*Ambrosia artemisiifolia*	More than 20 leaves/ 20 cm	100	21 (7 X 3 sites+ 5 Rovio	40	13.05.2015
TOTAL			500	172	**300**	**05.07.2015**

Table A4: Planting schedule for the cage non-choice experiments in Corbetta (Chapter 3).

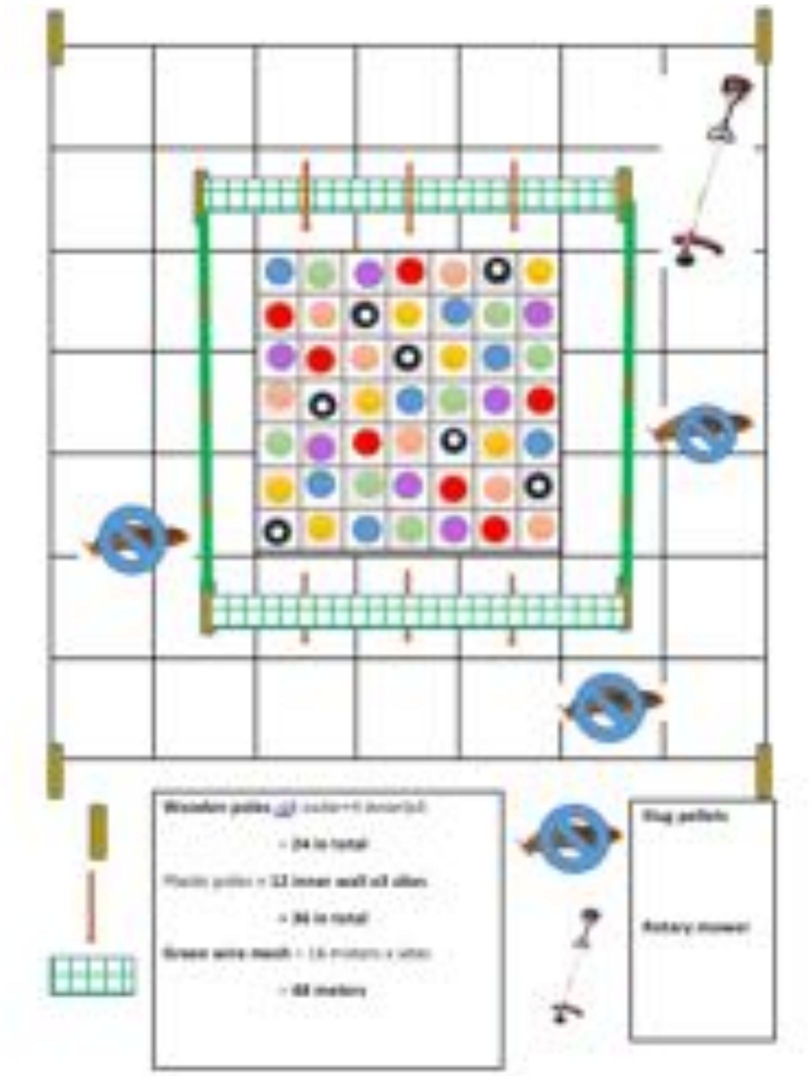
Code name	Plant Species	Stage/ Size	Number of Seeds planted	Number of plants required	Number of plants taken	Planting date
Girasol	*Helianthus annuus*	More than 20 leaves	40	18(6 plants for 3 cages)	25	13.05.2015
Ragweed	*Ambrosia artemisiifolia*	More than 20 leaves/ 20 cm	40	18(6 plants for 3 cages + rearing)	25	13.05.2015
TOTAL			120	51	**66**	**05.07.2015**

Table A5: Planting schedule for the 7 plant accession for the experimental setting for the third cohort in May for the choice experiment in a latin-square (Chapter 2).

Code name	Plant Species	Stage/ Size	Number of Seeds planted	Number of plants required	Number of plants taken	Planting date
Girasol	*Helianthus annuus*	2-4 leaves	100	21 (7 X 3 sites)	70	25.08.2015
Girasol	*Helianthus annuus*	6-8 leaves	50	21 (7 X 3 sites)	30	10.08.2015
Girasol	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites)	40	03.07.2015
Sunrich	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites)	40	03.07.2015
Iregui	*Helianthus annuus*	More than 20 leaves	60	21 (7 X 3 sites)	40	03.07.2015
Nyger	*Guezotia abbyssinica*	More than 20 leaves/ 20 cm	60	21 (7 X 3 sites)	40	03.07.2015
Ragweed	*Ambrosia artemisiifolia*	More than 20 leaves/ 20 cm	100	21 (7 X 3 sites)	40	03-07.2015
TOTAL			500	147	**300**	**10.09.2015**

Annex 3: Experimental setting for the preference test

ACTIVITY	ACTIONS	PEOPLE	MATERIAL
1 SETTING 7x7 m SQUARE	Extending rope	3	14 m Rope 90º guide 10 m rope
	Knock in Wooden Poles	2	4 wooden poles 1 wooden hammer
2 MOWING	Mow weed within the 7x7 square	1	Rotary mower Protection clothes
	Removing weed with the rack	2	2 Racks
3 SETTING 4x4 m SQUARE	Extending rope in triangle	3	12 m Rope 90º guide
	Extending extra 4 m rope	2	4 m Rope 90º guide
4 DEFINING LATING SQUARE	Moving 4 m rope with hook	2	2 mobile ropes (4 m) with marks and hooks
	Marking points	2	7 bamboo sticks marked with labels of 7 different colors for each row.
5 PLANTING	Digging	3	Hover and pick
	Transplanting	3	Pots
	Attaching a bamboo stick next to the plant	3	150 bamboo sticks
	Labelling	2	Labels Plastic wired strand Pencil
	Sparing Slug pellets	1	Slug pellets
	Collecting tissues	1	Plastic bag
6 BUILDING 4x4 m FENCE	Knock in Wooden Poles	2	4 wooden poles 1 wooden hammer
	Attach plastic With Green wired mesh	2	3 per pole.
	Plastic poles with green wired mesh	2	3 by side, every 50 cm.



Annex 4: Form for the preference experiment

Site: Date: Weather:

Remarks:

					Damage	Ophraella						
		Plant height [cm]	Plant width [cm]	Phenological stage	Ophraella damage %	# eggbatches	#eggs/batch	Larval stage 1-2	Larval stage 3	Pupae	Adults	Remarks
H. annuus	1											
Growth	2											
2-4 Leaves	3											
	4											
	5											
	6											
	7											
H. annuus	1											
Growth	2											
6-8 Leaves	3											
	4											
	5											
	6											
	7											

Annex 5: Distribution of *O. communa* life-stages among assessments

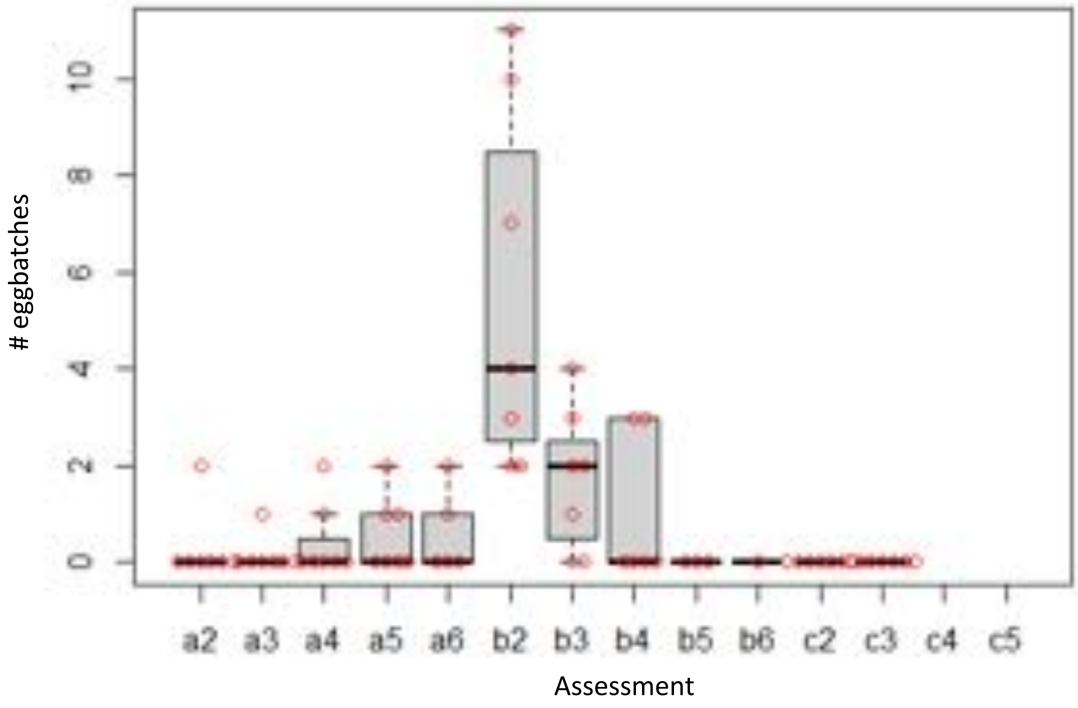


Figure A1. Distribution of egg-batches among *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square, each red circle represent one individual (see text for details).

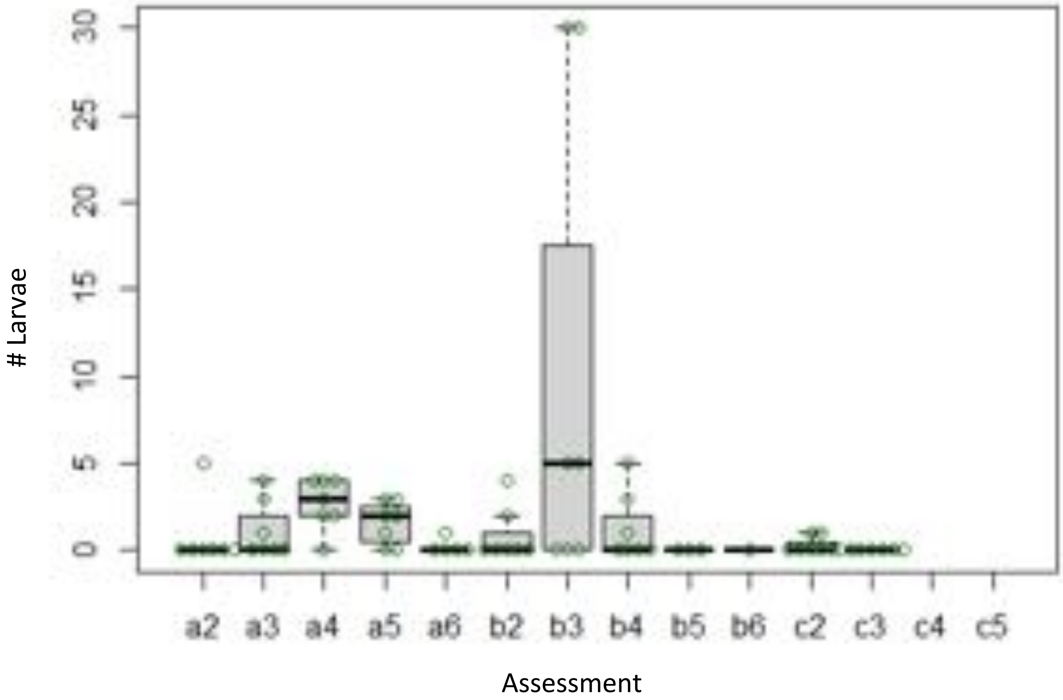


Figure A2. Distribution of L1 larvae among *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. The green circles in the graph represent each individual (see text for details).

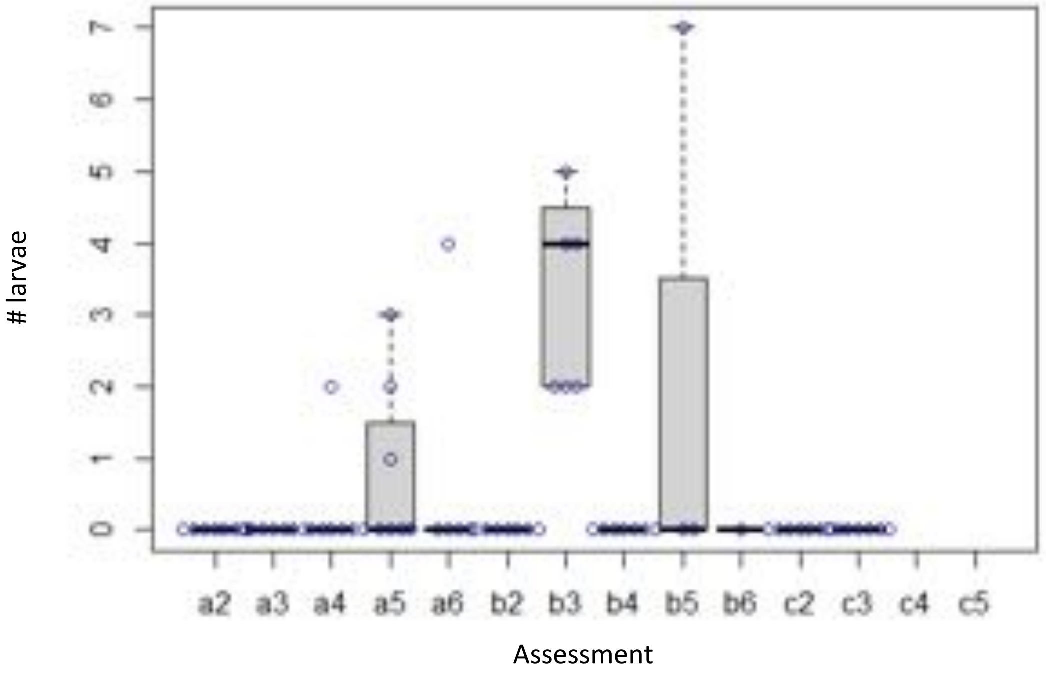


Figure A3. Distribution of L3 larvae among *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. The blue circles represent each L3 larvae (see text for details).

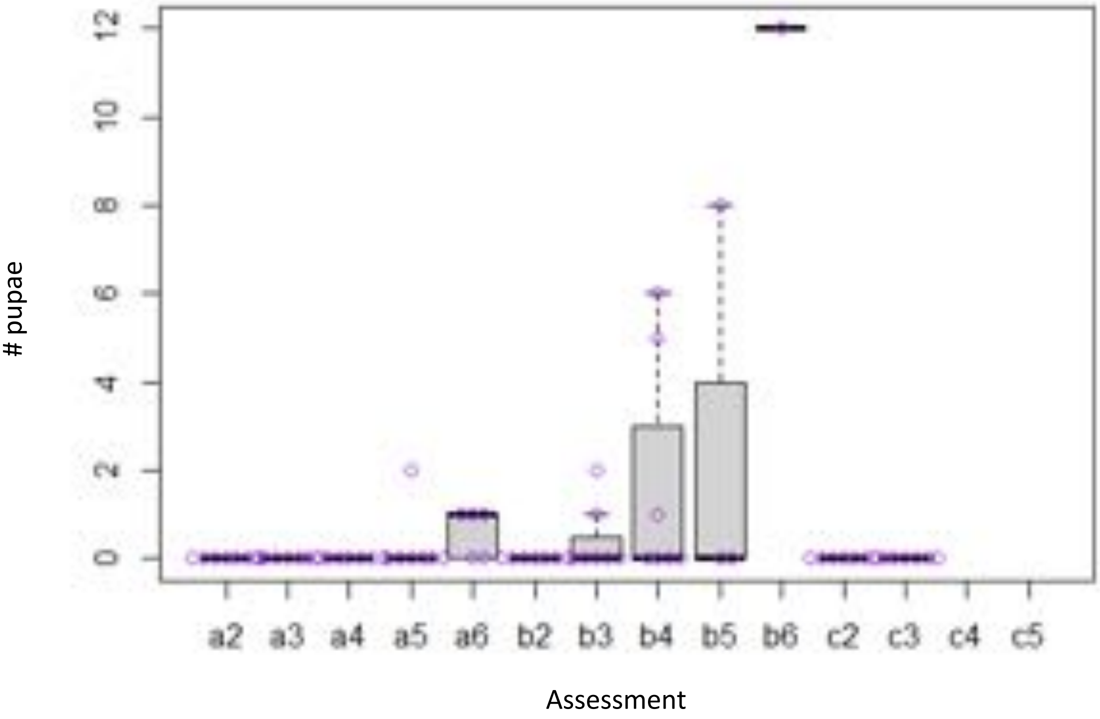


Figure A4. Distribution of pupae among *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Purple circles represent each pupa (see text for details).

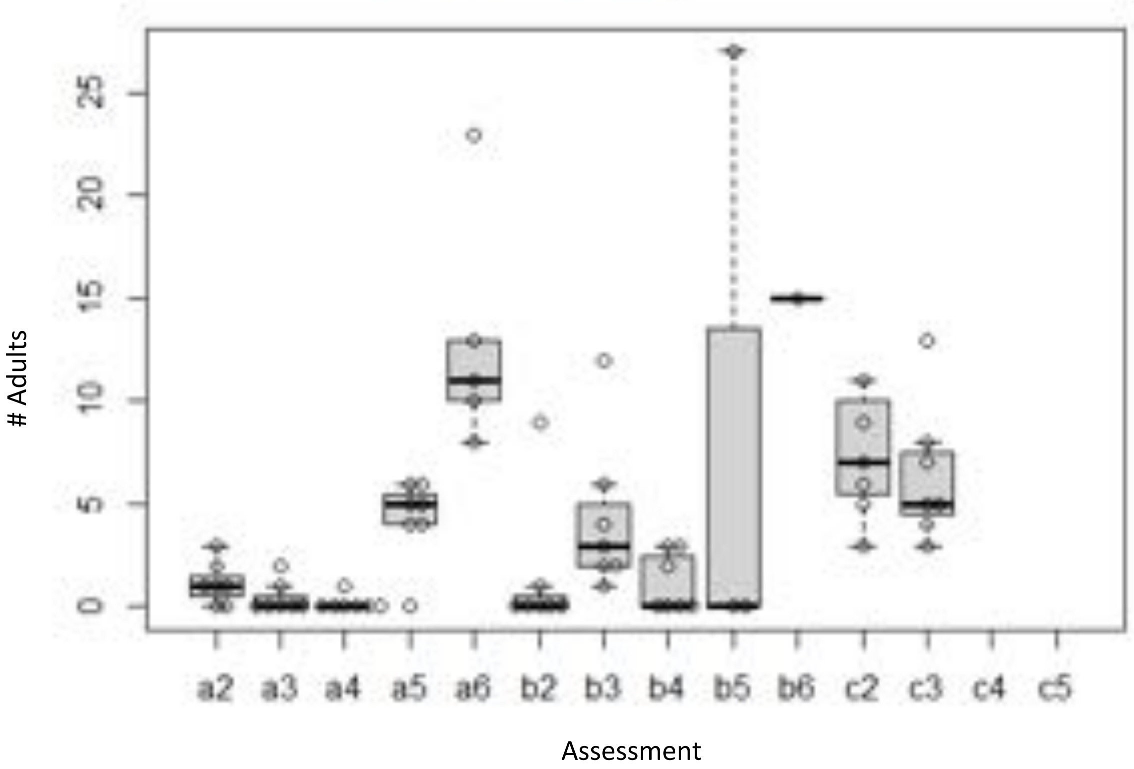


Figure A5. Distribution of adults among *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Black circles represent each adult individual (see text for details).

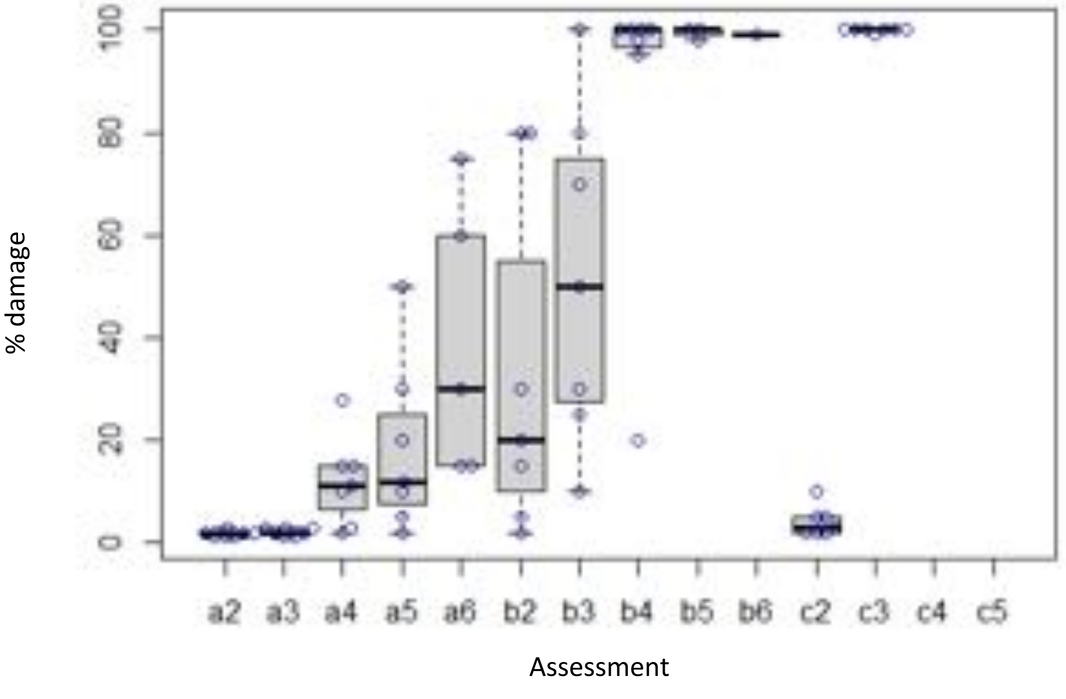


Figure A6. Distribution of the maximum damage found on *Ambrosia artemisiifolia* in Abbiategrasso throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Blue circles represent damage on each plant accession (see text for details).

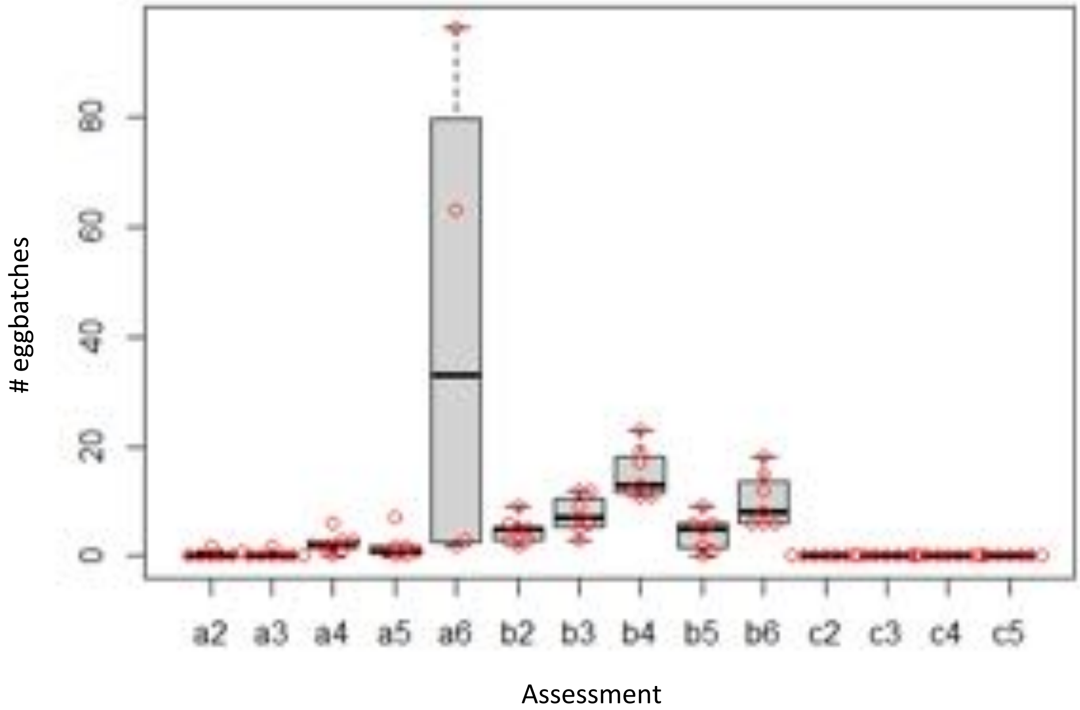


Figure A7. Distribution of eggbatches among *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Red circles represent each plant individual (see text for details).

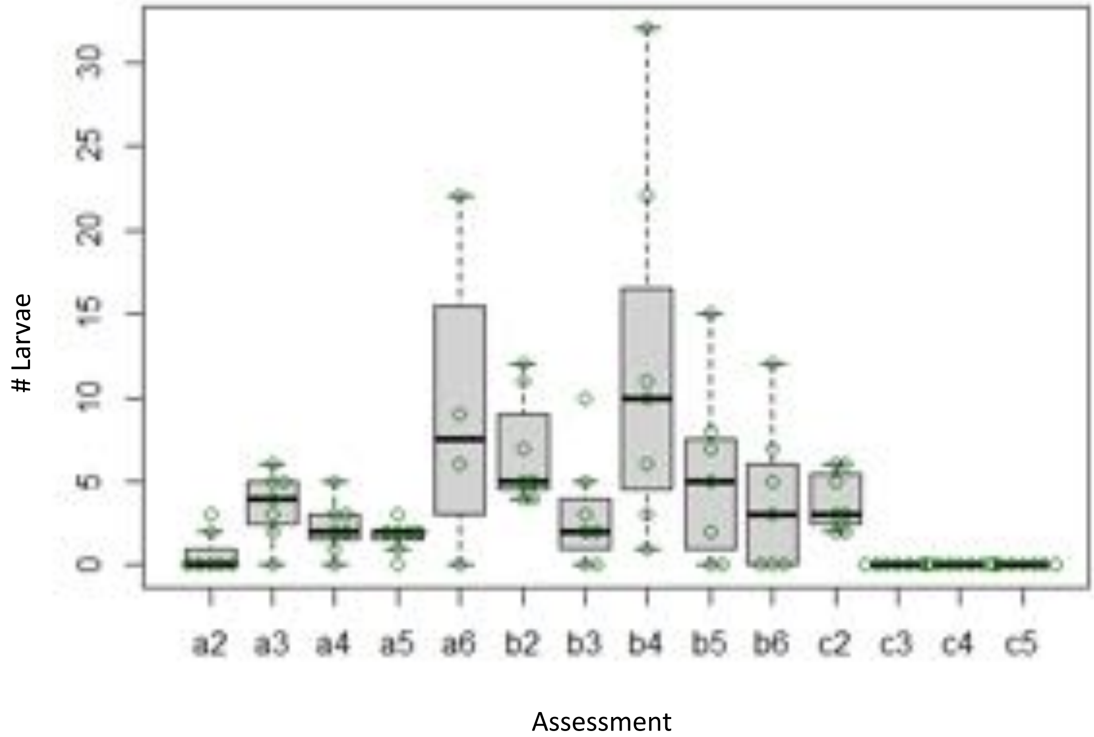


Figure A8. Distribution of L1 larvae among *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Green circles represent each L1 larvae (see text for details).

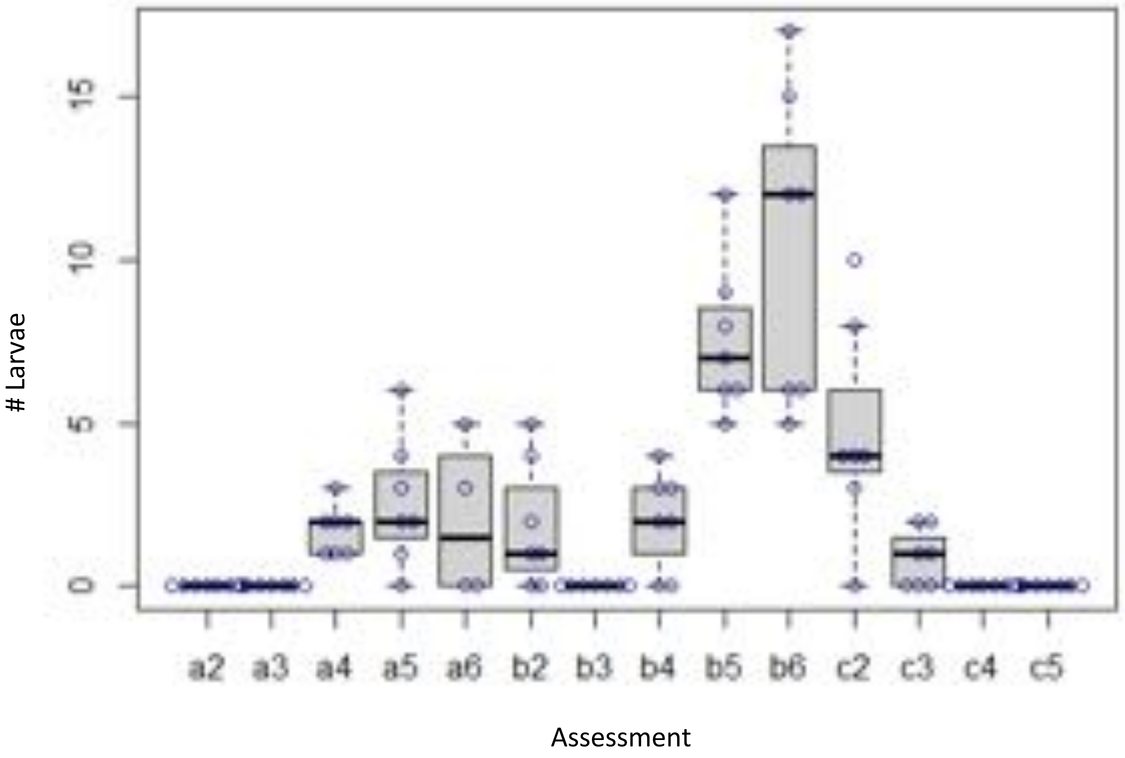


Figure A9. Distribution of L3 larvae among *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Black circles indicate each plant individual (see text for details).

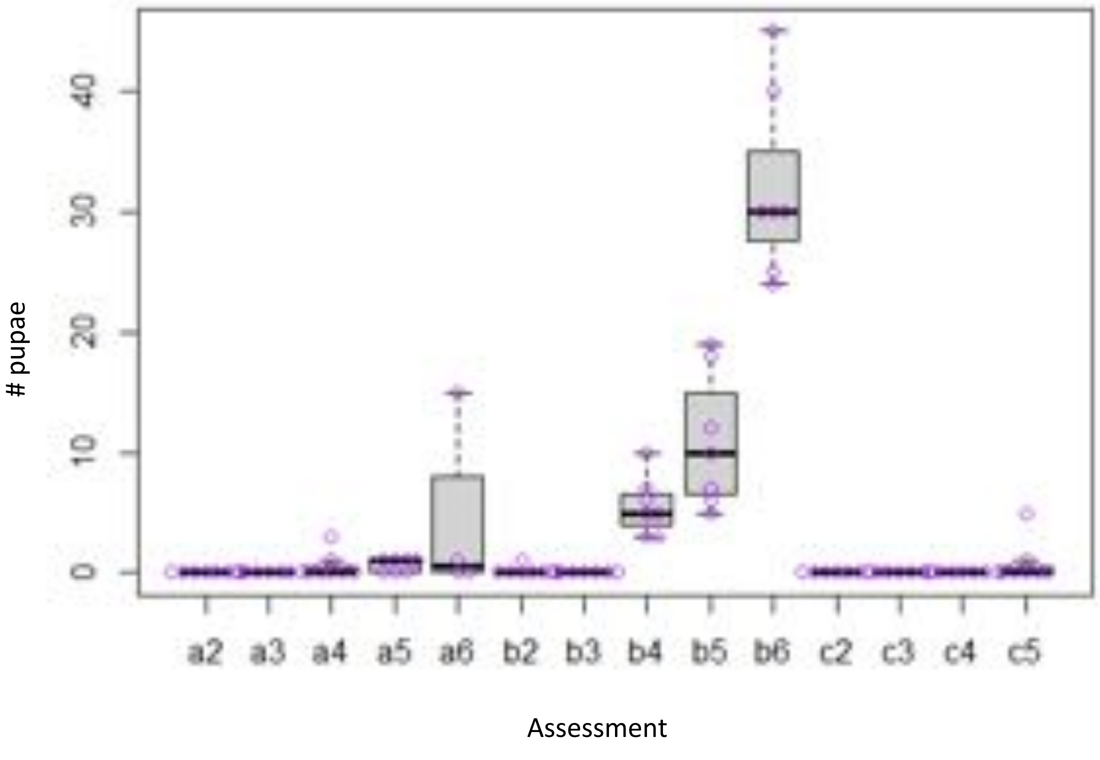


Figure A10. Distribution of pupae among *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Each purple circle represent a plant individual (see text for details).

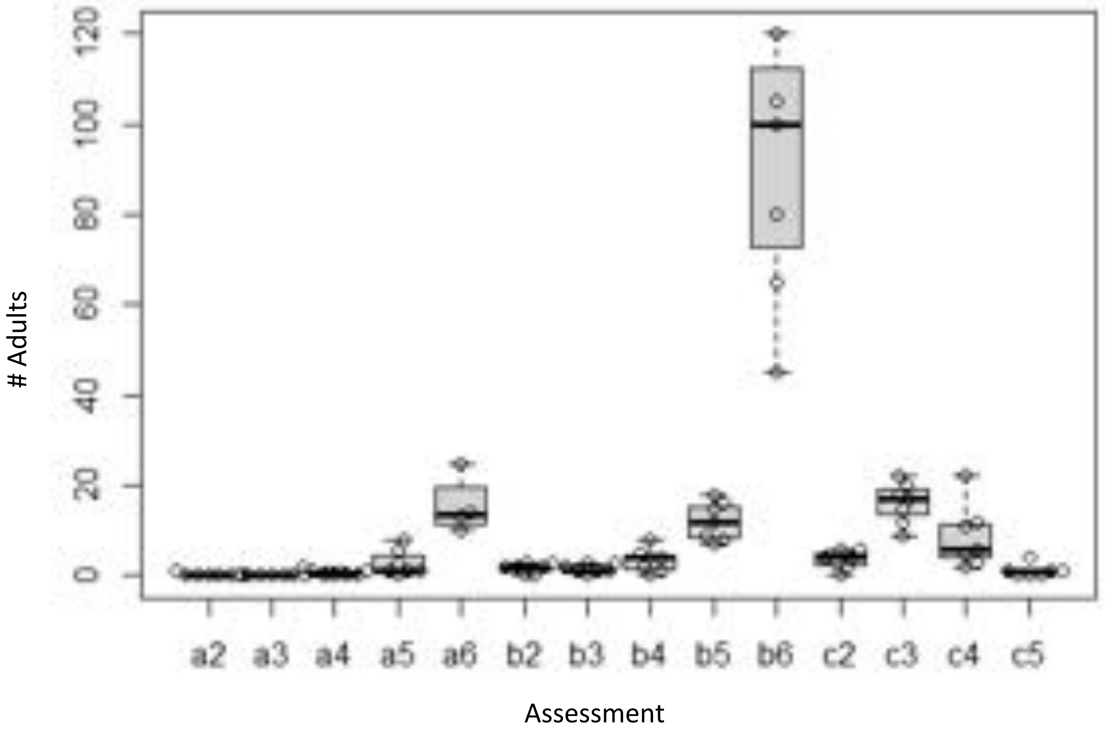


Figure A11. Distribution of adults among *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square Black circles show each plant individual (see text for details).

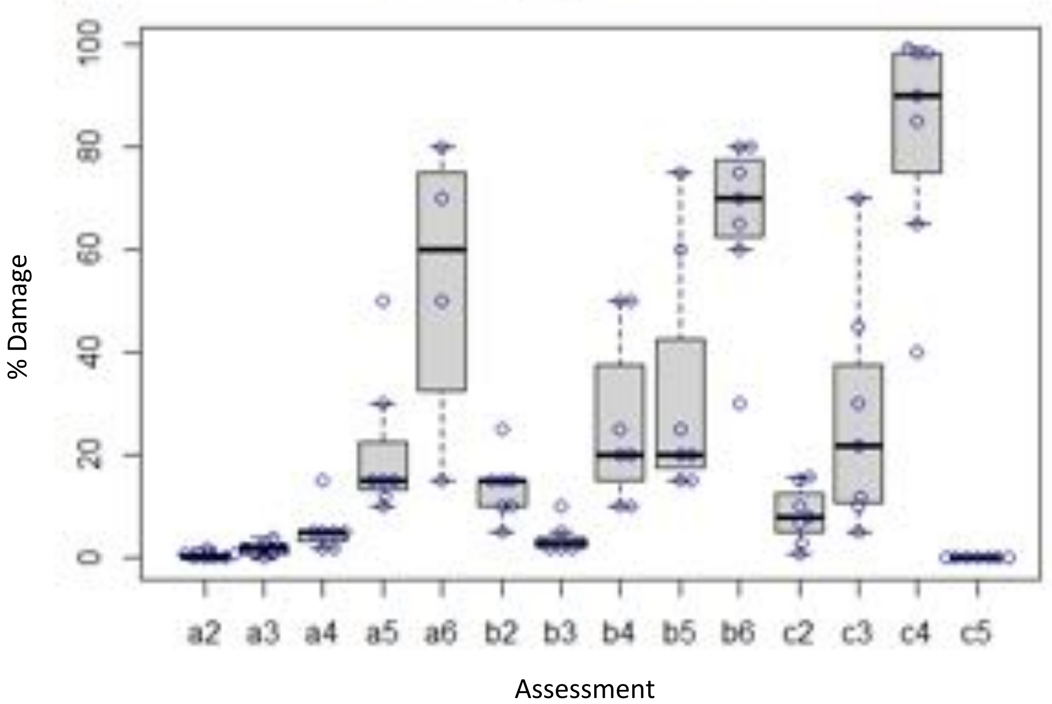


Figure A12. Distribution maximum damage on *Ambrosia artemisiifolia* in Magnago throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Blue circles indicate each plant accession (see text for details).

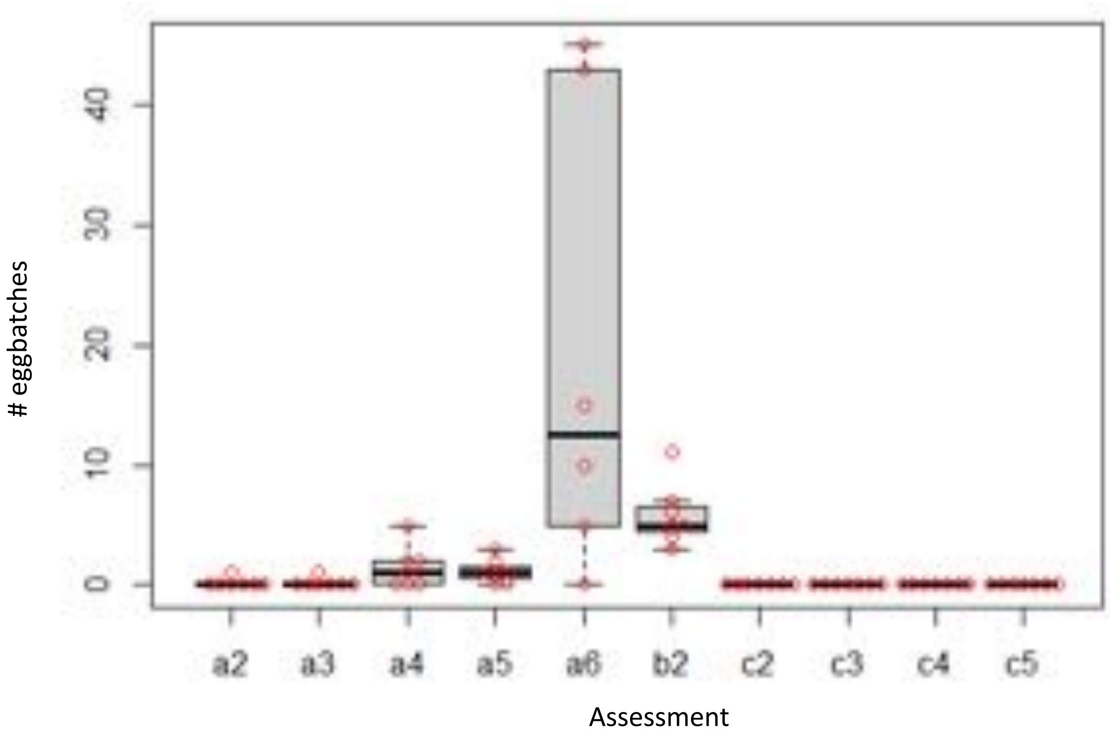


Figure A13. Distribution of eggbatches among *Ambrosia artemisiifolia* in Magenta throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Red squares represent each plant individual (see text for details).

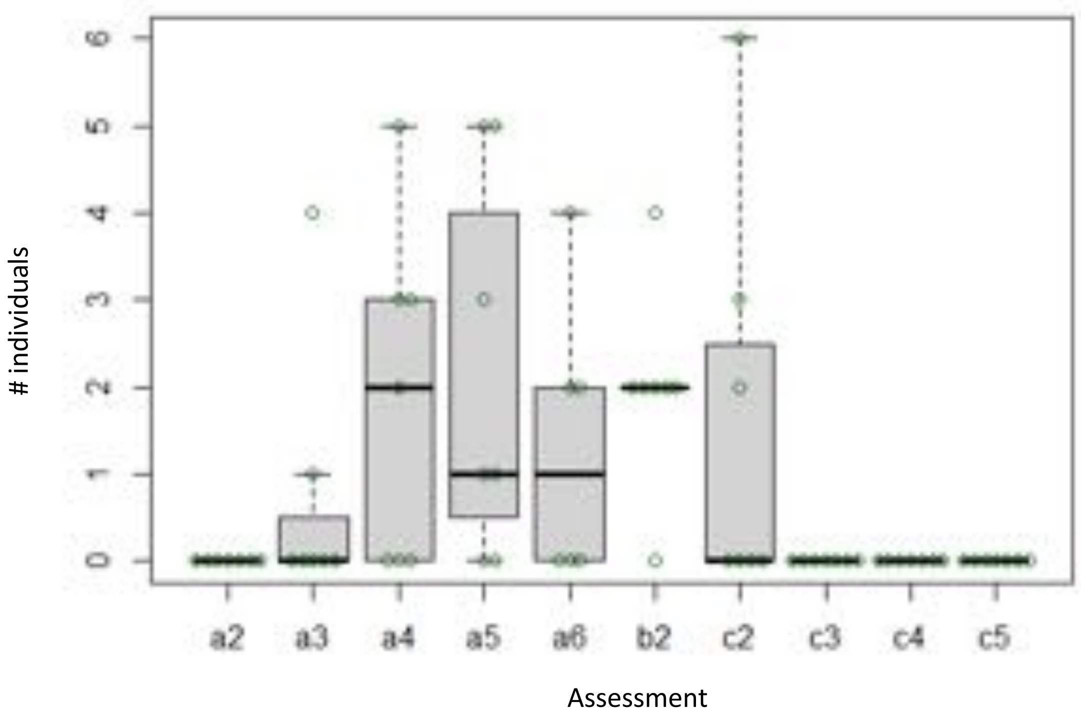


Figure A14. Distribution of L1 larvae among *Ambrosia artemisiifolia* in Magenta throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Each green circle represent a plant individual (see text for details).

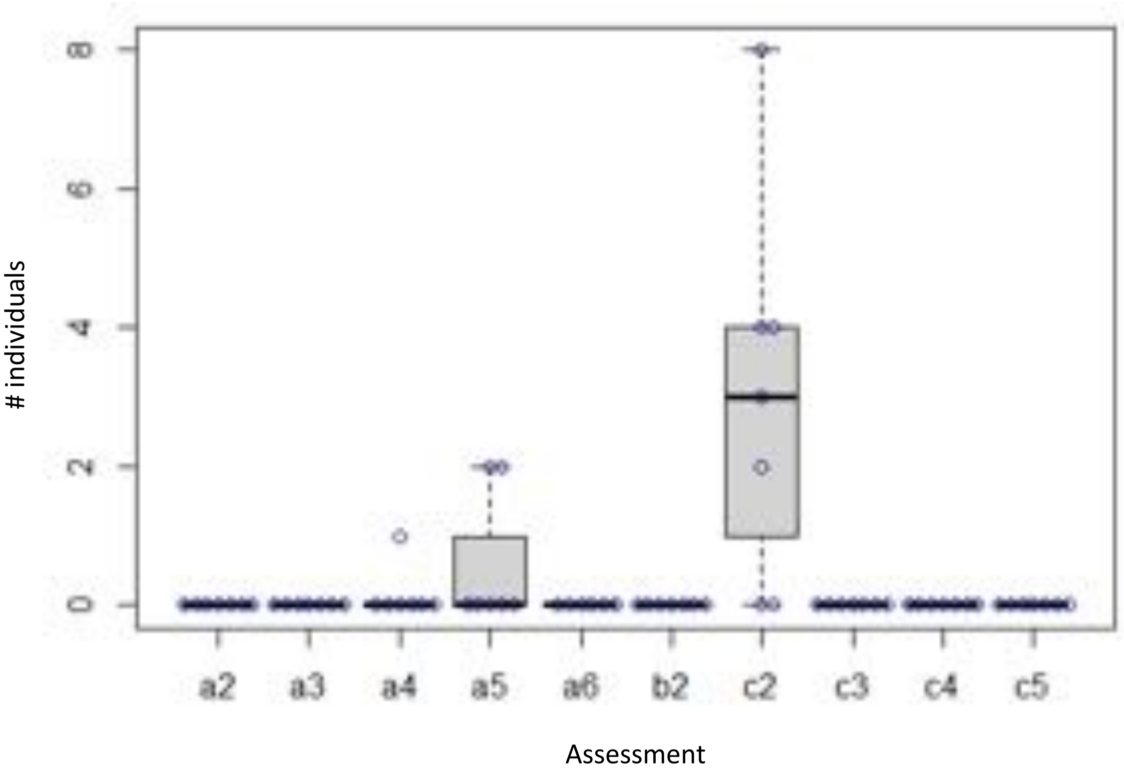


Figure A15. Distribution of l3 larvae among *Ambrosia artemisiifolia* in Magenta throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Each black circle represent a plant individual (see text for details).

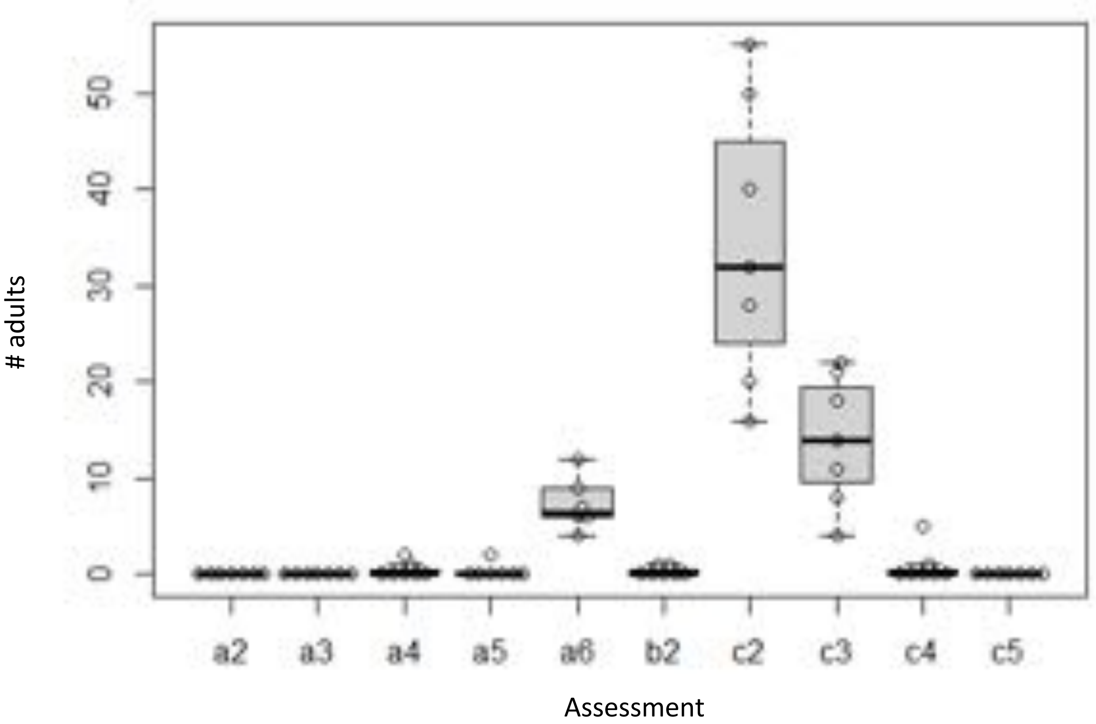


Figure A16. Distribution of adults among *Ambrosia artemisiifolia* in Magenta throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square showed by the circles in the graph (see text for details).

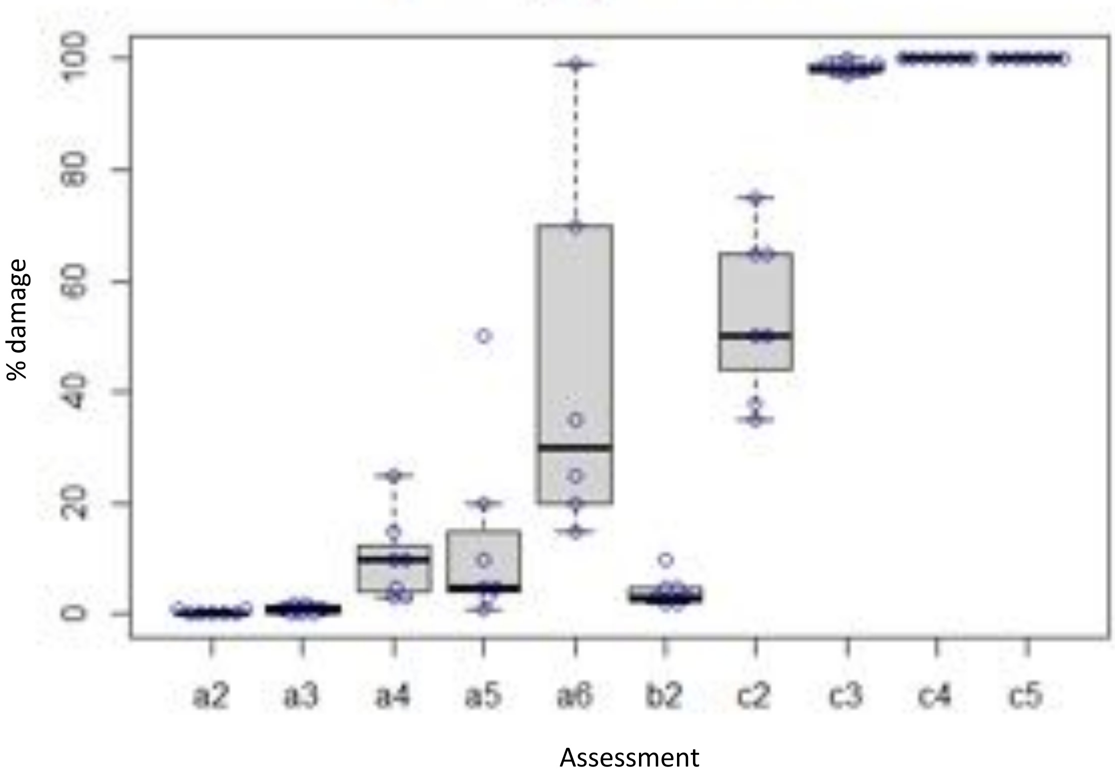


Figure A17. Distribution of maximum damage on *Ambrosia artemisiifolia* in Magenta throughout the 3 cohorts (a= first cohort, b=second cohort, c= third cohort). Data are based on 7 plant individuals per plant accession arranged in a latin-square. Black circles indicate each plant accession (see text for details).

Annex 6: Form for the performance experiment

Form: Performance on clip-on cages

CAGE 2 Weather Date

Nº Plant	Plant Species	Position	Family (F1-F10)	Locality of origin	Start date	Larva (yes/no)	Pupa (yes/no)	Adult (yes/no)	Clip-on cage moved (Yes/No)	Remarks
1		UP								
		DOWN								
2		UP								
		DOWN								
3		UP								
		DOWN								
4		UP								
		DOWN								
5		UP								
		DOWN								
6		UP								
		DOWN								
7		UP								
		DOWN								
8		UP								
		DOWN								
9		UP								
		DOWN								
10		UP								
		DOWN								
11		UP								
		DOWN								
12		UP								
		DOWN								

Annex 7: Insect family and plant species

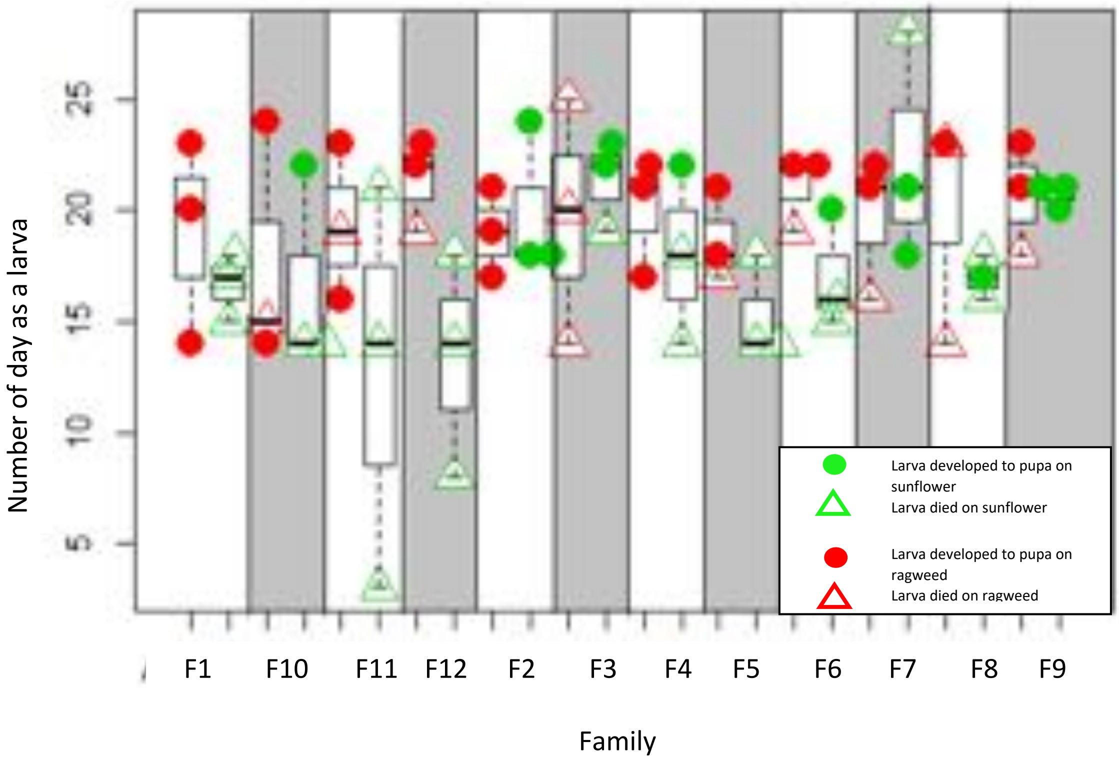


Figure 48: Distribution of the number of days that an individual spent as a larva before perishing (triangle) or developed into a pupa (circle) among 12 families of *Ophraella communa* collected in a wide range in Italy. For each family, three individuals were placed in *Ambrosia artemisiifolia (*in red) and three in *Helianthus annuus* (green). The bar represents the survival days for each insect family.

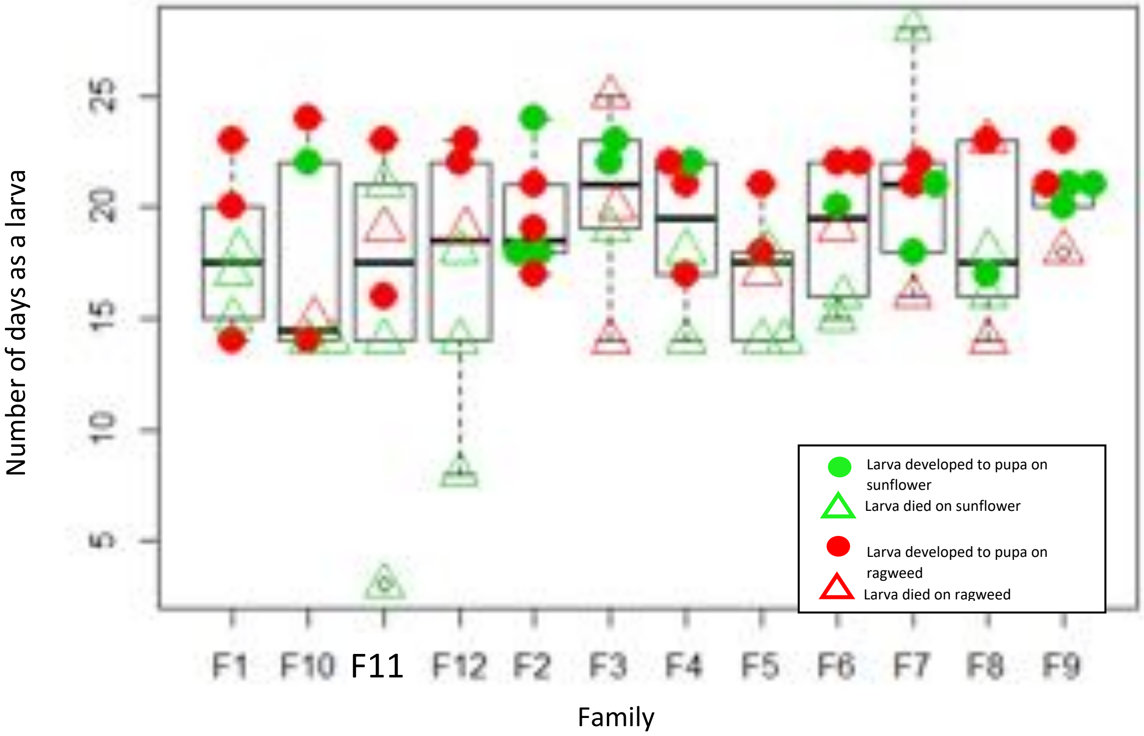


Figure 49: Distribution of the number of days that an individual survived as a larvae before perishing (triangle) or developing into a pupa (circle), among the 12 families and in 2 plant species: *Ambrosia artemisiifolia* in red and *Helianthus annuus* in green. Bars represent survival of the three larvae per family.

Annex 8

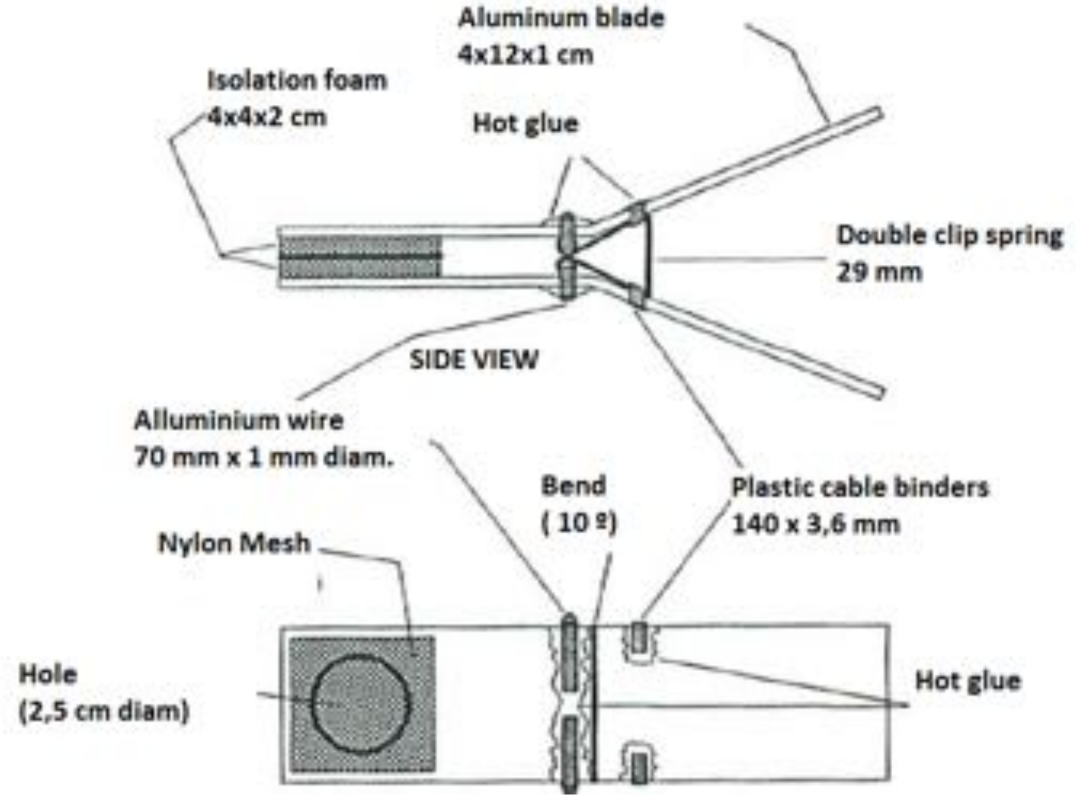


Figure 50. Mowry**'s** design (1993) modified to fit isolated *Ophraella communa* larvae on leaves of *Ambrosia artemisiifolia* (common ragweed) and *Helianthus annuus* (sunflower). The dimensions shown in the figure are the ones used for the experiment:

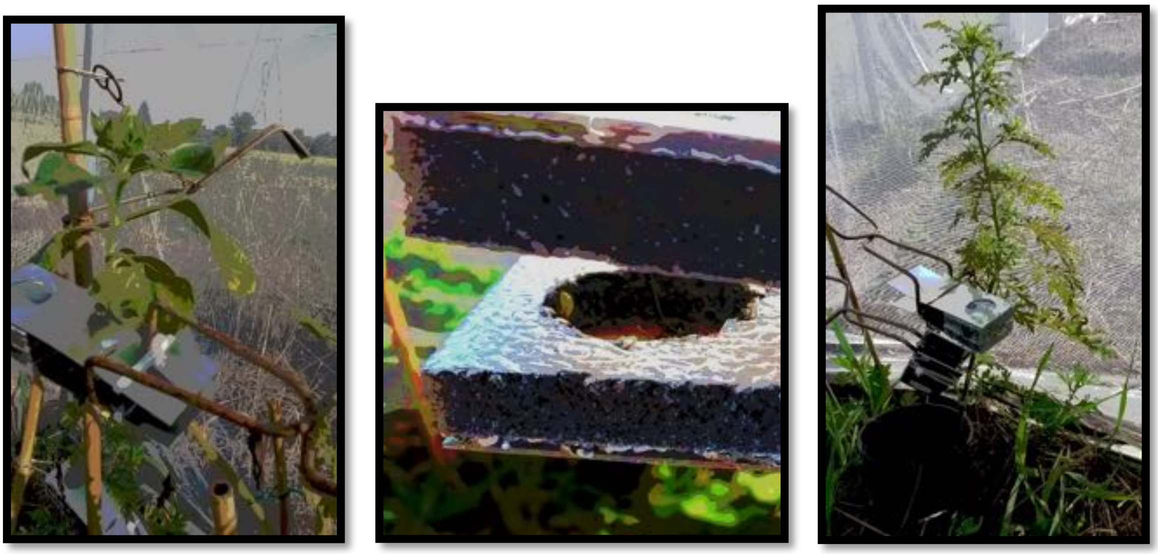


Figure 51. Pictures of the clip-on cages used in the experiment. On the left two used on sunflower, in the middle a larva inside is shown and the right one show the clip-on cages on ragweed. (Chapter 3)

Annex 9

LEAF DAMAGE SCALE

Level and damage intervals are only thought as a reference to score percentage overall damage. Please give a value in **percentage** (0-100%) when estimating the damage.

LEVEL	% DAMAGE	CHARACTERISTICS	PICTURE REFERENCE SINGLE LEAF	PICTURE REFERENCE WHOLE PLANT
O	0%	**NO damage** on the leaf		
A	1-5%	**MINIMAL** damage: Only few signs of feeding with less than 5% of the leaf of only few leaves.		
B	6-10%	**MARGINAL** damage: Minimal damage on most of the leaf plus some leaves with 5-10 %		
C	11-30%	**MODERATE** damage: 10-30% damage on multiple leaves, but fewer than half of the leaves affected.		
D	31-60%	**SIGNIFICANT** damage: At least half of all leaves with 10-50% damage and multiple leaves with more than 50% damage		
E	61-100%	**SEVERE** damage: Most of the leaves with more than 50% damage and multiple leaves with over 90% damage		

Annex 10: Preselected species for the non-target survey

Description species	Localities	Photo	Photo details
Ambrosia trifida: Annual weed, growing up to 2 meters. Stem woody at the base. Leaves oppositely arranged and blades variable in shape, from palmate with toothed edges to entire with few lobes. Female flowers growing in the base of the raceme and male growing at the end.	Mezzanina (Po River)		
Artemisia vulgaris: 40 – 140 cm tall. Plant aromatic, stem red. Leaves doubly pinnate, glabrous, and green in the upper surface, white tomentum underneath. Only yellowish disc flowers.in numerous capitula in a compact panicle. Flowering: July to September.	Common		
Asteriscus aquaticus: 10 – 50 cm tall. Stem and leaves covered with a wooly tomentum. Leaves entire, lanceolate, Flowerheads with yellow ray and disc florets. Flowering: From April to July	Few populations left, mainly meditarrean.		

Description species	Localities	Photo	Photo details
Bidens tripartita: 15 – 120 cm tall. Stem red. Leaves tripartite with dentate margin. Yellow flowerhead, bracts similar to leaves. Flowering: from July to October.	Parco le Folaghe (direction Pavia) Arround the little lakes formed from removing marmol or rocks (called cava or cave in italian)		
Buphthalmum salicifolium: 25 – 60 cm tall. Stem non-ramous. Alternate leaves, lanceolate. Terminal flowerheads with a rounded involucre, with equally long bracteae covered with pelt. Ray florets tridentate and disc florets of the same color as the ray florets.	- Monte Barro		
Carpesium cernuun: 20-60 cm tall. Stem pubescent. Leaves oblonge-lanceolate with a short petiole. Solitary terminal flower heads, with unequal coriaceous bracts. Disc florets green, ray florets absent. Flowering: July to August.	-Valle d’Ossola près de Trontano, between Luino et Ponte Tresa, east from Lugano Lake (Val Solda, Sta Margherita, Val d'Intelvi), Val Camonica, Haut-Adige et Val Venosta (Val Sarca, Cles in the V. di Non, along several station in the region of Bolzano and north up to Colmia di Barbiano		

Description species	Localities	Photo	Photo details
Centaurea nigescens subsp. ramosa: 40-100 cm tall. Stem erected and branching. Basal leaves ovate and the caulinar are linear. Flowerheads solitary, terminal, with a globular involucre, covered with bracts that end in a black triangle. Only ray florets.	Monte Barro.		
Conyza canadensis: 20-80 cm tall. Very ramous, Leaves lanceolate, entire, sessiles. Capitula white cylindrical, glabrous, numerous and in a dense panicle. Ray florets surpassing marginally the disc florets. Flowering: July to September.	Common next to crops.		
Helianthus annuus: 1- 4 meters. Erected stem, leaves are broad, coarsely toothed, rough and mostly alternate. Rayflowers yellow, red or orange. The disk flowers are arranged spirally.	CULTIVATED		

Description species	Localities	Photo	Photo details
Helianthus tuberosus: 1.5 – 3 m. Opposite leaves above but alternate below. Leaves are rough, hairy, ovoid-acute. Flowerheads with big ray florets comparing to the involucre. Disc florets yellow. Plant produes tubers.	Mezzanina (River Po) and next to old crops.		
Inula britannica: Plant with smell of garlic, 20- 90 cm, rameous stem, with lanceolate leaves, dentate to entire, sessiles. Yellow flowerheads, with longue ligules of 2 cm, and with the bracts of the involucre same sized. Flowering: July to August.	Frecuent in Southern Italy, not much in the North side. -Lasa and la Valle Isarco up to Bressanone. - Rivoltella e Sirmione. - Pozzolengo. (Bergamo) between 45-370 m.		
Inula conyzae: 50-100 cm tall. Rameous pubescent stem. Eliptic leaves with entire to denticulate margin, glabrous upper surface and hairy lower surface. Cylindrical flower heads, forming a thick panicule. Ray florets reddish, and disc florets yellowish. Flowering: July to October.	- Tomaselli (Bergamo) between 200 – 800 m.		

Description species	Localities	Photo	Photo details
Inula helenium: 60- 250 cm tall. Pubescent stem, generally rameous. Big lanceolated leaves, dentate, lower surface with tomentum. Yellow flowerheads, with long (3 cm) ray florets, Spatulated bracts of the involucre. Flowering: July to August.	- Pozzolengo, Orzinuovo Bergamo		
Inula helvetica: 50 – 150 cm tall. Stem pubescent, greyish. Leaves lanceolate with a strong smell of Melissa. The margin entire to finely dentate and the lower surface with greyish tomentum. Flowerheads yellow, in a corymb, ray florets spread. Flowering August to September.	Cuneo (Piemont)		
Inula hirta: 15-50 cm. Simple stems, covered with spreading hairs. Leaves subcoriaceus, sessiles, entires, elliptic. Solitary yellow flowerheads, ray florets spreading, longs of 2.5 cm, bracts ciliated. Flowering: June to july.	Monte Barro. 2.5 North of Maccagno (loc. Pioda). -Bergamaschi, Bergamo.		

Description species	Localities	Photo	Photo details
Inula montana: 10 to 30 cm. Plant covered with a long tomentum. Basal leaves oblongo-lanceolate. Solitary yellowe floweheads. Flowering: June to August.	Monte Organo (Brescia) Bonate Sopra (Bergamo) Ponte San Pietro (Bergamo)		
Inula salicina: 30 – 60 cm tall. Stem simple and glabrous. Leaves lanceolate, entire and with cilia in the margin. Petiole short and clasping. Solitary yellow flowerheads, ray florets spreading and thin. Flowering: July to August.	Pengo (Lombardía) Monte Orsa. Tagliata commune. Monte San Pellegrino (Bergamo). Selvino (Bergamo) Lenna (Pavia) Pontevico (Brescia)		
Inula spiraeifolia: 30-60 cm. Stem pubescent in the base and glabrous in the top. Leaves glabrous in the upper surface and pubescent in the lower surface. Flower heads solitary or in wide corymbs. Bracts of the involucre ciliated or glabrous. Flowering: July to August.	Maresana (Pavia) Andrara (Pavia) Oneta (Bergamo)		

Description species	Localities	Photo	Photo details
Picris hieracioides: 30- 70 cm. Stem erected, reddish, branching. Basal leaves in a roseate, and caulinar leaves alternate, sessile, dentate and lanceolate. Flower heads in terminal racemes, with only yellow ray florets. The bracts of the involucre imbricate	Monte Barro		
Pulicaria dysenterica: 30 – 60 cm. Leaves oval or lanceolate, with tomentum underneath, entire or slightly dentate. Petiole short and clasping. Flowerheads yellow with linear ray florets. Flowering: July to August.	Common in the landside.		
Pulicaria vulgaris: Stem 30 to 60 cm. Stem very ramous, all the plant pubescent. Leaves pubescent, undulate, entire, sessile. Flowerheads small, in corymbs. Ray florets surpassing slightly the involucre. External bracts shorter than the internal. Flowering: July to August.	Few populations, mostly Mediterranean.		

Description species	Localities	Photo	Photo details
Xanthium strumarium: 30 – 100 cm tall. Stem ramous, with short branches, greyish without thorns or spines. Leaves cordate to triangular with a long petiole. Capitula unisex, male in long flowerheads or solitary, female only with 2 florets. Involucre with hooked prickles bracts.	Open places, humid or next to streams.	 	
Inula graveolens: 50 – 80 cm tall. Stem erect, glandular hairs covering the linear sessile leaves. Flowerheads of 6 mm, with yellow ray flowers and yellow-reddish disc flowers. Flowering on fall, from September to December.	Riparian woodlands, margins of marshes, roadsides and disturbed places.		
Persicaria maculosa 30-80 cm tall. Erect annual herb with swollen joints. The leaf blades with dark points and fused stipules. Inflorescence a dense pink spike. Flowering from July to September.			

Annex 11

The SMARTER Ambrosia survey

Form: *Ophraella* non-target survey

Instruction

This form is to report presence of *Ophraella communa* on non-target plants. Use a different form for each plant species in case you encounter several non-target plants at a site.

If present, check also 50 *Ambrosia* plants per site within the area covered by the non-target plant(s). Please complete the entire form, the more details the better.

Personal data of collector

First name		Family name	
Email address			

Site name & date

Create a unique name for the site

Always use this name for reference to this site & date

Site name	*Nearest town + unique number*	Record date	*j j j j – m m – d d*

Site description (short description of site in words)

Location information

Country		Nearest town
GPS data	Latitude (N) (in decimal degrees, with negative sign for South)	
	Longitude (E) (in decimal degrees, with negative sign for West)	
	Altitude (alt) (in meters above sea level)	

Make digital pictures of:	**Tick if done**
1. **The site.** Save as jpg file with the name + ['site'] [date in jjjjmmdd]	
2. **Of the sampled non-target plant, against a white background.** Save as jpg file with the name + ['plant'] [date in jjjjmmdd]	
3. **Optional: other relevant pictures**	

Examined non-target plant (Tick the plant species that you are going to survey)

Helianthus annus	*Xanthium strumarium*	*Helianthus tuberosus*		*Inula graveolens*	*Conyza canadensis*	*Artemisia vulgaris*	
	Others:						

Non-target plant properties

Average plant height:

Phenology of most plants:
(veg. or flowering, etc.)

Density: number of plants per m^2

Population dimensions:
(length x width)

Abundance: Estimated total number plants in the population: 1-10 / 11-25 / 26-100 / 101-500 / 501-1000 / 1001-10000 / >10000

Ambrosia artemisiifolia properties:

Ambrosia present: yes/no

Average plant height:

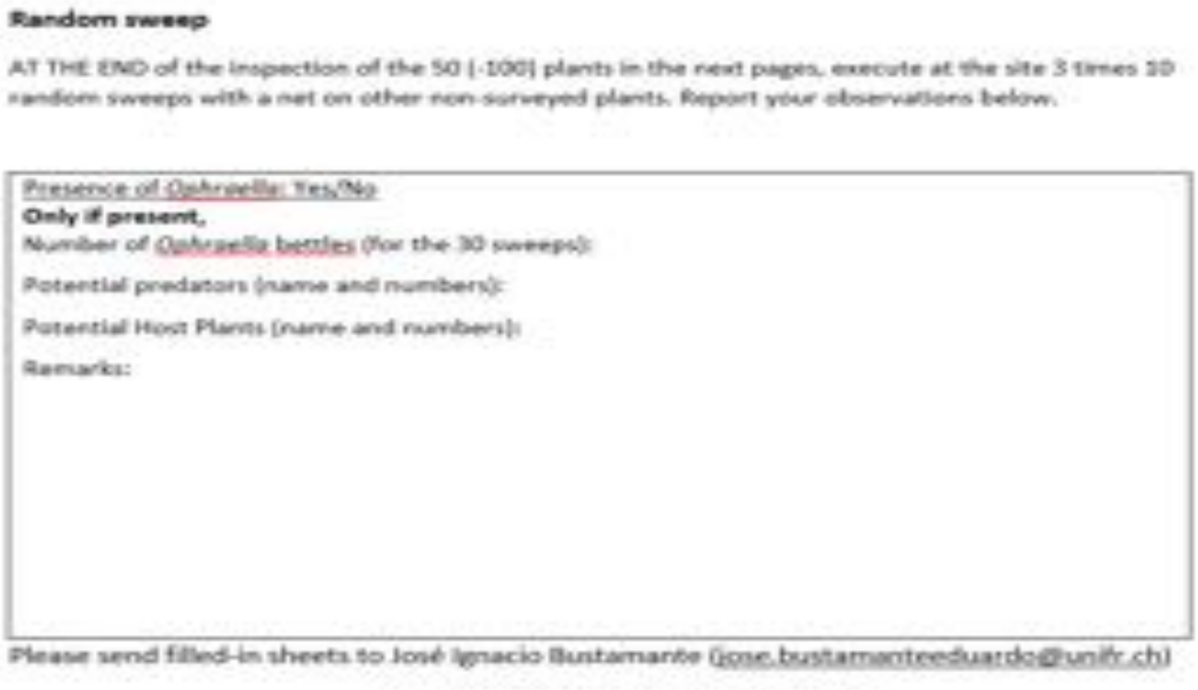
Most common stage of *Ambrosia* plants :
less than 10 leaves / more than 10 leaves / flowers formed / ripe seeds present / decaying or dead

Density: number of plants per m^2

Population dimensions:
(length x width)

Abundance: Estimated total number plants
1-10 / 11-25 / 26-100 / 101-500 / 501-1000 / 1001-10000 / >10000

Make a sketch of the site and the area examined (indicate presence of the monitored non-target and *Ambrosia* plants.



Random sweep

AT THE END of the inspection of the 50 (-100) plants in the next pages, execute at the site 3 times 10 random sweeps with a net on other non-surveyed plants. Report your observations below.

Presence of *Ophraella*: Yes/No

Only if present,

Number of *Ophraella* bettles (for the 30 sweeps):

Potential predators (name and numbers):

Potential Host Plants (name and numbers):

Remarks:

Please send filled-in sheets to José Ignacio Bustamante (jose.bustamanteeduardo@unifr.ch)

Observation of the non-target plants

Check 50 plants of your selected species along a transect when possible; if you survey a sunflower field, check 100 plants; if a field/meadow: enter the site from 4 different points; if a linear site (e.g. road, railroad) walk in a linear transect. Give a **percentage** to score damage (see damage scale)

Plant			**# *Ophraella***				**Damage**				
nr.	max. height	flowering Yes/No	egg batches	Larvae	pupae	adults	damage (0-100%)	**plant parts affected**			remarks
								leaves	stem	reprod.. structures	
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
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24											

Observation of *Ambrosia artemisiifolia* plants

Check 50 *Ambrosia* plants along a transect when possible. Give a **percentage** to score damage (see damage scale)

Ambrosia			***# Ophraella***				**Damage**				
nr.	max. height	flowering Yes/No	egg batches	larvae	pupae	adults	damage (0-100%)	**plant part affected**			remarks
								leaves	stem	reprod. structures	
1											
2											
3											
4											
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Annex 12

Non-target Survey: Overview

Table A6: This table summarizes the non-target species that were surveyed in Italy and Switzerland, as well as the number of plants and sites, and if damage and/or oviposition was found on them in any of the locations.

Plant species	Number of sites	Number of surveys	Number of Plants	Damage found	Oviposition	Remarks
Ambrosia artemisiifolia	8	8	400	Yes (10-100%)	Yes	In monte Barro and Parco delle cave not abundant
Helianthus annuus	2	3	260	Yes (0-25%)	Yes	Swiss fields not infested
Helianthus tuberosus	5	5	250	Yes (0-20%)	Yes	
Xanthium orientale	12	13	500+	Yes (0-60%)	Yes	Revised species
Ambrosia trifida	1	1	50	Yes (0-80%)	Yes	Found only in one site
Centaurea nigriscens	2	3	100+	Yes (0- 70%)	No	Only heavily damage in one survey
Buphthalmum salicifolium	1	1	25	Yes (0-70%)	No	Only found once
Xerolekia speciosissima	1	1	43	Yes (0-10%)	No	Only found once
Picris hieracioides	1	2	100+	No	No	
Bidens tripartita	3	3	100+	No	No	
Persicaria maculosa	2	2	100+	No	No	
Conyza Canadensis	6	6	250+	No	1 found	
Artemisia vulgaris	11	12	500+	Yes (young leaves)	1 found	A lot of adults sitting on it
Inula salicina	1	1	50+	No	No	
Pulicaria dysenterica	2	2	50+	No	No	
Inula sp.	1	1	50+	NO	No	
TOTAL	23	68	2500+			

Table A7: This table summarizes the results from the non-target species survey in presence of *Ophraella* and *Ambrosia*, showing information about the location, species, presences of *Ambrosia artemisiifolia* and *Ophraella communa*, as well as feeding damage of *Ophraella communa* (L= larval feeding; A= Adult feeding) and Oviposition, if eggbatches were found on the plants.

Location	**Species**	**Ragweed present**	***Ophraella* present**	***Ophraella* feeding**	***Ophraella* Oviposition**	**Remarks**
Vigevano	*Artemisia vulgaris*	Yes	Yes	NO	Yes	1 eggbatch found
Vigevano	*Conyza canadensis*	Yes	Yes	NO	Yes	3 egg batches found
Vigevano	*Xanthium orientale*	Yes	Yes	YES L + A	Yes	2 Surveys
Vigevano	*Ambrosia artemisiifolia*	Yes	Yes	YES L + A	Yes	Control Damage up to 100%
Vigevano	*Persicaria maculosa*	Yes	Yes	NO	NO	
Contone	*Helianthus annuus*	No	No	NO	NO	Field nº 1
Contone	*Helianthus annuus*	No	No	NO	NO	Field nº 2
Ponte della Becca	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	Plants far from Ambrosia without insects
Ponte della Becca	*Bidens tripartitum*	Yes	Yes	NO	NO	
Ponte della Becca	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	CONTROL
Mezzana corti	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Mezzana corti	*Helianthus tuberosus*	yes	Yes	Yes L+A	Yes	
Mezzana corti	*Ambrosia trifida*	yes	Yes	Yes L+A	Yes	
Mezzana corti	*Ambrosia artemisiifolia*	yes	Yes	Yes L+A	Yes	Control
Mezzana corti	*Bidens tripartitum*	yes	Yes	NO	NO	
Mezzana corti	*Artemisia vulgaris*	yes	Yes	NO	NO	
Mezzana corti	*Conyza Canadensis*	yes	Yes	NO	NO	
San Steffano	*Artemisia vulgaris*	Yes	Yes	NO	NO	
San Steffano	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	Control
San Steffano	*Conyza Canadensis*	Yes	Yes	NO	NO	
Abbiategrasso	*Helianthus tuberosus*	yes	Yes	Yes L+A	Yes	

Location	Species	Ragweed present	*Ophraella* present	*Ophraella* feeding	*Ophraella* Oviposition	Remarks
Abbiategrasso	*Xanthium orientale*	yes	Yes	Yes L+A	NO	
Abbiategrasso	*Conyza Canadensis*	yes	Yes	NO	NO	
Abbiategrasso	*Artemisia vulgaris*	Yes	Yes	NO	NO	
Abbiategrasso	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	Control
Monte Barro	*Centaurea nigrescens*	No*	Yes	Yes L+A	NO	3 Ambrosia near the road and on the experiments of Benno Augustinus
Monte Barro	*Picris hieracioides*	No*	Yes	NO	NO	3 Ambrosia near the road and on the experiments of Benno Augustinus
Monte Barro	*Buphthalmum salicifolium*	No*	Yes	Yes A	NO	3 Ambrosia near the road and on the experiments of Benno Augustinus
Monte Barro medow	*Xerolekia speciossisima*	No*	Yes	Yes A	NO	Still to confirm
Monte Barro	*Artemisia vulgaris*	No	Yes	Yes A	NO	
Parco delle cave	*Helianthus annuus*	yes	Yes	Yes L+A	Yes	Few plants of *Ambrosia* around
Pontecurone	*Xanthium orientale*	yes 2 plants	Yes	Yes L+A	Yes	Heavy infestation and damage!
Pontecurone	*Artemisia vulgaris*	yes 2 plants	Yes	No	No	
Ponte San Pietro	*Inula sp.*	Yes	Yes	No	No	
Ponte San Pietro	*Persicaria maculosa*	Yes	Yes	No	No	
Ponte San Pietro	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	Control; few plants only
Ponte San Pietro	*Helianthus tuberosus*	yes	Yes	Yes L+A	Yes	Heavy infestation
Ponte San Pietro	*Centaurea nigrescens*	Yes	Yes	No	No	
Ponte San Pietro	*Artemisia vulgaris*	Yes	Yes	NO	NO	
Ponte San Pietro	*Conyza Canadensis*	yes	Yes	NO	NO	
Corbetta	*Helianthus tuberosus*	Yes	Yes	Yes A	No	

Location	Species	Ragweed present	*Ophraella* present	*Ophraella* feeding	*Ophraella* Oviposition	Remarks
Corbetta	*Artemisia vulgaris*	Yes	Yes	NO	NO	
Corbetta	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Magenta	*Artemisia vulgaris*	Yes	Yes	NO	NO	
Magnago	*Artemisia vulgaris*	Yes	Yes	Yes A+L	NO	Slight adult and larval feeding on fresh leaves
Rovio	*Artemisia vulgaris*	Yes	Yes	Yes A+L	NO	Slight adult and larval feeding on young plants
Cassine	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Mezzanino	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Alba	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Alba	*Artemisia vulgaris*	Yes	Yes	No	No	
Alba	*Conyza Canadensis*	Yes	Yes	No	No	
Bareggio	*Artemisia vulgaris*	Yes	Yes	No	No	
Carvico	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	Heavy infestation and damage!; sweeping done
Carvico	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	Control, heavy infestation; 20 plants only
Liscate	*Xanthium orientale*	Yes	Yes	Yes L+A	Yes	
Liscate	*Persicaria maculosa*	Yes	Yes	NO	NO	
Lonato	*Xanthium orientale*	Yes	Yes	Yes L+A	No	Just 2 plants
Sirmione	*Pulicaria dysenterica*	Yes	Yes	No	No	
Sirmione	*Conyza canadensis*	Yes	Yes	No	No	
Sirmione	*Ambrosia artemisiifolia*	Yes	Yes	Yes L+A	Yes	Control; heavy infestation
Schilpario	*Inula sp.*	No	No	No	No	
Batistida Pancarana	*Inula salicina*	Yes	Yes	No	No	
Batistida Pancarana	*Xanthium orientale*	Yes	Yes	Yes A+L	Yes	
Batistida Pancarana	*Helianthus tuberosus*	Yes	Yes	Yes A	No	

Annex 13: Pictures of the preference experiment (Chapter 2)

Plants grown in a greenhouse in University of Fribourg (19.06.2015)

Transporting plants (09.09.2015)

Mowing to prepare the latinsquare in Rovio (10.07.2015)

Setting the latinsquare in Magnago (02.05.2015)

Preparing for transplanting, Abbiategrasso (03.05.2015)

Digging the holes every 50 cm, Abbiategrasso (03.05.2015)

Watering and applying slug pellets, Magenta (10.09.2015)

7x7 latinsquare set with a protective fence, Abbiategrasso (08.07.2015)

Larvae and adult on Girasole medium, Rovio Cohort 2 (19.07.2015)

Final assessment for cohort 1 in Magenta (03.08.2015)

Adults and larvae on Ambrosia flowerheads, Magnago (20.08.2015)

Adults feeding on *Guizotia abbyssinica*, Magenta, 20.10.2015

Adult feeding on Sunrich flowerhead, Magnago (21.10.2015)

Monitoring cohort three end of Autumn, Magnago, 21.10.2015

Pupae in Rovio Cohort 2 (19.07.2015)

Iregui damaged, Magenta (09.11.2015)

Annex 14: Pictures of the non-taget survey (Chapter 4)

Survey in a sunflower field in Ticino (06.08.2015)

Ambrosia trifida, Helianthus tuberosus and *Xanthium strumanium*, all hosts of *Ophraella communa* (11.08.2015)

Bidens tripartita, no *Ophraella* found (14.08.2015)

Pupae on a heavily damaged *A. trifida* (14.08.2015)

Counting eggbatches on an *A. trifida* (14.08.2015)

Adults feeding on *Buphthalmum salicifolium,* Monte Barro (10.08.2015)

Checking adults on *Conyza canadensis*, River Ticino (04.08.2015)

Survey in Monte Barro for *Picris hieracioides* (12.09.2015)

Predators also found in *Xanthium strumanium* (11.08.2015)

Adult sitting on *Artemisia vulgaris* (04.08.2015)

Survey in Monte Barro for *Centaurea nigriscens* (10.08.2015)

Larva on *Helianthus tuberosus,* Abbiategrasso (13.08.2015)