UNIVERSITÉ DE FRIBOURG
UNIVERSITÄT FREIBURG

# Is *Ophraella communa* a risk for non-target plant species?

Master Thesis
Gaëlle Kadima

Supervised by:
Heinz Müller-Schärer

University of Fribourg (CH)
Ecology and Evolution

## Abstract

*Ambrosia artemisiifolia* L. or common ragweed is native to North America and became invasive around the world causing allergic reactions to humans through its pollen and yield losses to crops as a weed. Chemical and mechanical methods are already developed and used to control the weed but sustainable control methods to reduce its abundance and minimize its spread are missing in Europe. A biological control insect already used in China is the leaf beetle *Ophraella communa,* native to North America*.* In 2013, it was also found in Europe, assumed to be accidentally introduced. Before introducing natural enemies as biological control agents or in our case spreading the beetle on purpose into ragweed infested areas, host specificity tests are essential to minimize the damage to non-target plant species.

We studied oviposition preference, larval and adult feeding, larval development and adult fecundity under natural conditions using different experimental designs under choice and no-choice situations, and in an early and late cohort along the ragweed growing season. Experiments were carried out in five study sites in Italy, one study site in Switzerland and one study site in China, using besides *A. artemisiifolia* the test plant species *Ambrosia trifida, Artemisia annua, Helianthus annuus, Helianthus tuberosus and Zinnia elegans*. Under choice conditions a very low amount of *O. communa* egg batches was found on non-target plants and none of the stages were found on *A. annua* and *Z. elegans*. Most egg batches observed on sunflowers were found in the experiments in Langfang/Beijing on the sunflower variety Extrasol (n=22) in the Reverse Interspersion experiment (with a high relative number of the non-target plants compared to the target plants) and less on the variety Girasol (n=8) in the Latin square experiment. Only 1-2 egg batches were found on sunflowers in the other choice tests and study sites. *Ophraella communa* larval survival and presence of pupae and adults were significantly higher on *A. artemisiifolia* than on the non-target plants. In general, abundance of adults increased throughout the season, most prominently in the Latin square experiment in Rovio, where mean *O. communa* adult load increased from 17.75 in the first cohort to 49.67 in the second cohort. Here, *O. communa* was able to severely damage *H. annuus* plants late in the season (end of September), but oil sunflower is already harvested by end of August. In all three open field experiments in Langfang/Beijing, sunflowers were significantly more damaged than *A. artemisiifolia*. The *H. annuus* varieties in the Reverse Interspersion experiment in Langfang/Beijing had all a damage level of 4 (>90% damaged leaf tissue). In the Interspersion experiment in Rovio, *A. artemisiifolia* and the sunflower varieties Extrasol, Girasol and Italy were severely damaged. In the Interspersion experiments in Italy *A. artemisiifolia* was significantly more damaged than the non-target plant species and the *H. annuus* varieties Girasol and Extrasol were slightly more damaged

than the other non-target plants. *Helianthus tuberosus* was only moderately damaged. Under no-choice conditions through egg transfer, between 17-81% developed to small and big larvae on *A. artemisiifolia.* The larval development on non-target plants was very low. Most small larvae on non-target plants were found on *H. annuus* LG5687 (n=28) and no larvae were found on *A. annua.* Damage caused to the non-target plants in the no-choice test was not higher than in the choice-tests, therefore it cannot be concluded that damage is highest under no-choice conditions. Results of our study with *E. strenuana* in Langfang/Beijing showed a negligible number of galls in sunflowers (only 1 gall), but a considerable amount of galls (n=32) on *A. trifida* in the Reverse Interspersion experiment.

The various experimental setups providing different field situations allowed getting a more differentiated overview, but overall, predicting the risk to the non-target plant species, especially to *H. annuus* remains difficult. The fewer *A. artemisiifolia* and the more *O. communa* present in the open-field experiments, the higher was the damage to non-target plants. Thus, the interaction of the abundance of *A. artemisiifolia* and *O. communa* greatly determines the risk to non-target plants.

**Key words:** *Ambrosia artemisiifolia*, *Ophraella communa*, *Epiblema strenuana*, non-target plant species, classical biological control, risk assessment

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# 1. Introduction

## 1.1 Classical biological control of invasive plant species

As a result of human globalization with increased trade, transport and travel the introduction of plant species to a non-native area increased significantly in the past 200 years in most parts of the world (Pyšek et al., 2009). These introduced, non-indigenous or alien species can be beneficial for humans, e.g. most crop species, or become invasive and be one of the major threats to biodiversity (Blossey, 1999). They can affect the new environment in an ecologically, environmentally or economically damaging way (Wittenberg and Kenis, 2005). Though, in Europe in contrast to other continents, recognition of the negative impact of invasive plant species just began to increase recently (Hulme and Drake, 2009). One of the most prominant invasive plant species in Europe is *Ambrosia artemisiifolia* (Asteraceae). Chemical and mechanical methods have recently been developed and are used to control *A. artemisiifolia* (Buttenschøn et al., 2009; Essl et al., 2015), but sustainable control methods to reduce its abundance and minimize its spread are missing in Europe. For example in Australia biological control is a successful solution to manage *A. artemisiifolia* sustainably (Gerber et al., 2011). Biological control involves the release of a natural enemy to control a target species, which is a pest in a given environment, with the aim to reduce the existence of the pest species. There are two different types of biological control. The augmentative biological control is defined, when a native biological control agent is released once or several times within a season to control a pest species. In classical biological control, the agent is released with the goal to establish and to control the target species permanently. The advantage of biological control is not just the environmentally beneficial replacement of pesticides (De Bach, 1964; Huffaker, 1957), but also the low economic cost/benefit ratio and the long-term reduction of invasive species (Louda et al., 2003). On the other hand, some ecologists value deliberate release of non-native species to be a threat to the structure and dynamic of communities. For instance the irreversibility of an introduction, the potential host switching or adaptation to new hosts and the possible dispersal of agents are main concerns (Simberloff and Stiling, 1996; Strong and Pemberton, 2000, 2001). The problem of most natural enemies feeding on *A. artemisiifolia* is their relatively wide host range (non-target effect = biosafety issue) and only moderate damage to *A. artemisiifolia* (limited target effect = efficacy issue). The presently most promising biological control agents in China are the leaf beetle *Ophraella communa* (Coleoptera: Chrysomelidae) and the stem-galling moth *Epiblema strenuana* (Lepidoptera: Tortricidae) (HongSong et al., 2013). Gerber et al. (2011) recently made a literature survey for

potential biological control agents to be used in Europe, and prioritized them with regard to their biosafety and effectiveness (Fig. 1).

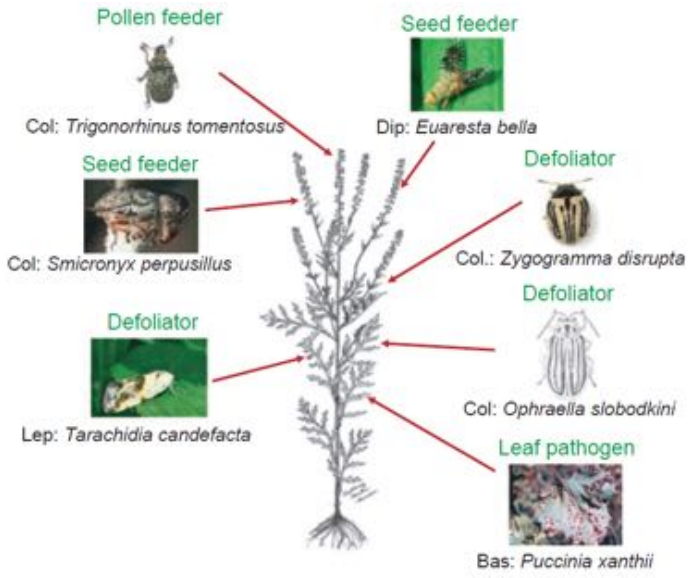


**Figure 1 :** Potential biological control agents for *Ambrosia artemisiifolia* in Europe. Bas: Basidiomycota, Col: Coleoptera, Di: Diptera, Lep: Lepidoptera. (Source: Gerber et al, 2011).

Host specificity tests are essential before introducing natural enemies as biological control agents into an infested area. Oviposition preference, larval and adult feeding as well as larval development or adult fecundity under natural conditions, but also different experimental designs like choice or no-choice situations are of importance for host specificity tests (Heard and Van Klinken, 1998; Müller-Schärer and Schaffner, 2008). Choice tests can be separated into normal choice test where the non-target plants are provided with the target plant, and choice minus control where the non-target plants are provided without the target plant (Heard and Van Klinken, 1998). There are different types of choice tests (cf. 2.1.3) that can be performed in the open field or in a controlled area such as laboratory experiments. Choice tests mainly concentrate on test plants species where the potential biological control agent was observed to have fed, oviposit or completed its life cycle under no-choice conditions. No-choice tests, on the other hand, can be separated into sequential and simultaneous test, depending on whether the target species and the non-target species are offered in sequence to the same insect or at the same time to different insects (Heard and Van Klinken, 1998). No-choice tests intend to identify fundamental larval host ranges, which is the range of plant species that support complete development (Schaffner, 2001). The importance of no-choice larval development tests has been disputed (Cullen, 1990). On the one hand these results are assumed to be essential to identify the fundamental larval host range, but on the other hand it is criticised that

the results are unhelpful because biological control agents can be eliminated even though they would be safe in practice. It is because the range of plant species on which larvae are able to complete their life cycle is often wider than the oviposition range of female beetles (Schoonhoven et al., 1998). This has also been discussed by Schaffner (2001). Few larvae were able to complete their life cycle on non-target test plants that were not recorded as field host plants under no-choice conditions. Some of those test plants were not even from the same host genus. In this study, we carried out a simultaneous no-choice egg transfer test to assess larval development of *O. communa*. These tests help to predict most non-target effects and false results (Marohasy, 1998). Non-target effects of biological control agents involve the harm of species other than the target species such as competitive displacement of non-target species (Cook et al., 1996).

## 1.2 Study system

*Ambrosia artemisiifolia* L. or common ragweed is from the family Asteraceae, the tribe Heliantheae and the subtribe Ambrosiinae. It is native to North America including Mexico, the United States and especially the eastern parts of Canada. It has been introduced around the world and became invasive causing allergic reactions to humans and yield losses to crops (Bagarozzi and Travis, 1998; Bassett and Crompton, 1975; Essl et al., 2015; Richardson et al., 2000). It is a dicotyledonous, monoecious and wind-pollinated plant species with many staminate flowers and a lot of pollen grains that are the primary cause for allergenic hay fever histamine reactions, eczema or asthma (Bagarozzi and Travis, 1998). It is an annual plant that grows best in full light (Wenyuan, 1991) and reproduces only through seeds and germinates in April. The male flowers are in spike-like racemes at the top of the plant producing a lot of pollen, from the beginning of July until fall. Female flowers are in lower axil with each single flower creating achene-like fruits starting in the middle of August until the end of the life cycle. The ragweed plant does not survive the frost (Bohren et al., 2008), but its seeds are able to overwinter (Brandes and Nitzsche, 2007) (Annex 1). As a pioneer species with a high level of allelopathic activity it is in different provincial statutes even considered as a “noxious weed” occurring in disturbed habitats such as along motorways and river banks, but also in open lots or uncultivated crop fields (Bagarozzi and Travis, 1998; Essl et al., 2015; Teshler et al., 2002). As agriculture increased, *A. artemisiifolia* started to spread widely and became a real pest in the eastern parts of Canada (Teshler et al., 2002). It also began to disperse in other countries and became invasive in China, Japan, Australia and some parts of Europe including Switzerland (Essl et al., 2015; Wan and Ding, 1993; Wittenberg and Kenis, 2005). Actually, in western Europe it was already discovered in the mid-1800s and in eastern Europe in the 1900 but started to become a problem not until the 1920s (Csontos et al., 2010). It invaded countries in central Europe (Hungary, Slovakia and

Austria), southern Europe (Italy and southern France), eastern Europe (Ukraine, European part of Russia) and south-eastern Europe (Romania, Serbia and Croatia) (Hulme and Drake, 2009). Populations in France have been discovered to have similar genetic variability as those in the native range. The introduced range had surprisingly even higher within-population variation than in North America, but subsequent results showed that it was due to introduction of seed mixtures containing different North American populations (Genton et al., 2005). The main concern in Europe is its allergenic pollen, which affects 15% (e.g. Germany, Denmark and The Netherlands) to 60% (Hungary) of the population (Rybnicek and Jager, 2001; Taramarcaz et al., 2005). Also the caused significant yield losses especially in sunflower, sugar beet, maize, soya beans and cereal crops are severe problems (Kazinczi et al., 2008). *Ambrosia artemisiifolia* is difficult to control because it can grow new stems below cutting height and it can easily adapt to mowing even though it can be easily uprooted (Vincent and Ahmim, 1985). But based on a prioritisation scheme created by Sheppard et al. (2006) it is in the top 20 of the most promising species for classical biological control in Europe. The achieved success of biological control in Australia and Asia (Zhou et al., 2010) is most promising to be also transferred successfully to Europe (Gerber et al. 2011).

*Ophraella communa* is an oligophagous leaf-eating beetle (Chrysomelidae) from the subfamily Galerucine (LeSage, 1986) and the tribe Galerucini (Futuyma and McCafferty, 1990). It is native to North America and Mexico (Welch, 1978). The beetle is multivoltine, i.e. has several generations within a year (Zheng et al., 2011). The pyriform yellow eggs of *O. communa* are laid in batches on the leaves of the host plants and hatch after 5-6 days (Welch, 1978) (Fig. 2). The time of development of the three larval stages is each 12-14 days long. At the beginning, the larval feeding causes “shot-hole”-damage, after that they disperse and skeletonize the leaves. The third larval stage forms a cocoon usually on the upper part of the leaves and away from the feeding area (Fig. 2). This cocooned non-feeding prepupal stage lasts for 3-4 days. The pupal stage itself is 3-4 days. This means that the total development time, from egg deposition to the adult stage, is 21.8±0.86 days (Welch, 1978)The adults mate usually 1-2 days after hatching. The female beetles oviposit 5 days after emergence or, if it is the last generation they overwinter and oviposit in the followed summer (Goeden and Ricker, 1985; Welch, 1978). During the last decade *O. communa* has been introduced to several countries all over the world. In East Asia it has been first discovered in 1996 in Japan (Takizawa et al., 1999) and Taiwan (ChinLing and MouYen, 1998). Later it has been discovered in 2000 in Korea (Kwon et al., 2001) and in 2001 in Nanjing, China (Meng and Li, 2005; Shiyake and Moriya, 2005). In 2013, *O. communa* has been found in Europe only in the south of the Alps (in Ticino, southern Switzerland and Lombardia, Piemonte and Emilia-Romagna, northern Italy) (Müller-

Schärer et al., 2014). *Ambrosia* is reported to be its preferred native host plant (Funk et al., 1995; Futuyma and McCafferty, 1990; Palmer and Goeden, 1991). Because of observations in Japan that have shown the excellent vagility and host-discriminating ability of *O. communa* (Yamazaki et al., 2000) the beetle is considered to be a promising biological control agent against ragweed. But even though *O. communa* is an oligophagous beetle, especially feeding on *A. artemisiifolia* (LeSage, 1986; Teshler et al., 2002) and on few other members of the subtribe Ambrosiinae (Gerber et al., 2011; Miyatake and Ohno, 2010; Yamazaki et al., 2000), it has a history of host use, especially in introduced ranges (Funk et al., 1995; Futuyma et al., 1995; Palmer and Goeden, 1991). Whereas in Japan and China experiments showed that it is able to feed on *Ambrosia trifida,* this behaviour has never been reported in the native northern American region (LiJie et al., 2005; Moriya and Shiyake, 2001; Takizawa et al., 1999; Watanabe and Hirai, 2004; Yamazaki et al., 2000). Preadaptation might be a reason why *O. communa* is able to feed on non-target plant species not reported in the native region (Marohasy, 1996). Therefore detailed studies of its host-specificity are important. The newly recently started European research network SMARTER (Sustainable management of *Ambrosia artemisiifolia* in Europe; EU-COST FA1203, Müller-Schärer and Lommen, 2014) is offering an optimal framework to respond quickly to the recent establishment of *O. communa* in Europe (Müller-Schärer et al., 2014). For instance, the risk to *Helianthus annuus* remains unclear.


**Figure 2 :** *Ophraella communa* life stages on *A. artemisiifolia* **A**: egg batch; **B**: larva; **C**: pupa; **D**: adult. Source: http://cse.niaes.affrc.go.jp/yamamura/RG_stages_e.htm

*Epiblema strenuana* Walker (1863) or ragweed borer belongs to the order *Lepidoptera* and to the family *Tortricidae*. The gall-building moth is native to North America including the Unites States, Canada and Mexico (Brown, 1973a; Brown, 1973b; Heinrich, 1923; Mackay, 1959; Muesebeck, 1935; Rice, 1936). It was introduced from Mexico to Australia in 1982, where it was approved as a promising classical biological control agent for different *Ambrosia* weed species (McClay, 1987). Then it was introduced to China in 1990 (Wan and Ding, 1993). In 2008, *E. strenuana* was found in Western Hedera, Israel on *Ambrosia tenuifolia* and *Ambrosia confertiflora* probably imported from the United States via the import of plant material such as grains (Yaacoby and Seplyarsky, 2011). *Epiblema strenuana* lays eggs singly or in small groups which are fixed to the stem (McClay, 1987) or the leaves (Yaacoby and Seplyarsky, 2011). The larvae first feed on the leaves, then bore into the stems and begin to feed on the terminal meristem. They build galls, where the pupae are produced. Plants attacked by the moth were observed to have growing shoots which became yellowish, and in which the apical dominance was broken (Yaacoby and Seplyarsky, 2011). Attacked *A. artemisiifolia* were reported to have reduced size, abundance and pollen production (Gerber et al., 2011). Choice and no-choice tests in China showed that *E. strenuana* has a restricted host range. The moth was observed to complete its life cycle on *A. artemisiifolia*, *A. trifida*, *P. hysterophorus* and *Xanthium* sp. (Wan and Ding, 1993).

**Figure 3 :** *Epiblema strenuana* life stages: Left: Adult on *Ambrosia confertiflora*. Right: emergence of *E. strenuana* from pupae. Laboratory rearing, PPIS Diagnostic service, 2009.

Five different non-target study species with different level of relatedness and different use were used for my experiments (Table 1). *Ambrosia trifida* is from the same genus and is most closely related to *Ambrosia artemisiifolia*. *Helianthus annuus* and *Helianthus tuberosus* are from the same tribe Heliantheae as ragweed but from the subtribe Helianthinae. *Zinnia elegans* is as well from the same tribe but in the less closely related subtribe Zinninae. According to their phylogenetic relationship to *A. artemisiifolia,* they are considered to have the highest risk of being attacked by *O. communa* (Briese, 2003). Only *Artemisia annua* is from another tribe but from the same Asteraceae family (Table 1).

*Ambrosia trifida* or giant ragweed is an annual plant species that is native to North America and belongs to the family Asteraceae (Bassett and Crompton, 1982) and the tribe Ambrosiinae. It occurs in southern Canada and midwestern and eastern United States of America in meadows, roadsides or river valleys, but also in fertile fields (Abul-Fatih et al., 1979; Bassett and Crompton, 1982; Hunt and Bazzaz, 1980). It considered to be one of the most problematic annual weeds during summer (Gibson et al., 2005). Field observations in Japan showed that *O. communa* is not only feeding on *A. artemisiifolia,* but on *A. trifida*, too, especially when the target weed *A. artemisiifolia* was not available anymore (Emura, 1999; Yamazaki et al., 2000). It was also observed that *O. communa* was laying a few eggs on this non-target plant (Watanabe and Hirai, 2004). No-choice tests in a laboratory in Japan showed that adults preferred feeding on *A. artemisiifolia* although larval performance was higher on *A. trifida* (Yamazaki et al., 2000). In Korea a trial was made using *O. communa* to control *A. trifida*. Hence, *A. trifida* is considered to be a host plant for *O. communa* and *O. communa* could play a role in controlling *A. trifida* in the northern parts of China (Lee et al., 2007). *Ambrosia trifida* is also increasingly becoming invasive in Europe, with already large established populations in Lombardia, Northen Italy (pers. observation).

*Helianthus annuus,* sunflower, is native to America and a widespread crop in the world. It belongs to the family Asteraceae, the tribe Heliantheae and the subtribe Helianthinae. There are many different varieties of *H. annuus* (Heiser Jr, 1954). For instance the different *H. annuus* varieties used in the experiments were the variety Extrasol and LG5687 from France or Girasol from Switzerland. Field tests in the USA showed no record of the beetle on sunflowers but no-choice tests under laboratory conditions showed the opposite. Extensive feeding and the completion of the life cycle of the beetle lead to the result that the introduction of *O. communa* as a biocontrol agent was regarded as too risky for Australia (Palmer and Goeden, 1991). No-choice tests in Canada showed that larvae can damage sunflowers significantly, but that the survival rate is low and that adults avoid sunflowers so that the risk to sunflowers is negligible (Dernovici et al., 2006). Different tests in China showed that

there is a negligible amount of oviposition and no risk for oviposition on sunflowers. But tests on leaf discs showed the ability to complete the life cycle on eight different sunflower varieties (Zhenjun Cao, 2011; Zhou et al., 2010). Choice and no-choice tests in China found *E. strenuana* larvae able to form galls and complete their life cycle on *H.annuus*. But because the larvae were only found on weakened *H. annuus* plants and only few adults emerged from these galls and because no oviposition was ever observed on *H. annuus* it was concluded that *H. annuus* is not a suitable host for *E. strenuana* (Wan and Ding, 1993).

*Helianthus tuberosus* L., topinambour or Jerusalem artichoke, is native to America and a temperate zone crop plant species (Kays and Nottingham, 2007). Field reports from Japan recorded occasional feeding of *O. communa* adults on *H. tuberosus* (Watanabe and Hirai, 2004). Laboratory choice feeding tests in China showed feeding of adults but no feeding of larvae on *H. tuberosus*. Larvae could develop into adults but the females did not oviposit on *H. tuberosus* and larval survival and weight of pupae was lower than on *A. artemisiifolia* (Hu and Meng, 2007).

*Zinnia elegans* L. cv. Canary bird is an ornamental plant species from the family Asteraceae and the tribe Heliantheae, it is native to North America and Mexico and has been widely cultivated as an ornamental plant (Torres, 1963). In a laboratory choice cage experiment in the USA no feeding by *O. communa* adults or larvae and no female oviposition was observed on *Z. elegans* (Palmer and Goeden, 1991).

*Artemisia annua* L. is an annual plant that belongs to the family Asteraceae and to the tribe Anthemideae. It is native to Asia and Eastern Europe, has naturalized in the United States and is widely distributed throughout temperate regions (Bailey, 1976; Simon et al., 1984). *Artemisia annua* has been used for many centuries in Chinese medicine. Its substance artemisin is considered to ease the fever of malaria (Klayman et al., 1984). Field surveys in Japan showed no presence of *O. communa* on *A. annua*. Laboratory studies testing larval performance and feeding choice of *O. communa* larvae in Japan showed feeding on *A. annua,* but no suitability in terms of larval survival (Yamazaki et al., 2000).

**Table 1 :** Overview of the relatedness, the source and the importance of the study species used in the choice and no-choice tests.

| Study species | Tribe | Life cycle | Origin | Source | Importance |
|---|---|---|---|---|---|
| *A. artemisiifolia* | Heliantheae | Annual | N-America | Geneva | Invasive |
| *A. trifida* | Heliantheae | Annual | N-America | China | Invasive |
| *A. annua* | Anthemidae | Annual | Asia | Botanical garden, University of Zurich | Medicinal |
| *H. annuus* Extrasol | Heliantheae | Annual | America | France | Crop plant |
| *H. annuus* Girasol | Heliantheae | Annual | America | Switzerland | Crop plant |
| *H. annuus* Italy | Heliantheae | Annual | America | Italy | Crop plant |
| *H. annuus* LG5687 | Heliantheae | Annual | America | France | Crop plant |
| *H. tuberosus* | Heliantheae | Perennial | N-America | Gartencenter Wyss, Zuchwil | Crop plant |
| *Z. elegans* | Heliantheae | Annual | S-America | Migros, Biel Bienne | Ornamental |

## 1.3 Study aim and hypothesis

Since the beetle *O. communa* has been introduced in Europe and been able to establish in 2014, the question emerged if this is a chance for the control of ragweed in Europe or if it is a risk for the local ecosystem, especially for sunflower. The aim of my master thesis was to get an overview of the effect of the newly introduced beetle to non-target plant species. Considering the variable results and conclusions in previous experiments in other countries it was difficult to hypothesise the damage in Europe on non-target species especially for sunflowers. Most pre-existing results showed that different stages of *O. communa* can be found on non-target plant species, but in a negligible, harmless quantity. It is assumed that larval survival will be very low on the non-target plant species. For the choice experiments in the open field it is supposed that there will be significantly more *O. communa* on the target plant species than on the non-target plant species. Comparing the different choice experiments it is hypothesized that the more *A. artemisiifolia* plants are available for the beetle the less beetle will be found on the non-target plant species and hence the lower will be the damage on those plants. It is also hypothesized that *O. communa* will be able to increase its population throughout the summer, build-up several generations and lead to a highly damaged and decreasing population of *A. artemisiifolia*. *Epiblema strenuana* is assumed to be only able to form galls on *A. artemisiifolia*. For the no-choice egg transfer experiment it is hypothesized that significantly more larvae will develop on *A. artemisiifolia* than on non-target species because they would not have enough food and hence die of starvation. Furthermore, it is assumed that damage to the non-target plant species will be higher in the no-choice test compared to the choice tests because the female beetles would not lay eggs on non-target plants in nature and because of that the larval damage would be lower.

# 2. Choice test: Open-field experiments

## 2.1 Material and Method

### 2.1.1 Growth conditions of the study species

Some study plant species were directly collected in the field others were reared in the greenhouse (Table 1). The plant species reared in the greenhouse at the University of Fribourg were either in a TKS2 (Flora gard) and sand mixture (2:1) or in a ProTer+ProType4 (ProTer4, Landi) and sand mixture (2:1). Then, the seedlings with a height of approximately 5cm were potted into 1L pots before they were put in big boxes to be transported by car to the study sites. Every sunflower variety, except LG5687, was also sown and grown next to an allotment garden in Corbetta, Italy as a backup. These latter ones were only used for the first cohort (cf. below), because they were destroyed by insects and heavy rain later in August. The plants were planted in the field with a height between 20-40cm and had no previous damage from or presence of *O. communa*. The *O. communa* adults released in the experiments in Italy and Switzerland were directly collected at the study sites. The sunflower varieties used for the experiments in Langfang/Beijing China, were sown in pots outside the experimental Station. *Ambrosia trifida* and *A. artmisiifolia* were sown in an outdoor and then transplanted into bigger pots before they were planted in the study site. The beetles used in the experiments in China were mostly collected as adults, only few were collected as larvae and reared in cages in the greenhouse of the biological invasion research group in Langfang, Beijing. Most of the collected *Epiblema strenuana* were in their larval stage in *A. artemisiioflia* stems and some were collected as adults.

### 2.1.2 Study sites

Abbiategrasso, Busto Arsizio, Magnago and Magenta in Italy, Rovio in Switzerland and Langfang/Beijing in China were used as study sites (Table 3; Annex 3; Annex 4). These study sites have been chosen because *A. artemisiifolia* and *O. communa* have been observed the previous year. The sites in Abbiategrasso and in Magnago were abandoned meadows next to a factory. Both had an intermediate abundance of *O.communa* and *A. artemisiifolia*. The study sites in Busto Arsizio were a 10x10m square inside an experimental site of Rodolpho Gentilini, University of Bicocca, Milan. One field was between a road and the Parco alto Milanese and the other in the middle of the Parco alto

Milanese. Both had a high density of *A. artemisiifolia* and *O. communa* and were next to crop fields. The study site in Magenta was inside a crop field with a very low abundance of *A. artemisiifolia* and *O. communa* in 2014. The study site in Rovio was a steep meadow next to a hotel where sheep were grazing. The study site itself was separated with a fence to avoid damage on study plants by the sheep and further spread of seeds by sheep. It had an intermediate abundance of *A. artemisiifolia* and *O. communa*. The study site in Langfang/Beijing was an experimental field inside the University area (Annex 4).

### 2.1.3 Experimental setup

Three different open-field study designs were built (Table 2). An Interspersion and a Reverse Interspersion experiment were performed in a natural environment (Table 2). Natural means that the surroundings of the study sites were not manipulated in any way, except the planting of test plants. The interspersion experiment was to examine the potential damage of *O. communa* on non-target species in a field full of *A. artemisiifolia*, whereas the Reverse Interspersion was supposed to mimick the opposite situation. In the Reverse Interspersion experiment, the preference of *O. communa* on non-target species was assessed in a field full of non-target species and only few ragweed plants. A similar experiment was the open-field Latin square experiment (Table 2). In the open-field Latin square experiment the study sites were prepared to have the most neutral and similar start situation for all plant species. Neutral means that only the test plants were supposed to be inside the experimental plot and all other plants were removed. The aim of the Latin square study design was to mimic the situation during and after the colonisation by *O. communa*. The question was to know how the beetles would react to the decreasing ragweed population and if they would prefer to migrate to the adjacent non-target species, if they would look for another ragweed population and leave the experimental plot or if they would die of starvation. The Latin square experiment was separated in two cohorts. The first cohort was from July-August 2014, where the experiment was expected to be in the presence of *A. artemisiifolia* and *O*. *communa*. The second cohort was from August- September 2014, where *A. artemisiifolia* was expected to be killed by *Ophraella*, and thus absent. Because of plant feeding from wild animals, the study side in Abbiategrasso was completely destroyed. After building it up again and after a second destruction we decided not to use this study site anymore. Every plant species or plant variety in the study designs in Italy and Switzerland were labelled with different colours to be easily recognized in the field (Annex 5).

**Table 2 :** Overview of the study designs and the expected damage by *O. communa* to non-target species. The non-target plant species in the Iinterspersion experiment are supposed to have the least damaged tissue. It is supposed that the damage to the non-target plant species in the Latin square is lower in the presence of *A. artemisifolia* than in absence of A. *artemisiifolia*. The non-target plant species in the Reverse Interspersion are assumed to be much more damaged than in the Interspersion experiment. The most damaged non-target plants are assumed to be in the no-choice egg transfer experiment (cf. chapter 3).

| Experiment | Mimicked situation | Expected damage |
|---|---|---|
| Interspersion | Non-target species in an *A. artemisiifolia* field |  |
| Open-field Latin square w/ *A. artemisiifolia* | Colonisation by *O. communa* | |
| Open-field Latin square w/out *A. artemisiifolia* | Dispersal by *O. communa* (after *A. artemisiifolia* control) | |
| Reverse Interspersion | Crop field before *A. artemisiifolia* control | |
| Egg transfer | Larval development | |

**Table 3** : Overview of the study sites used for the choice and no-choice tests. The first cohort was from July to August 2014 and the second cohort was from August to September 2014 (Annex 13).

| Study sites | GPS coordinates | Habitat description | Experiment | Cohort (duration in weeks) |
|---|---|---|---|---|
| Abbiategrasso, IT | 45.38319, 8.92802 | Abandoned field | Latin square<br>Egg transfer | Early (4), late (4)<br>Early (4), late (4) |
| Busto Arsizio 1, IT | 45.59834, 8.86926 | Crop field | Interspersion | Early (8) |
| Busto Arsizio 2, IT | 45.59412, 8.87059 | Crop field | Interspersion | Early (8) |
| Magenta, IT | 45.45953, 8.87472 | Crop field | Latin square<br>Egg transfer | Early (4), late (4)<br>Early (4), late (4) |
| Magnago, IT | 45.57073, 8.78546 | Abandoned field | Latin square<br>Interspersion<br>Egg transfer | Early (4), late (4)<br>Early (8)<br>Early (4), late (4) |
| Rovio, CH | 45.93087, 8.98377 | Meadow | Interspersion<br>Latin square | Early (8)<br>Early (4), late (4) |
| Langfang/Beijing, CN | 39.057509, 116.327605 | Meadow | Interspersion<br>Reverse Interspersion<br>Latin square | Early (10)<br>Early (10)<br>Early (10) |

#### 2.1.3.1 Setup, monitoring and measurements Latin square experiment

The non-target sunflower varieties *H. annuus* Extrasol, Girasol, LG5687 and an Italian variety, the species *H. tuberosus*, *A. annua* and *Z. elegans* were used in the Latin square experiments in Italy (Annex 2). The setup of the experiment was on the 15$^{th}$ of July 2014 in Magnago and on the 17$^{th}$ of

July 2014 in Magenta. A 12x12m plot was mown with a hand mower and the test plants were planted in a 7x7m plot in the middle of this 12x12m plot. Each plant had a distance of one meter from one to another. The alignment of the non-target test plants was random but every species or variety appeared once per row and column and the target species was planted in a diagonal, always starting from the left head to the bottom right (Fig. 4). The design was thus 8 plant species x individuals (reps) = 64 plants in total. The plants were fixed to bamboo sticks to avoid the stem to break or the plant to fall to the ground. On the 22nd of July 2014 120 Beetles were released out of plastic tubes in the middle of the Latin square (Annex 13). The exposure was weekly from the 30th of July to the 20th of August 2014 in Magnago and 22nd of July 2014 in Magenta. In the final assessment the plants were cut and weighted for the fresh biomass. At the same day, all non-target plants were replaced in the exact same holes as the previous ones for the second cohort of the experiment. This time, no *A. artemisiifolia* plants were planted. The exposure was again weekly for four weeks until the 16th of September in Magnago and the 17th of September in Magenta. Again, all plants were cut and weighted for their fresh biomass during the final assessment. At the beginning it was planned to have satellite plots during the second part of the experiment but because there were still many *A. artemisiifolia* plants present around the study design, we decided not to plant any additional ones.

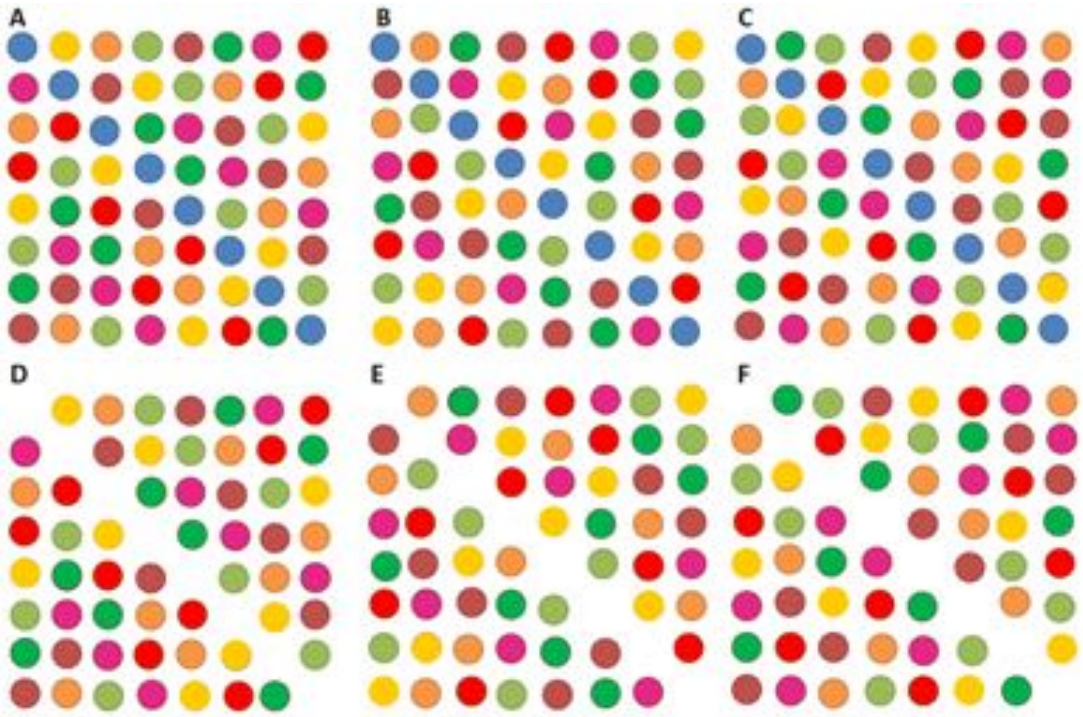


**Figure 4** : Set design for the open-field Latin square experiments in Italy. The blue circles are *A. artemisiifolia* plants, the greenish and reddish circles are the non-target species (seven species). Every row and column contains each species once. There are eight plants per species. A/D: Open-field latin square in Magnago. B/E: Open-field Latin square in Magenta. C/F: Open-field latin square in Abbiategrasso. A-C: The open-field latin squares which were set up in the early season July/August where *O. communa* and *A. artemisiifolia* were assumed to be present. E-F: The open-field latin squares from the late season in August/September, where *O. communa* was assumed to be present and *A. artemisiifolia* was assumed to be absent.

The setup of the Latin square experiment in Rovio, Switzerland, was on the 4th of August 2014. A 5x5m plot was mown with a hand mower. Inside the plot, a 3x3 meter plot was used to plant four plants per *H. annuus* variety (Extrasol, Girasol and Italy) and four *A. artemisiifolia* plants, having one meter distance from one to another (Fig. 5). Like in the Latin square experiments in Italy, *A. artemisiifolia* was planted in a diagonal starting from the left head going to the bottom right. The *H. annuus* varieties were planted randomly but always one per row and column (Fig. 5). 30 *O. communa* adult beetles were released in the centre of the Latin square on the 4th of August 2014 (Annex 13). The exposure of the first cohort was from the 11th of August to the 1st of September 2014. There was a weekly assessment and for the final assessment on the 1st of September 2014, the plants were cut and the fresh biomass was taken. After that, the non-target plants were replaced for the second cohort. The exposure of the second cohort was from the 8th of September to the 1st of October 2014. There was a weekly assessment but no assessment in the third week.

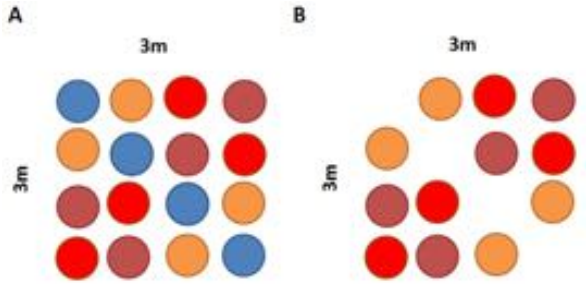


**Figure 5 :** Latin square experiment in Rovio, Switzerland. The blue dots are *A. artemisiifolia* plants. The mat red dots are *H. annuus* Girasol, the bright red dots are *H. annuus* Italy and the orange dots are *H. annuus* Extrasol. **A:** Latin square in the first cohort where *A. artemisiifolia* and *O. communa* are present. **B:** Latin square in the second cohort in absence of *A. artemisiifolia.*

In Langfang/Beijing in China, the setup of the Latin square experiment was on the 8th of July 2014, at which ten plants per species were planted in the centre plot (Fig. 6). The grass inside the plot and one meter around the plot was mown to have a clear field. Some *Xanthium sibiricum* plants were still in the experimental field but they were cut to make sure that they're not bigger than the test plants. The exposure was from the 11th of July to the 30th of September 2014 in an experimental plot of 50x50meters (Fig. 6). The initial assessment of these plants was on the 11th of July 2014. Height, phenological stage (vegetative, flowering or seed producing), overall Ophraella damage level and the amount of *O. communa* and *E. strenuana* galls were assessed. One day after, 100 *O. communa* adults were released in the centre of the central plot and hundred *E. strenuana* in galls were distributed randomly inside the central plot (Annex 13). On the 13th of July the first assessment was made but

without height measuring. On the 14th of July eight non-target plants per species (two per species at each corner) were planted in a satellite plots ten meters from the centre plot. On the 15th of July they were assessed for the first time where the height was only measured for the non-target plants from the satellite plots. The target satellite plots, with four *A. artemisiifolia* plants (plant number 11-26) per corner, were setup on the 19th of July 10m away from the non-target satellite plots. On the 20th of July they were assessed for the first time including all other test plants. There was a daily assessment from the 13th of July to the 20th of July 2014 and then weekly from the 28th of July until the 28th of September 2014. On the 30th of September the fresh biomass of all plants was taken.

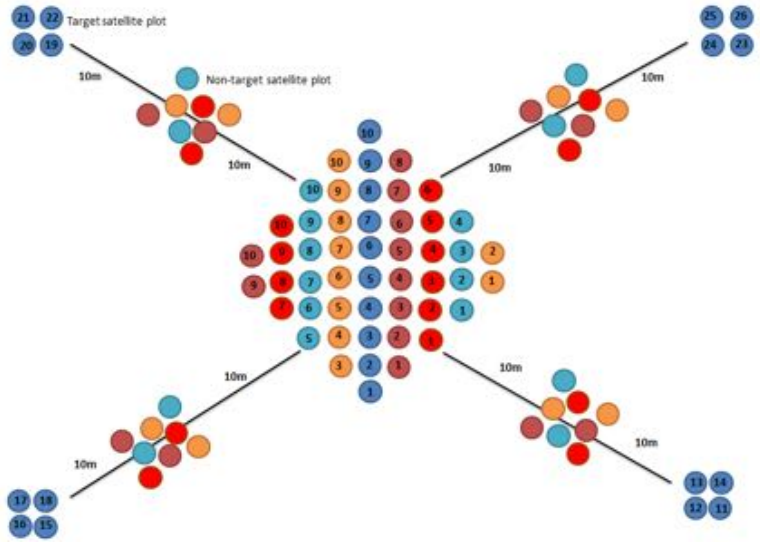


**Figure 6 :** Latin square experiment in Langfang/Beijing, China. The dark blue circles are *A. artemisiifolia* plants and the light blue circles are *A. trifida.* The mat red circles are Girasol, bright red is Italy and orange is Extrasol. The non-target satellite plots are ten meters away from the centre plot while the target satellite plots are 10m away from the non-target satellite plots.

#### 2.1.3.2 Setup, monitoring and measurements Interspersion experiment

The non-target plant species *A. annua*, *H. tuberosus*, *Z. elegans* and *H. annuus* (varieties Extrasol, Girasol and Italy) were used for the Interspersion experiment in Italy (Annex 2). The setup of both study sites in Busto Arsizio was on the 22nd of July. Eight plants per plant species or sunflower variety were planted randomly dispersed inside a 10x10m field. Because there was another experiment inside this field that should not be disturbed I decided to plant them in a row. That way the study plants were easier to find again. The alignment of the rows was randomized with Excel. I also added bamboo sticks next to every plant to make them even more visible (Fig. 7). The weekly assessment started on the 31st of July for eight weeks until the 16th of September. The plants were cut and

weighted for the final assessment. The setup of the Interspersion experiment in Magnago was on the 19th of July 2014. The plants were planted randomly in a 10x15m area and marked with bamboo sticks. The weekly assessment was from the 30th July for eight weeks until the 16th of September 2014. The setup of the interspersion experiment in Rovio, Switzerland, was on the 4th of August. *Ambrosia artemisiifolia* and the non-target plants *H. annuus* Extrasol, Girasol, Italy were used (Annex 2). Twelve plants per species/variety were planted randomly inside the experimental plot between the Latin square experiment and the population dynamic experiments. The first assessment was one week later on the 11th of August. The exposure was during eight weeks until the 1st of October 2014. The seventh week was left out because it was considered to be unnecessary to have a weekly assessment at the end. Again, all plants were cut and weighted for the final assessment. The exposure of the interspersion experiment in China was from the 13th of July to the 28th of September in a 15x15m field. Before the test plant species *A. artemisiifolia*, *A. trifida*, *H. annuus* Extrasol, Girasol and Italy were planted (Annex 2), two hundred *O. communa* adults and twenty *E. strenuana* adults were distributed randomly on the 8th of July to already have an established population of the potential biological control agents (Annex 13). To increase the population of *E. strenuana* thirteen more were released on the 10th of July and even hundred more on the 12th of July. On the 13th of July 10 test plants per species were planted and measured for the first time. Until the 20th of July the assessment was performed daily then from the 28th of July on it was weekly until the 28th of September. On the 30th of September only the fresh biomass of the fifty test plants was measured.

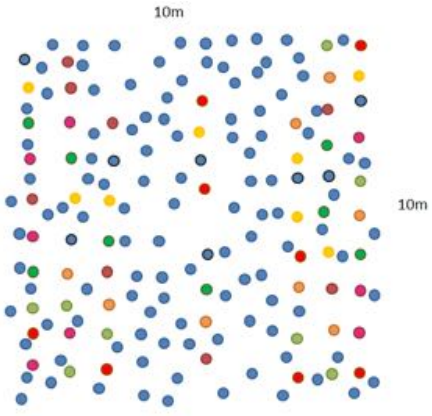


**Figure 7 :** Interspersion experiment in Italy (symbol picture). The plants were planted randomly inside the plots. The blue dots indicate the surrounding *A. artemisiifolia* plants and the blue dots with black frames are the *A. artemisiifolia* plants that were planted as control plants. The coloured dots indicate the different non-target plants.

### 2.1.3.3 Setup, monitoring and measurements Reverse Interspersion experiment

One hundred non-target test plants per species were planted on the 29th of June in a block, having 25 plants in one line and four columns per non-target species (Fig. 8). On the 09th of July fifteen *A. artemisiifolia* plants were planted randomly inside the study plot. The initial assessment was on the 11th of July. The next day hundred *O. communa* adults were released in the centre of the plot and 100 *E. strenuana* were released randomly inside the study plot (Annex 13). The first assessment was on the 13th of July and again twenty *O. communa* adults were released in the centre of the study plot. On the 14th of July two *O. communa* adults were put on every *A. artemisiifolia* test plant. From the 13th to the 20th of July there was a daily assessment. From the 28th of July to the 28th of September the assessment was only weekly. On the 30th of September 2014 the weight in gram was measured for every test plant. The fifteen *A. artemisiifolia* plants were assessed weekly. Only 10 of the 100 non-target plants per species were assessed weekly. They were chosen randomly.

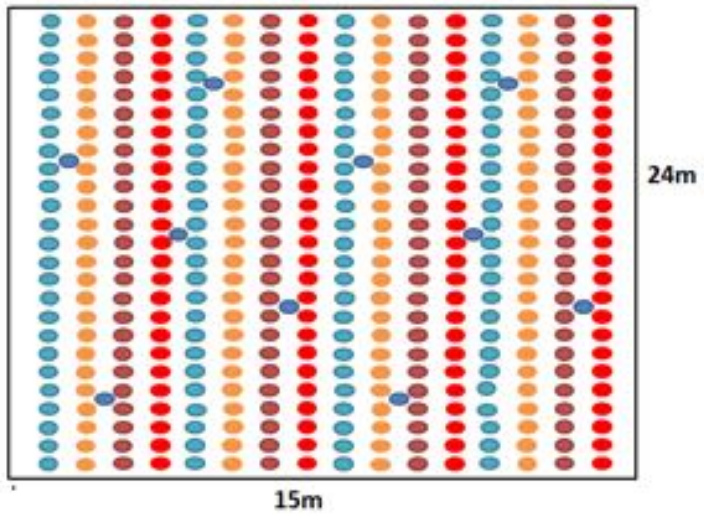


**Figure 8 :** Reverse Interspersion experiment in Langfang/Beijing 2014. There were sixteen columns with each twenty five non-target test plants. Every non-target test plant species had four columns. Ambrosia artemisiifolia was planted randomly inside the experiment (dark blue dots).

### 2.1.3.4 Monitoring and statistical analyses

The monitoring and statistical analysis was the same in all choice test designs. Besides to the assessment of the abundance of the biological control agents, plant performance (morphology and developmental stage) as well as site characteristics were listed (Annex 14). The presence of other insects or predators was also recorded. At the end of every cohort the fresh biomass was weighted. The statistical analysis was calculated with JMP (JMP® Pro 11.1.1, SAS Institute Inc. Cary, NC, 1989-

2013) and the graphs were created with Microsoft® Excel 2010. A full ANOVA (analysis of variance) model was used to calculate the effect of the sites, experiments, plant species and the cohort on the distribution of all *O. communa* stages and *E. strenuana* galls. Because no *O. communa* was found on *A. annua* and *Z. elegans*, they were excluded from the statistical analysis. Every site was tested separately. The cohorts were tested separately and then compared. A weekly assessment interval of four weeks was chosen to have the possibility to observe at least one generation time, as we assumed that the development time would be c. 22 days (Welch, 1978).

To analyse the oviposition preference of the female *O. communa* beetles, we counted all egg batches found on the test plants. Assuming that the larvae would hatch after 5-6 days (Goeden and Ricker, 1985; Welch, 1978) only full egg batches were counted to have a cumulative number of laid egg batches. A distribution index of the egg batches was calculated for each plant species indicating the relative attractiveness for oviposition of each plant species (Palmer, 1986). For this, the number of egg batches found on a plant individual was divided by the total number of egg batches found in the experiment.

$$\text{Distribution index of egg batches} = \frac{absolute\ number\ of\ egg\ batches\ on\ plant}{absolute\ number\ of\ egg\ batches\ in\ experiment}$$

Second, larval development and larval survival was assessed. Assuming that the three larval stages have a development time between 12-14 days (Goeden and Ricker, 1985; Welch, 1978) and 9-12days (Müller-Schärer et al., 2014) the larval stages 1, 2 and 3 were separated in small larvae (larval stage 1 and 2) and big larvae (larval stage 3). Therefore, the probability to count larvae twice was minimized. There could still be a bias because larval stage 1 larvae are difficult to find. A distribution index was calculated for the cumulative number of larvae per plant indicating the relative attractiveness for each plant species (Palmer, 1986). The absolute number of larvae on a plant individual was divided by the total number of larvae in the experiment:

$$\text{Distribution index of larvae} = \frac{absolute\ number\ of\ larvae\ \ on\ plant}{absolute\ number\ of\ larvae\ in\ experiment}$$

The completed life cycle of *O. communa* was indicated by the number of full pupae found on the test plant species. It was assumed that adults would emerge after 6-8 days from the cocoon and that all pupae would become adults (Welch, 1978). A distribution index of the pupae was calculated for each plant species indicating the relative attractiveness of each plant species (Palmer, 1986). The absolute number of full pupae found on a plant individual was divided by the total number of full pupae found in the experiment:

Distribution index = $\frac{absolute\ number\ of\ pupae\ on\ plant}{absolute\ number\ of\ pupae\ in\ experiment}$

Finally, the *O. communa* adult load was analysed. All adults found on the test plant species were counted for all assessments giving an absolute number of adults found on test plants. The mean load of *O. commua* adults was calculated with the cumulative number of adults found on every plant individual divided by the absolute number of assessments where adults were found in the experiment:

Mean adult load = $\frac{cumulative\ number\ of\ adults\ on\ plant}{number\ of\ assessment\ with\ adults}$

The feeding damage of the plant individuals caused by *O. communa* feeding stages was estimated and rated on a scale similar to dedscribed in Briese et al. (2002). It was assumed that all *O. communa* feeding stages found on the test plants were eventually feeding on the test plant.

- Level 0 = 0% damage
- Level 1 = 1-10% damaged plant tissue
- Level 2 = 11-50% damaged plant tissue
- Level 3 = 51-90% damaged plant tissue
- Level 4 = more than 90% damaged plant tissue.

The abundance of *E. strenuana* in the study in China was estimated as the number of galls found in the test plants. The absolute number of galls found on the test plant individual divided by the absolute number of galls in the experiment was calculated to give a distribution index for each test plant species (Palmer, 1986). The distribution index showed the relative attractiveness of each plant species for *E. strenuana*.

Distribution index of galls = $\frac{absolute\ number\ of\ galls\ \ on\ plant}{absolute\ number\ of\ galls\ in\ experiment}$

## 2.2 Results

### 2.2.1 Oviposition preference of *O. communa*

#### 2.2.1.1 Latin square

The number of egg batches found on the study plants decreased continuously during the summer season in Magenta, Magnago and Rovio (Annex 7). The egg batch distribution between the study sites did not vary significantly during the first cohort ($p < 0.08$). Most egg batches were found in Magenta (n=156) followed by Magnago (n=136) and Rovio (n=42). The calculated egg batch distribution indicated that significantly more egg batches were found on the target plant species than on the non-target plant species, in presence of *A. artemisiifolia* ($p < 0.0001$; Fig. 9a). Except of two egg batches found on two different *H. annuus* Girasol in the fourth assessment and one egg batch found on one *H. annuus* LG5687 on the second assessment in Magenta, no egg batches were found on the non-target plants. During the second cohort, in absence of *A. artemisiifolia*, only one egg batch was found on a *H. annuus* Girasol plant in Magenta and no egg batch was found on any other non-target plant (Fig. 9b). The egg batch distribution was not significant between the non-target plant species ($p < 0.41$).

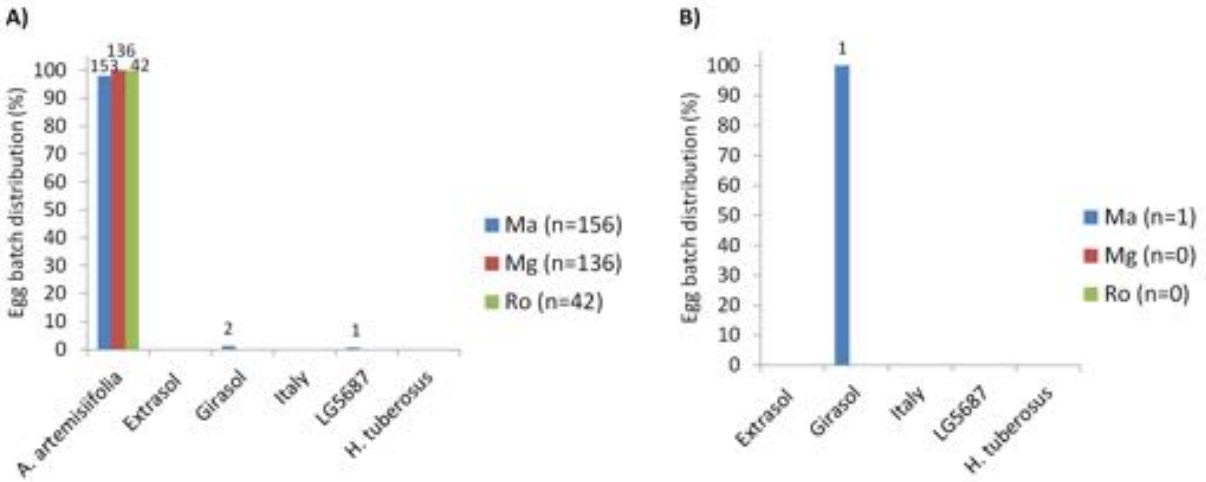


**Figure 9 :** Distribution of egg batches among the study plant species *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687), and *H. tuberorus* within the sites in the Latin square experiments in Magenta (Ma), Magnago (Mg) and Rovio (Ro). There were 8 plants per species in Magenta (Ma) and Magnago (Mg) and 4 plants per species in Rovio (Ro). The plant species *H. annuus* LG5687 and *H. tuberosus* were not used in Rovio (Ro). The number above the column indicates the absolute number of egg batches found on the plants during the exposure. A) Egg batch distribution during the first cohort in presence of *A. artemisiifolia*. B) Egg batch distribution during the second cohort in absence of *A. artemisiifolia.*

In the Latin square experiment in Langfang/Beijing the number of egg batches increased throughout the season (Fig. 10; Annex 9). Less egg batches were found in the central plot (402 egg batches) than in the satellite plot (455 egg batches) but the difference was not significant ($p < 0.24$). There was a significant difference of the egg batch distribution among the plant species ($p < 0.0001$) in the central and the satellite plot. Significantly more egg batches were found on *A. artemisiifolia* than on the non-target plant species. In the central plot 91.29% of the egg batches were found on *A. artemisiifolia*, 6.47% on *A. trifida* and 2.24% on the sunflower varieties Girasol and Italy. In the satellite plot 98.03% of the egg batches were found on *A. artemisiifolia*, 0.88% on *A. trifida* and 1.09% on sunflower variety Girasol (Fig. 10; Table 4).

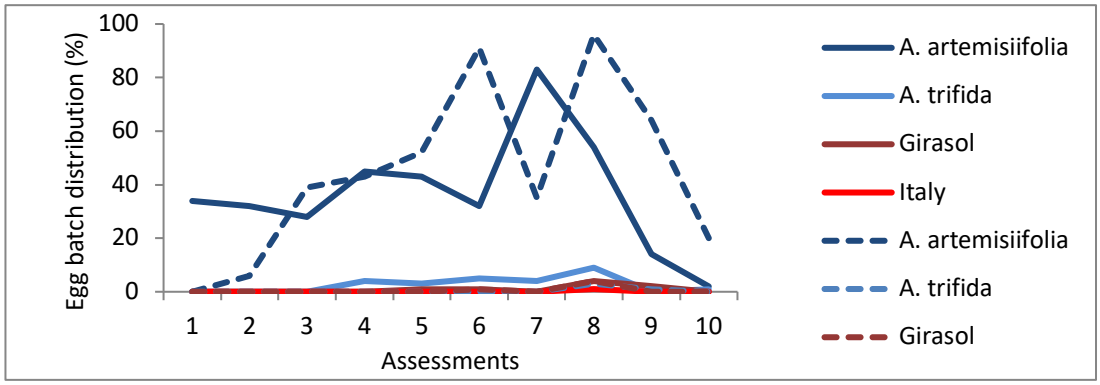


**Figure 10 :** Egg batch distribution among the test plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Girasol and Italy) within the sites in the Latin square experiment in Langfang/Beijing, China 2014. There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (Fig. 6). The full lines represent the plants in the central plot and the dashed lines represent the plants in the satellite plot.

**Table 4 :** Distribution (in %) of all *O. communa* stages among the test plant species A*. artemisiifolia, A. trifida, H. annuus* (varieties Extrasol, Girasol, Italy) within the sites in the Latin square experiment in Langfang/Beijing, China, 2014. Ten plants per species were in the central plot and eight non-target plants per species respectively 16 *A. artemisiifolia* plants were in the satellite plot. The letter "n" represents the total number of *O. communa* found during the exposure from 20th of July to the 28th of September 2014.

| Ophraella stage | Plot | *A. artemisiifolia* | *A. trifida* | Extrasol | Girasol | Italy |
|---|---|---|---|---|---|---|
| Egg batches | Central | 91.29% (n=367) | 6.47% (n=26) | 0 | 1.99% (n=8) | 0.25% (n=1) |
| | Satellite | 98.02% (n=446) | 0.88% (n=4) | 0 | 1.1% (n=5) | 0 |
| Small larvae | Central | 98.67% (n=893) | 0.55% (n=5) | 0 | 0.77% (n=7) | 0 |
| | Satellite | 96.08% (n=687) | 3.92% (n=28) | 0 | 0 | 0 |
| Big larvae | Central | 92.09% (n=384) | 3.36% (n=14) | 0.24% (n=1) | 4.32% (n=18) | 0 |
| | Satellite | 97.54% (n=278) | 0.70% (n=2) | 0 | 1.75% (n=5) | 0 |
| Pupae | Central | 92.06% (n=510) | 7.22% (n=40) | 0.18% (n=1) | 0.36% (n=2) | 0.18% (n=1) |
| | Satellite | 99.62% (n=786) | 0.38% (n=3) | 0 | 0 | 0 |
| Adults | Central | n=1506 | n=113 | n=2 | n=40 | n=2 |
| | Satellite | n=588 | n=82 | 0 | n=19 | 0 |

#### 2.2.1.2 Interspersion and Reverse Interspersion

The Interspersion experiment in Italy and Switzerland showed similar results to the Latin square experiment. The number of egg batches found on the test plants declined during the summer (Annex 8). The calculated egg batch distribution in Italy and Switzerland indicated that there was no significant difference between the study sites but significantly more egg batches were found on the target than on the non-target plant species ($p < 0.0001$). In total 165 egg batches were found on the test plants in Italy, 49 egg batches in Busto Arsizio 1, 69 in Busto Arsizio 2 and 47 egg batches in Magnago (Fig. 11). Except of 2 egg batches found on *H. annuus* Extrasol and 1 egg batch found on *H. annuus* LG5687 in Busto Arsizio 2 (BA2) all egg batches were found on *A. artmemisiifolia*. In Switzerland, 138 egg batches were found in total, 136 egg batches on *A. artemisiifolia* (98.55%), 1 egg batch on *H. annuus* Girasol (0.72%) and 1 egg batch on *H. annuus* Italy (0.72%).

In total, only 11 egg batches were found on the 50 test plants in the Interspersion experiment in Langfang/Beijing, China. 10 egg batches on *A. artemisiifolia* (90.09%) and 1 egg batch on *H. annuus* Extrasol (9.1%) but there was no significant difference of the egg batch distribution among the test plant species ($p < 0.33$). The egg batch abundance was steady and low from the beginning to the end of the exposure (Annex 9). In the Reverse Interspersion experiment significantly more egg batches ($p < 0.0001$) were found on the fifteen *A. artemisiifolia* plants (191 egg batches) than on the non-target plant species. Except of 22 egg batches found on *H. annuus* Extrasol (10.28%) and 1 egg batch found on *H. annuus* Girasol (0.47%) no egg batches were found on the other non-target plant species in the Reverse Interspersion experiment in China (Fig. 11).

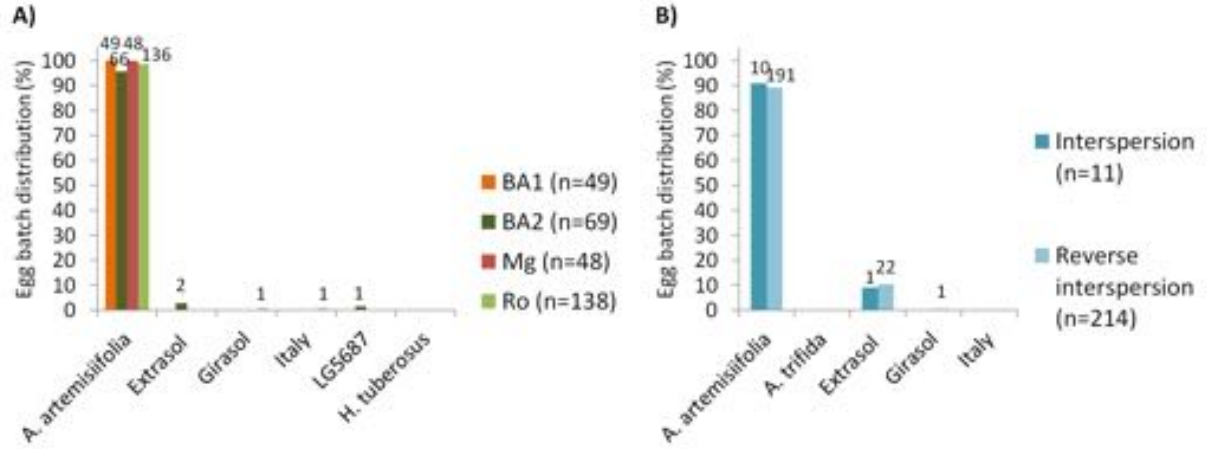


**Figure 11** : A) Distribution of egg batches in the Interspersion experiment in both Busto Arsizio sites (BA1 and BA2), Magnago (Mg) and Rovio (Ro) among the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy, LG5687) and *H. tuberosus*. There were 8 plants per species in Busto Arsizio (BA1 and BA2) and Magnago (Mg) and 12 plants per species in Rovio (Ro). B) Distribution of egg batches in the Interspersion experiment and Reverse Interspersion experiment in Langfang/Beijing, China, among the study plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy). There were 10 plants per species in the Interspersion experiment and 100 non-target plants (only 10 plants per species were assessed) respectively 15 *A. artemisiifolia* plants in the Reverse Interspersion experiment. The number above the column indicates the absolute number of egg batches found during the exposure in the summer 2014 (Annex 13).

### 2.2.2 Abundance of *O. communa* larvae and pupae

#### 2.2.2.1 Latin square

Abundance of *Ophraella* larvae

Significantly more small and big larvae were found on *A. artemisiifolia* than on the non-target plant species during the first cohort, in presence of *A. artemisiifolia*, in Italy and Switzerland ($p < 0.0001$). Most small larvae were found in Magnago (n=314), then in Rovio (n=76) and then in Magenta (n=54). The same was for the big larvae (Fig. 12). During the second cohort, in absence of *A. artemisiifolia*, no significant difference of the abundance of small and big larvae was observed among the non-target plants in Switzerland and Italy. In Magenta, no larvae were found on the non-target plants in absence of *A. artemisiifolia*. In Magnago, only small larvae and no big larvae were found in absence of *A. artemisiifolia* and in Rovio two small larvae and three big larvae were found on non-target plants in absence of *A. artemsiifolia* (Fig. 12). In Langfang/Beijing significantly more small and big larvae were found on *A. artemisiifolia* than on the non-target plant species in the central and the satellite plot (Fig. 13). But there was no significant difference between the central plot and the satellite plot. Table 4 gives the distribution of the larvae among the test plant species. Most small larvae were found on the 5$^{th}$ assessment and most big larvae were found on the 6$^{th}$ assessment (Fig. 13). In total 1620 small larvae and 702 big larvae were found during the season.

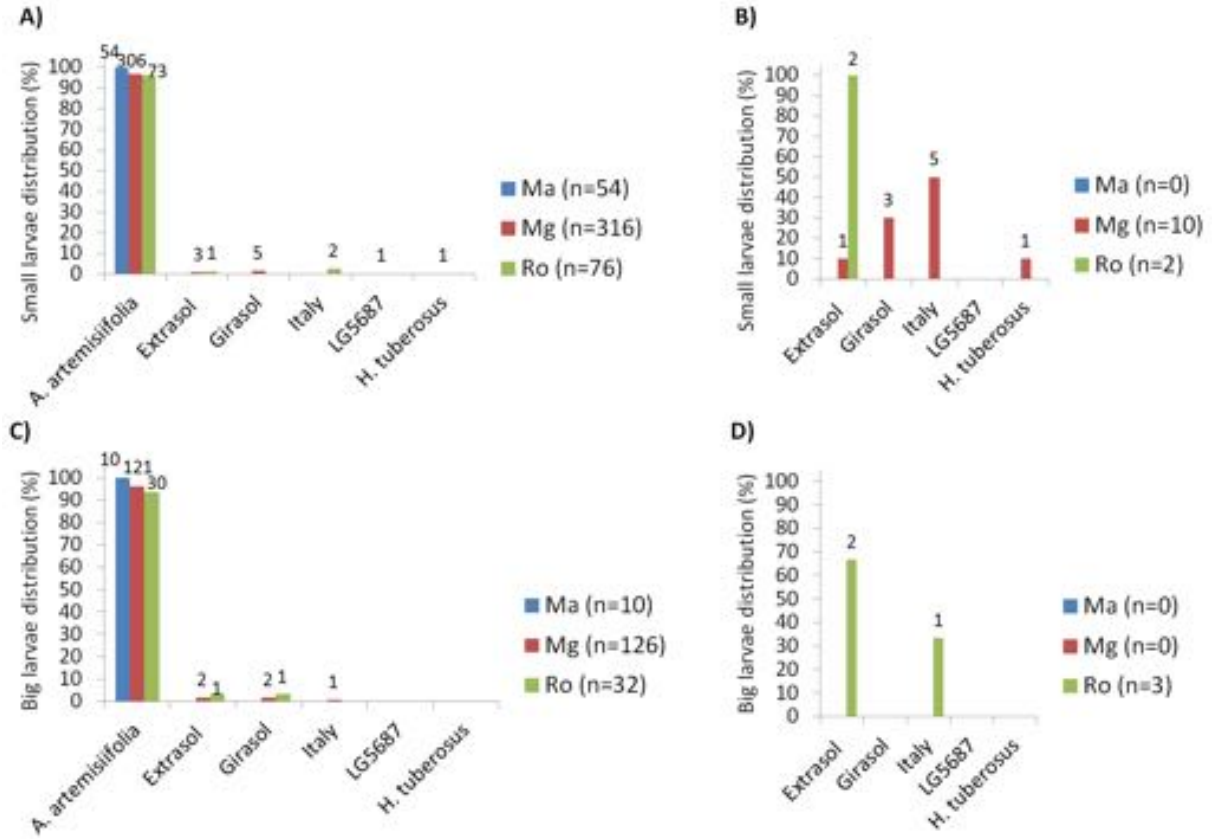


**Figure 12 :** Larval distribution among the test plant species *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* within the sites in the Latin square experiments in Magenta (Ma), Magnago (Mg) and Rovio (Ro) in 2014. The number above the column indicates the absolute number of larvae found on the test plants during the exposure. There were 8 plants per species in Magenta (Ma) and Magnago (Mg) and 4 plants per species in Rovio (Ro). A) Small larval distribution in presence of *A. artemisiifolia* during the first cohort. B) Small larval distribution in absence of *A. artemisiifolia* during the second cohort. C) Big larval distribution in presence of *A. artemisiifolia* during the first cohort. D) Big larval distribution in absence of *A. artemisiifolia* during the second cohort.

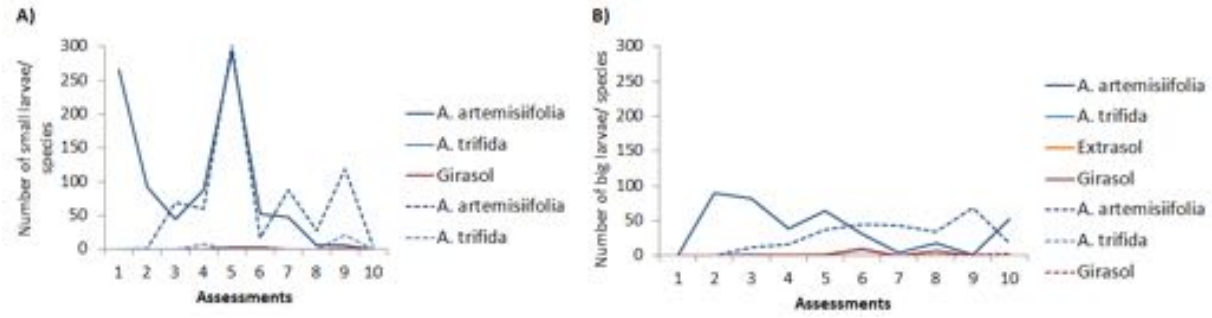


**Figure 13 :** A) Abundance of small larvae on the test plant species *A. artemisiifolia, A. trifida and H. annuus (*varieties Extrasol, Girasol and Italy) within the sites in the Latin square experiment in Langfang/Beijing, China 2014. B) Big larvae abundance on the test plant species *A. artemisiifolia, A. trifida and H. annuus (*varieties Extrasol, Girasol and Italy) in the Latin square experiment in Langfang/Beijing, China 2014. The full lines represent the plants in the central plot and the dashed lines represent the plants in the satellite plot. There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (Fig. 6). The exposure was from the 20th of July to the 28th of September 2014 (Annex 13).

Abundance of *Ophraella* pupae

Except of one pupa that was found on *H. annuus* Girasol in Magnago during the first cohort, in presence of *A. artemisiifolia*, no pupae were found on any other non-target plant in Italy and Switzerland (Fig. 14). In Langfang/Beijing, China, significantly more pupae were observed on *A. artemisiifolia* than on the non-target plant species ($p < 0.0001$) but there was no significant difference of the distribution of the pupae between the central and the satellite plot ($p < 0.91$). Table 4 gives the distribution of the pupae among the test plant species. There was a significant difference of the absolute number of pupae between the assessments ($p < 0.0001$). Most pupae were found on the 7th assessment (n=356) and no pupae were found on the 1st assessment (Fig. 15).

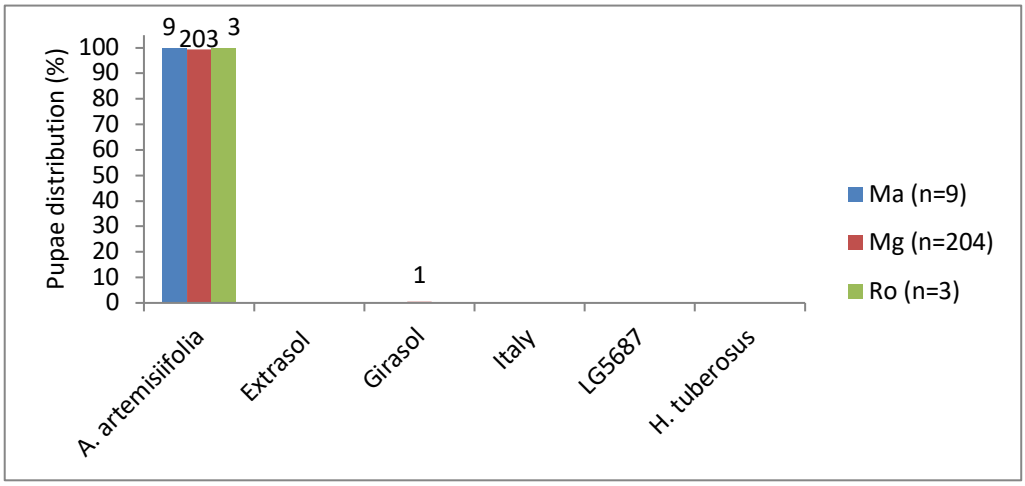


**Figure 14 :** Distribution of full pupae among the test plant species *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* within the sites in the Latin square experiment during the exposure on the first cohort in presence of *A. artemisiifolia* in the study sites Magenta (Ma), Magnago (Mg) and Rovio (Ro). The number above the column indicates the absolute number of full pupae during the exposure.

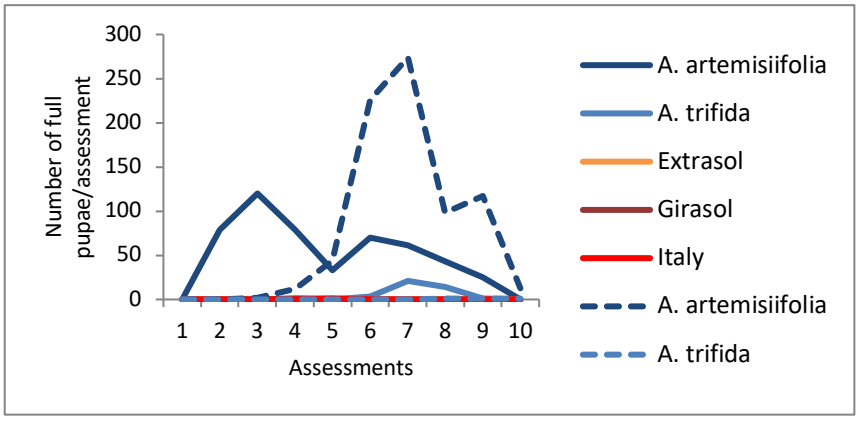


**Figure 15 :** Distribution of *Ophraella* pupae among the test plants *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) within the sites in the Latin square experiment in Langfang/Beijing, China 2014. There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (cf. Fig. 6). The full lines represent the plants in the central plot and the dashed lines represent the plants in the satellite plot.

#### 2.2.2.2 Interspersion and Reverse Interspersion

Abundance of *Ophraella* larvae

At the end of the season almost no larvae were observed on the test plants in the Interspersion experiment in Italy and Switzerland (Annex 8). The study sites did not differ significantly in the abundance of small larvae ($p < 0.06$). Significantly more small and big larvae were observed on *A. artemisiifolia* than on the non-target plant species ($p < 0.0001$). Most small larvae were observed in Rovio (n=347) then in Busto Arsizio 1 (n=177), Busto Arsizio 2 (n=162) and in Magnago (n=129). Most big larvae were found in Rovio (n=159), then in Busto Arsizio 2 (n=39), Busto Arsizio 1 (n=35) and in Magnago (n=29). In all study sites in Italy and Switzerland most larvae found on the non-target plants were on H. annuus Girasol (Fig. 16).

In the Interspersion experiment in Langfang/Beijing 100% of the small larvae (n=41) and 96% of the big larvae (n=47) were found *A. artemisiifolia.* Expect of one big larva found on *A. trifida* and one on *H. annuus* Girasol no other big larvae were found on non-target plants (Fig. 16). The abundance of small larvae ($p < 0.01$) and big larvae ($p < 0.002$) was significantly different between the assessments. Most larvae were found on the 7$^{th}$ assessment (Annex 9). The time line of the Reverse Interspersion experiment showed only a significant difference of the small larvae abundance among the assessments ($p < 0.0017$) but no significant difference of the abundance of big larvae ($p < 0.063$). Most larvae were found on the 5$^{th}$-6$^{th}$ assessments but the abundance showed no pattern (Annex 9). A significant difference of the larval distribution among the test plants species was observed ($p < 0.0001$). 98.77% of the small larvae were found on *A. artemisiifolia* (n=806) and 1.23% on *H. annuus* Extrasol (n=10). 100% of the big larvae were found on *A. artemisiifolia* (n=336).

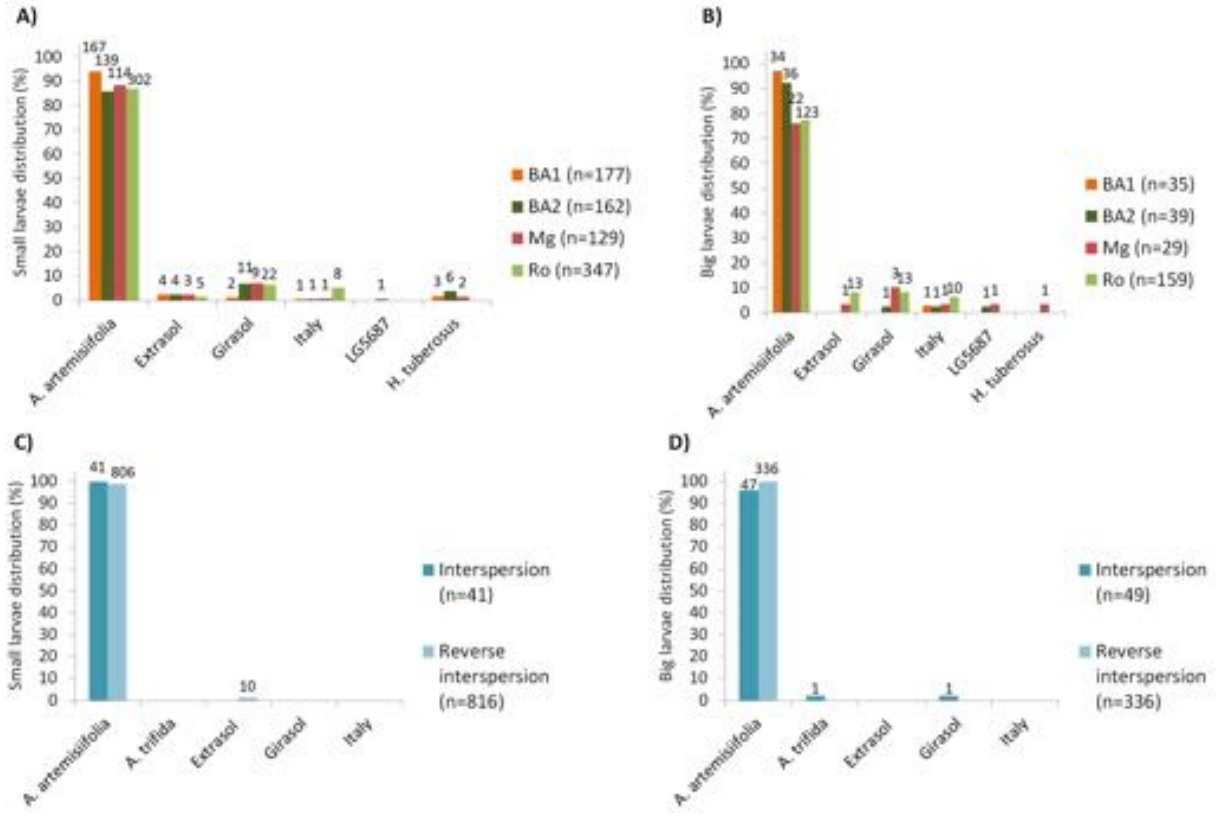


**Figure 16 :** A/B) Distribution of small and big larvae in the Interspersion experiment in both Busto Arsizio sites (BA1 and BA2), Magnago (Mg) and Rovio (Ro) among the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy, LG5687) and *H. tuberosus*. There were 8 plants per species in Busto Arsizio (BA1 and BA2) and Magnago (Mg) and 12 plants per species in Rovio (Ro). C/D) Distribution of small and big larvae in the Interspersion experiment and Reverse Interspersion experiment in Langfang/Beijing, China, among the study plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy). There were 10 plants per species in the Interspersion experiment and 100 non-target plants (only 10 plants per species were assessed) respectively 15 *A. artemisiifolia* plants in the Reverse Interspersion experiment. The number above the column indicates the absolute number of larvae found during the exposure in the summer 2014 (Annex 13).

## Abundance *of Ophraella* pupae

Except of slight significance in Busto Arsizio 2 ($p < 0.04$) no significant difference of the abundance of pupae was observed between the assessments in the study sites in Italy and Switzerland. Most pupae were observed between 4$^{th}$-6$^{th}$ assessment weeks. In the first assessment no pupae were observed except of one pupa in Busto Arsizio 1. On the final assessment again no pupae were observed on the test plants except of one pupa in Busto Arsizio 2 (Annex 8). No significant difference of the pupae abundance was observed between the study sites ($p < 0.56$). There was a significant difference of the pupae distribution among the test plant species in Italy and in Switzerland ($p < 0.0001$). Significantly more pupae were found on *A. artemisiifolia* than on the non-target plants (Fig. 17). In Busto Arsizio1 93.33% of the pupae were found on *A. artemisiifolia* (n=14) and 6.67% on *H. annuus* Extrasol (n=1). In Busto Arsizio2 86.96% of the pupae were found on *A. artemisiifolia* (n=20), 8.7% on *H. tuberosus*

(n=2) and 4.35% each on *H. annuus* Italy (n=1). In Magnago 92.86% of the pupae were found on *A. artemisiifolia* (n=14) and 7.14% on *H. annuus* Italy (n=1). In Rovio 96.3% of the pupae were found on *A. artemisiifolia* (n=26) and 3.7% on *H. annuus* Girasol (n=1).

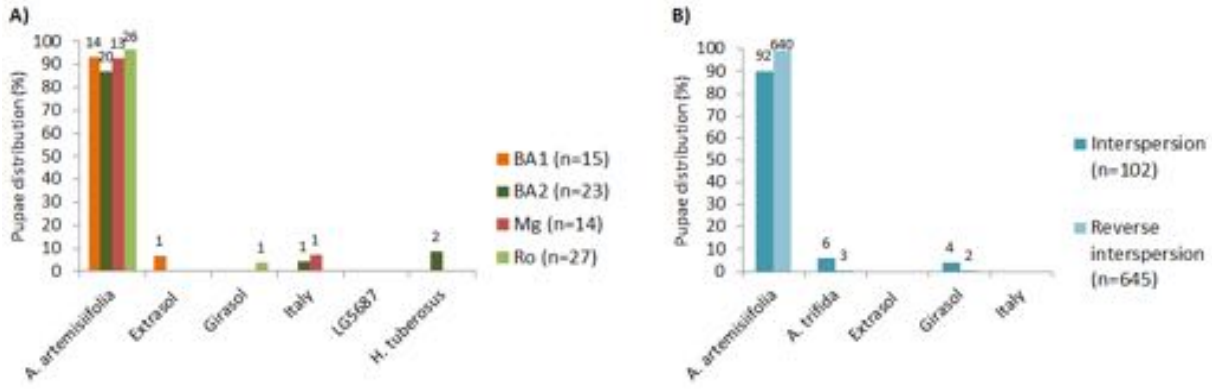


**Figure 17 :** A) Distribution of full pupae in the Interspersion experiment in both Busto Arsizio sites (BA1 and BA2), Magnago (Mg) and Rovio (Ro) among the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy, LG5687) and *H. tuberosus*. B) Distribution of full pupae in the Interspersion experiment and Reverse Interspersion experiment in Langfang/Beijing, China, among the study plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy). The number above the column indicates the absolute number of full pupae found during the exposure in summer 2014 (cf. Annex 13).

In Langfang/Beijing a significant difference of the abundance of *Ophraella* pupae between the assessments was observed in the Interspersion experiment ($p < 0.002$) and the Reverse Interspersion ($p < 0.008$). In the Interspersion experiment most pupae were found on the 8$^{th}$ assessment but a lower peak on the 4$^{th}$ assessment was observed as well. In the Reverse Interspersion experiment most pupae were found on the 7$^{th}$ assessment (Annex 9). The pupae distribution among the test plant species was significantly different in the Interspersion experiment and the Reverse Interspersion experiment ($p < 0.0001$). Significantly more pupae were found on *A. artemisiifolia* than on non-target plant species (Fig. 17). In the Interspersion experiment 90.7% of the pupae were found on *A. artemisiifolia* (n=92), 5.88% on *A. trifida* (n=6) and 3.92% on *H. annuus* Girasol (n=4). In the Reverse Interspersion experiment 99.24% of the pupae were found on *A. artemisiifolia* (n=640), 0.47% on *A. trifida* (n=3) and 0.31% on *H. annuus* Girasol (n=2).

### 2.2.3 Adult load of *O. communa*

#### 2.2.3.1 Latin square

The adult load between the test plant species in the Latin square in the study sites Magnago, Magenta and Rovio was significantly different ($p < 0.0001$). Adults were found at every assessment and the adult load was significantly higher on *A. artemisiifolia* than on the non-target plant species. The adult load declined from the first to the second cohort in Magenta and Magnago (Fig. 18a; Annex 7). In Rovio, the adult load increased significantly from the first to the second cohort ($p < 0.008$; Annex 7), but it did not differ significantly between the non-target plant species. No significant difference of the adult load between the study sites was observed in the first cohort ($p < 0.08$). In the second cohort a significant difference of the adult load was observed between the study sites ($p < 0.0001$). The adult load was significantly higher in Rovio (n=49.67) than in Magenta (n=4.5) and Magnago (n=3.67) (Fig. 18b). The adult load did also increase troughtout the exposure in the Latin square experiment in Langfang/Beijing (Annex 9). A significant difference of the adult load was observed between the test plant species in the central and the satellite plot ($p < 0.0001$). The adult load was significantly higher on *A. artemisiifolia* than on the non-target plant species. In general, more adults were observed in the central plot (n=1663) than in the satellite plot (n=689) (Fig. 19).

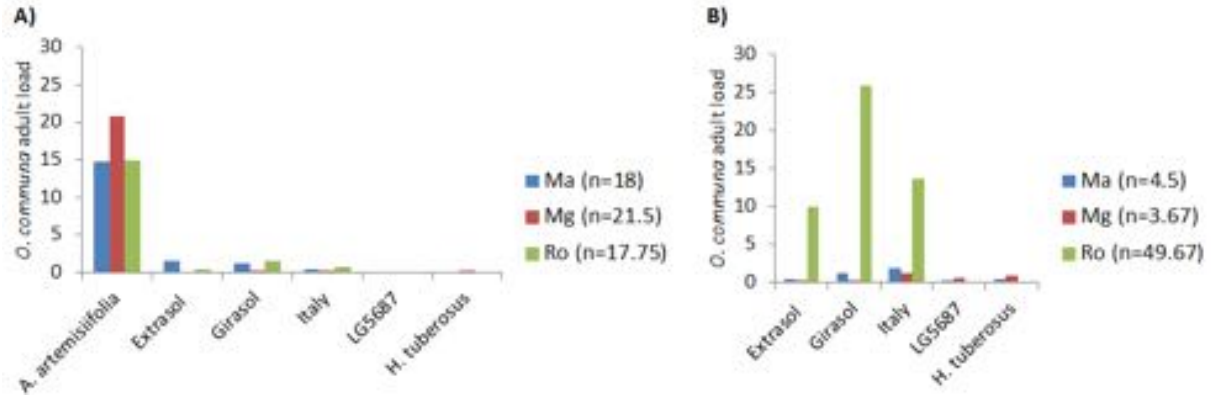


**Figure 18 :** *O. communa* adult load on the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* within the sites in the Latin square experiment in Magenta (Ma), Magnago (Mg) and Rovio (Ro). There were 8 plants per species in Magenta (Ma) and Magnago (Mg) and 4 plants per species in Rovio (Ro). The *H. annuus* variety LG5687 and *H. tuberosus* were not used in Rovio (Ro). A) *O. communa* adult load during the first cohort in presence of *A. artemisiifolia* (cf. Annex 13). B) *O. communa* adult load during the second cohort in absence of *A. artemisiifolia* (cf. Annex 13).

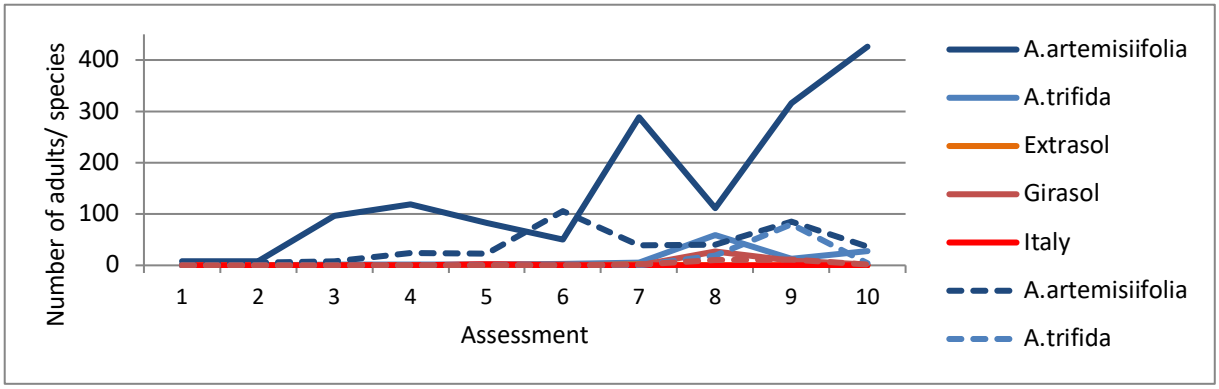


**Figure 19** : *O. communa* adult load within the sites in the Latin square experiment in Langfang/Beijing from the 20th of July to the 28th of September 2014 (Annex 13). The adult load was significantly higher on *A. artemisiifolia* than on the non-target plant species *A. trifida* and *H. annuus* (varieties Extrasol Girasol and Italy) ($p < 0.0001$). The difference between the central and the satellite plot was not significant. The full lines represent the plants in the central plot and the dashed lines represent the plants in the satellite plot. There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (Fig. 6).

#### 2.2.3.2 Interspersion and Reverse Interspersion

*Ophraella communa* adults were found at every assessment of the Interspersion experiment in Italy and Switzerland except on the 2nd assessment in Magnago (Fig. 20a). The adult abundance increased slightly during the exposure in Busto Arsizio 2 and Magnago and decreased in Rovio (Annex 8). The adult load was significantly higher on *A. artemisiifolia* than on the non-target plant species ($p < 0.0001$). The highest adult load was observed in Rovio (n=96.95), then Busto Arsizio 1 (n=19.14), Busto Arsizio 2 (n=15.97) and Magnago (n=15.28). In the Interspersion and Reverse Interspersion experiment in Langfang/Beijing the adult load was significantly higher on *A. artemisiifolia* than on the non-target plants as well ($p < 0.0001$). No adults were observed on *H. annuus* Italy in Langfang/Beijing (Fig. 20b). The adult abundance on the test plants was significantly different between the assessments in the Interspersion experiment in Langfang/Beijing ($p < 0.002$). The abundance increased during the season with a peak on the 9th assessment (n=24) but on the final assessment only 1 adult was found on *A. artemisiifolia*. The adult abundance in the Reverse Interspersion experiment did also increase in the beginning but declined on the 6th assessment to then increase again (Annex 9). The difference between the assessments was not significant ($p < 0.06$).

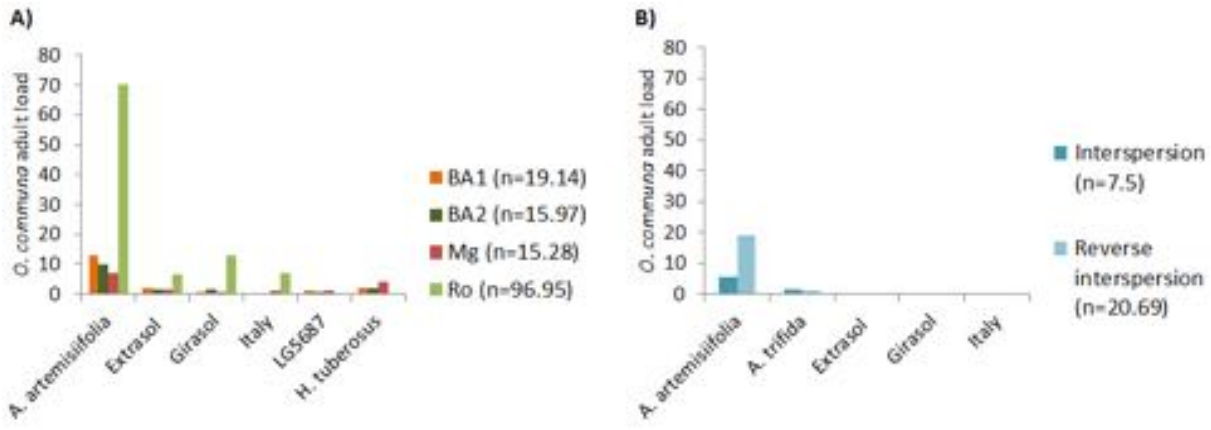


**Figure 20 :** A) *O. communa* adult load on the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG 5687) and *H. tuberosus* within the sites in the Interspersion experiment in both Busto Arsizio sites (BA1 and BA2), Magnago (Mg) and Rovio (Ro). The *H. annuus* variety LG5687 and *H. tuberosus* were not used in Rovio (Ro). There were 8 plants per species in Busto Arsizio (BA1 and BA2) and Magnago (Mg) and 12 plants per species in Rovio (Ro). B) *O. communa* adult load on the test plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) within the sites in the Interspersion and Reverse Interspersion experiment in Langfang/Beijing. There were 10 plants per species in the Interspersion experiment and 100 non-target plants (only 10 plants per species were assessed) respectively 15 *A. artemisiifolia* plants in the Reverse Interspersion experiment.

### 2.2.4 Damage to test plants

#### 2.2.4.1 Latin square

The damage to *A. artemisiifolia* after four weeks of exposure during the first cohort (Annex 13) in the Latin square experiment in Italy and Switzerland was significantly higher than the damage to the non-target plants ($p < 0.0001$). The non-target plants *H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* in Magnago were less damaged in the second cohort than in the first cohort (Fig. 21). The non-target plants *H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* in Magenta were on average more damaged in the second cohort than in the first cohort. The non-target plants *H. annuus* (varieties Extrasol, Girasol and Italy) in Rovio were significantly more damaged during the second cohort in absence of *A. artemisiifolia* (Fig. 21b).

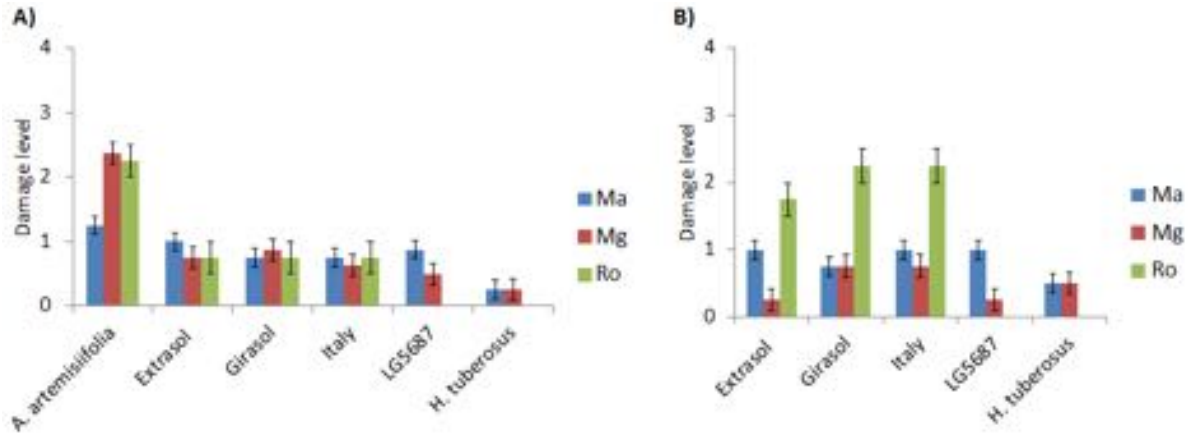


**Figure 21 :** A) Mean damage level of the study plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* after four weeks of exposure in presence of *A. artemisiifolia* within the sites in the Latin square experiment in Magenta (Ma), Magnago (Mg) and Rovio (Ro). B) Mean damage level of the study plants *H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* after four weeks of exposure in absence of *A. artemisiifolia* within the sites. There were 8 plants per species in Magenta (Ma) and Magnago (Mg) and 4 plants per species in Rovio (Ro). The *H. annuus* variety LG5687 and *H. tuberosus* were not used in Rovio (Ro). The error bars indicate the least significant difference (LSD).

The mean damage level was significantly different between the test plants *A. artemisiifolia, A.trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) after 10 weeks of exposure in the Latin square experiment in Langfang/Beijing. The non-target plants *H. annuus* Extrasol, Girasol and Italy had a significantly higher damage level than *A. trifida* and *A. artemisiifolia* in the central and the satellite plot (Fig. 22). The sunflowers in the central plot all had a mean damage level of 4 while *A. trifida* had a mean damage level of 2.9 and *A. artemisiifolia* a mean damage level of 3. The sunflowers in the satellite plot had a damage level between 3.36 and 3.88, while *A. trifida* had a mean damage level of 2.63 and *A. artemisiifolia* a mean damage level of 2.38 (Fig. 22).

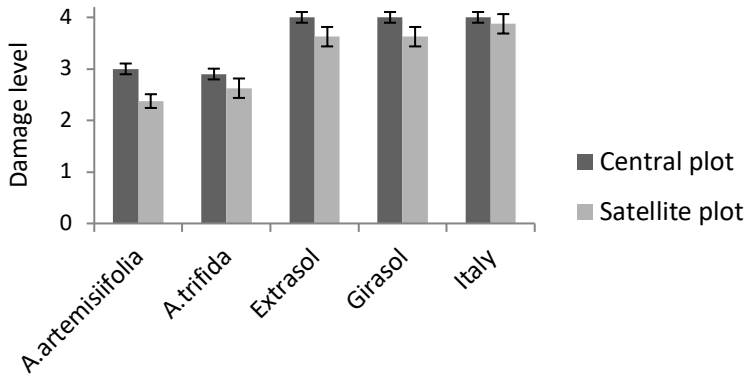


**Figure 22 :** Mean damage level of the test plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) in the Latin square experiment in Langfang/Beijing on the final assessment on the 28th of September 2014 (Annex 13). There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (Fig. 6). The error bars indicate the least significant difference (LSD).

#### 2.2.4.2 Interspersion and Reverse Interspersion

After 8 weeks of exposure (Annex 13) *A. artemisiifolia* was significantly more damaged than the non-target plant species in the study sites in Italy ($p < 0.0001$). The *H. annuus* varieties Girasol and Extrasol were slightly more damaged than the *H. annuus* varieties Italy and LG5687 and *H. tuberosus*, but no non-target plant was highly damaged (Fig. 23a). No significant difference of the mean damage level between the test plant species was observed in Rovio ($p < 0.2904$). After 8 weeks of exposure in Rovio (Annex 13), all *A. artemisiifolia* plants were completely destroyed and the non-target plants were highly damaged as well (Fig. 23a). After 11 weeks of exposure in the Interspersion experiment in Langfang/Beijing (Annex 13) a significant difference of the mean damage level between the study plants was observed ($p < 0.0001$). The varieties *H. annuus* Extrasol and Girasol were more damaged than *A. artemisiifolia* (Fig. 23b). Least damaged was *A. trifida*. All non-target plant species in the Reverse Interspersion experiment were significantly more damaged than *A. artemisiifolia* ($p < 0.0001$). The *H. annuus* varieties had all a damage level of 4 (Fig. 23b).

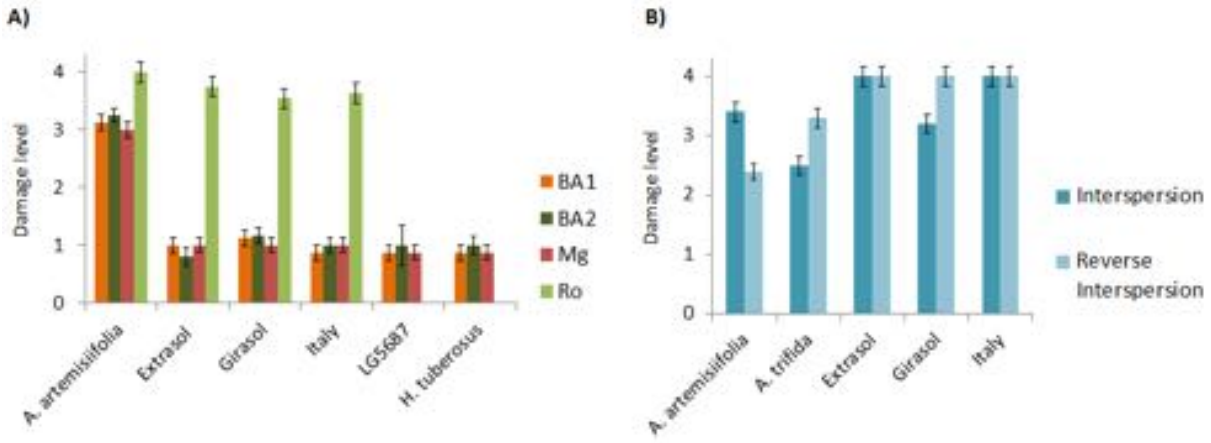


**Figure 23 :** A) Mean damage level of the test plants *A. artemisiifolia, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687) and *H. tuberosus* on the final assessment within the sites in the Interspersion experiment in Busto Arsizio (BA1 and BA2), Magnago (Mg) and Rovio (Ro). There were 8 plants per species in Busto Arsizio (BA1, BA2) and Magnago (Mg) and 12 plants per species in Rovio (Ro). B) Mean damage level of the test plants *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) on the final assessment within the sites in the Interspersion and Reverse Interspersion in Langfang/Beijing. There were 10 plants per species in the Interspersion experiment and 100 non-target plants (only 10 plants per species were assessed) respectively 15 *A. artemisiifolia* plants in the Reverse Interspersion experiment. The error bars indicate the least significant difference (LSD).

### 2.2.5 Abundance of *E. strenuana* galls

#### 2.2.5.1 Latin square

There was a significant difference of the *E. strenuana* gall distribution among the test plant species in the central and the satellite plot ($p < 0.0001$). Except of three galls found on *A. trifida* in the central plot, no galls were found in the non-target plant species (Fig. 24, Table 5). More galls were found in the satellite plot than in the central plot, but the difference between the satellite (n=52) and central plot (n=34) was not significant ($p < 0.13$).

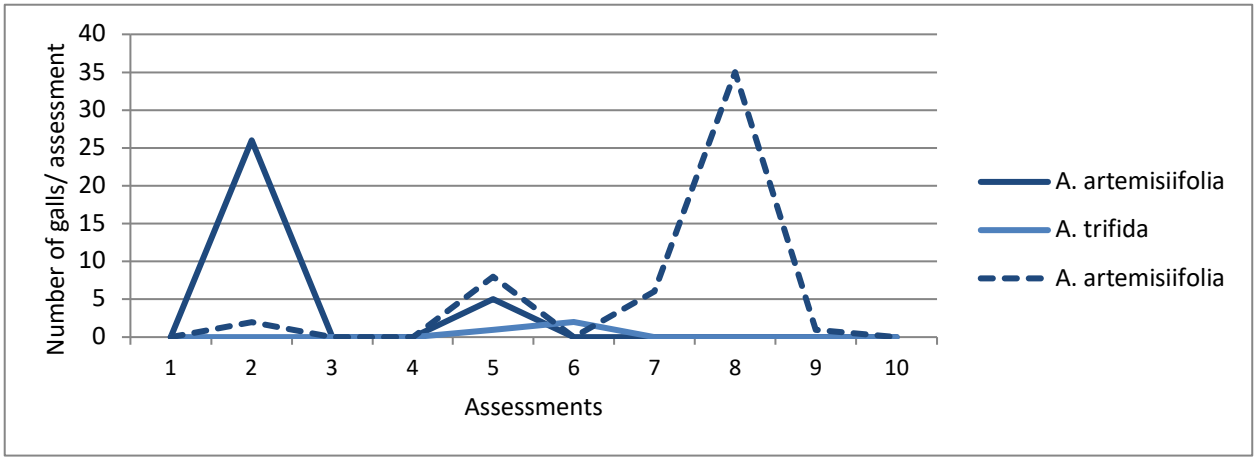


**Figure 24 :** *E. strenuana* gall distribution during the exposure from the 20th of July to the 28th of September 2014 (Annex 13) within the site in the Latin square experiment in Langfang/Beijing. There were 10 plants per species in the central plot and 8 non-target plants respectively 16 *A. artemisiifolia* plants in the satellite plot (Fig. 6). The full lines represent the plants in the central plot and the dashed line represents the plants in the satellite plot.

**Table 5 :** *E. strenuana* galls found per plant species during the summer season 2014 (Annex 13) in the choice tests in Langfang/Beiing. The letter "n" indicates the absolute number of galls found on the study species during the exposure.

| **Plant species** | **Open-field Latin square** | | **Interspersion** | **Reverse Interspersion** |
|---|---|---|---|---|
| | Central plot | Satellite plot | | |
| *A. artemisiifolia* | 91.18% (n=31) | 100% (n=52) | 96.67% (n=29) | 69.81% (n=74) |
| *A. trifida* | 8.82% (n=3) | 0 | 0 | 30.19% (n=32) |
| Extrasol | 0 | 0 | 3.33% (n=1) | 0 |
| Girasol | 0 | 0 | 0 | 0 |
| Italy | 0 | 0 | 0 | 0 |

### 2.2.5.2 Interspersion and Reverse Interspersion

There was a significant difference of the distribution of the *E. strenuana* galls among the test plant species in the Interspersion and the Reverse Interspersion experiment in Langfang/Beijing ($p < 0.0001$). From the 133 released *E. strenuana* adults in the Interspersion experiment 30 new galls were found. Except of 1 gall found in *H. annuus* Extrasol no galls were found in other non-target plants. From the 100 released *E. strenuana* adults in the Reverse Interspersion experiment 106 new galls were formed (Fig. 25). 69.81% of the galls were found in *A. artemisiifolia* (n=74) and 30.19% in *A. trifida* (n= 32) (Table 5).

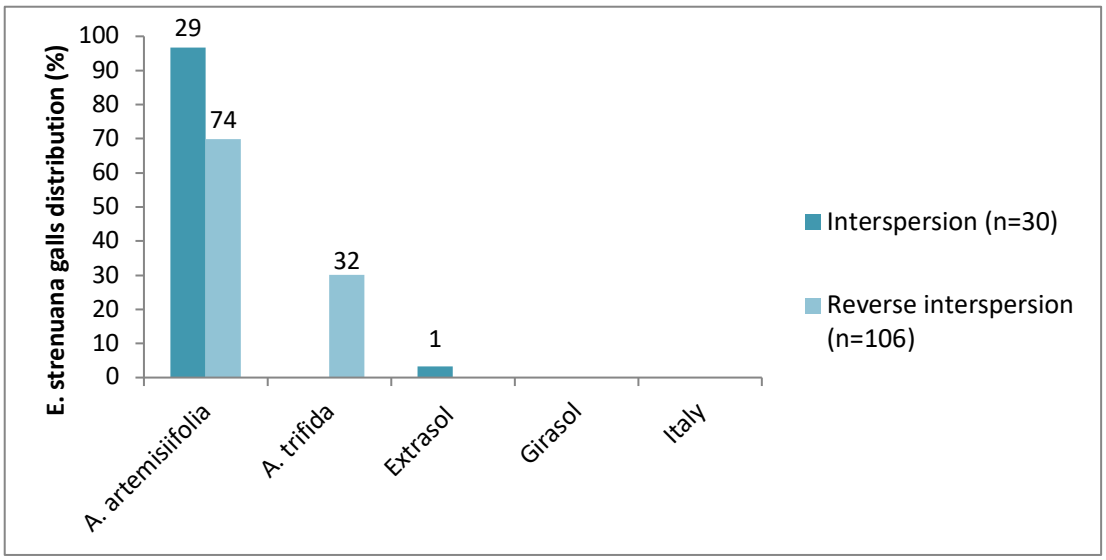


**Figure 25 :** Distribution of *E. strenuana* galls among the test plant species *A. artemisiifolia, A. trifida* and *H. annuus* (varieties Extrasol, Girasol and Italy) within the site in the Interspersion and Reverse Interspersion experiment in Langfang/Beijing in the summer 2014 (Annex 13). There were 10 plants per species in the Interspersion experiment and 100 non-target plants (only 10 plants per species were assessed) respectively 15 *A. artemisiifolia* plants in the Reverse Interspersion experiment. The numbers above the columns indicate the absolute number of galls found in the test plants.

## 2.3 Discussion

### 2.3.1 Oviposition preference of *O. communa*

As hypothesized, a very low amount of egg batches of *Ophraella* was found on the non-target plants. Except in the Reverse Interspersion experiment in Langfang/Beijing, where in total 22 egg batches were laid on *H. annuus* Extrasol only few egg batches were laid on the sunflowers in the different open field tests. It is assumed that the relatively high number of egg batches found on *H. annuus* Extrasol in the Reverse Interspersion experiment was because of the high relative numbers of the non-target plants compared to the target plants and compared to the relative numbers of the non-target plants in the other open field experiments. Expect of 22 egg batches laid on *A. trifida* in the central plot and 4 egg batches laid on *A. trifida* in the non-target satellite plot of the Latin square experiment in Langfang/Beijing, no egg batches were found on *A. trifida* in the Interspersion and Reverse Interspersion experiment. We expected that more egg batches would be laid on *A. trifida* because of the results of different experiments and field surveys in Korea that showed all stages of *O. communa* on *A. trifida* and even mention the potential of *O. communa* as a biological control agent for *A. trifida* (Lee et al., 2007). In general, the low amount of egg batches found on the non-target plants species in the open field can be compared with the results of the multiple-choice oviposition

test of Zhenjun (2011) in the open field in China where significantly more eggs were laid on *A. artemisiifolia* compared to the non-target plant species such as *H. annuus* and *A. trifida*.

Oviposition decreased during the season in the open field in Italy and Switzerland. In the Latin square experiment and the Reverse Interspersion experiment in Langfang/Beijing the number of egg batches laid on the target plant species increased at the beginning, but decreased then as well. The number of egg batches in the Interspersion experiment in Langfang/Beijing was very low from the beginning. Only 10 egg batches were found on *A. artemisiifolia* during the season even though 200 *O. communa* adults were released randomly at the beginning of the season. But from these 10 egg batches 92 larvae reached pupation. What is unexpected is that from the total 102 pupae found in the Interspersion experiment 6 pupae were found on *A. trifida* and 4 pupae were found on *H. annuus* Girasol even though no egg batches were laid on and only 1 larva was found on each of those plants. It can be assumed that the larvae that developed to pupae on those non-target plants were either not found during the assessment or migrated after the assessment. Therefore it was difficult to determine the number of generations during the season with regards to the egg batch abundance.

### 2.3.2 Abundance of *O.communa* larvae and pupae

The results are in line with our hypothesis. In all open field experiments we found significantly more larvae on *A. artemisiifolia* than on the non-target plant species and more small larvae than big larvae were found on the non-target plants. Only in the Interspersion experiment in Langfang/Beijing 41 small larvae but 49 big larvae were found during the season and in the Latin square experiment in Rovio two small larvae but three big larvae were found during the assessment of the second cohort. This may result from the fact that small larvae are difficult to find. The larval distribution in this study confirms the assumption that larval survival is lower on the non-target plant species and confirms earlier experiments such as the field survey of Hu and Meng in China (2007) and the field and greenhouse experiments of Dernovici in Canada (2003). In each experiment the larval survival was significantly higher on *A. artemisiifolia* than on the non-target plant species such as *H. annuus* or *H. tuberosus*.

Nevertheless, a few *O. communa* were able to complete their life cycle on the non-target plant species *A. trifida*, *H. annuus* and *H. tuberosus,* but survival from larvae to pupae was very low. Not every small larva reached the third larval stage and only few were able to pupate. Few pupae were found on *H. annuus* and only two pupae were found on *H. tuberosus*. Except of the Reverse Interspersion experiment most pupae were found one week after the peak of the big larvae

abundance. This confirms the development time of *O. communa* (Welch, 1978). Much more pupae than big larvae were found on *A. artemisiifolia* in the Latin square in Magnago. A possible explanation could be larval movement. There are no previous studies investigating larval movement, but observation during the Master thesis of Stéphanie von Bergen showed that larvae were able to migrate to other plants. The big larvae on the non-target plants could have migrated to the neighbour A. *artemisiifolia* plants to pupate.

### 2.3.3 Adult load of *O.communa*

*Ophraella communa* adults were found on every non-target plant species even on the two non-target plant species *Artemisia annua* and *Zinnia elegans* that are not included in the results. As we hypothesized the adult load was significantly higher on the target than on the non-target plants. It was also expected that *O. communa* would be able to build up high populations especially in the Interspersion experiment, where a high abundance of *A. artemisiifolia* was present. Another expectation was that the more *A. artemisiifolia* would be around the less adults would be found on the non-target plants. This could not be confirmed in every choice test. The adult load increased highly during the season in the Latin square experiment in Rovio, where adult load was significantly higher in absence of *A. artemiisifolia.* The adult load also increased in the Latin square experiment in Langfang/Beijing. On the other hand, a decrease of the adult load throughout the season was observed in the Interspersion experiment in Rovio. This result could be explained with the migration of the *O. communa* adults to far away *A. artemisiifolia* populations. In the Interspersion experiments in Busto Arsizio 2, Magnago and in Langfang/Beijing the adult load only increased slightly. In exchange, adult load was as expected to be significantly lower in absence of *A. artemisiifolia* in the Latin square experiments in Magenta and Magnago. The adult load in the Interspersion experiment in Busto Arsizio 1 and the Reverse Interspersion was fluctuating throughout the season.

### 2.3.4 Damage to test plants

At the end of the season *O. communa* was able to severely damage *H. annuus* plants. *Helianthus tuberosus* was only moderately damaged. In all three open field experiments in Langfang/Beijing, sunflowers were significantly more damaged than *A. artemisiifolia*. Similar results were observed in the Interspersion experiment in Rovio. All plants including the non-target plants were severely damaged with a mean damage level > 3.5 (Annex 10). The higher damage to the non-target plants in the Interspersion experiment in Langfang/Beijing and Rovio compared to the light damage to the

non-target plants in the Latin square experiment in Magenta and Magnago was unexpected, because the damage was expected to be lowest in the Interspersion experiment. The high damage to the non-target plants in the Reverse Interspersion experiment and in the Latin square, in absence of *A. artemisiifolia*, was expected because of the very low amount or even missing *A. artemiisolifa* (Table 2). It was also unexpected that the number of *O. communa* adults in the Latin square in Langfang/Beijing was over six times as big as the number of adults found in the Reverse Interspersion, but both choice tests had exactly the same pattern. In both cases the damage to the non-target plants was higher than the damage to *A. artemisiifolia*. Overall, the results show a risk of *O. communa* feeding damage to non-target plant species. But it was difficult to evaluate the damage because of the somehow imprecise assessment of damage level using only a few scores, and the uncertainty that only *O. communa* damage was included in the results. Therefore, the damage assessed in this study should not be the most important component to answer the question if there is a risk to non-target plants.

### 2.3.5 Abundance of *E. strenuana* galls

Compared to the results from studies of McFadyen in Australia (1992), where no galls were found in *H. annuus*, the results from this study is similar to the study from Wan in China (1995), who found few galls in *H. annuus*. The results of this study showed that 1 larva was able to form a gall in the *H. annuus* variety Extrasol. But the few galls found in *H. annuus* in the study of Wan (1995) were all in weakened plants. No oviposition by *E. strenuana* adult females was ever observed on *H. annuus*. Therefore it is unlikely that *E. strenuana* is able to cause economic damage to *H. annuus*. Even though *A. trifida* was recorded as host plant of *E. strenuana* in North America (McClay, 1987) only 3 galls were found in *A. trifida* in the Latin square experiment in Langfang/Beijing. More galls (n=32) were found in *A. trifida* in the Reverse Interspersion experiment. The difference between the two choice tests could be because of the higher frequency of *A. trifida* in the Reverse Interspersion than in the Interspersion experiment. The use of *E. strenuana* as the only biological control agent for *A. artemisiifolia* is questionable, as studies in China showed that the moth is not able to effectively control *A. artemisiifolia* on its own (Wan et al., 2002).

# 3. No-choice test: Egg transfer experiment

## 3.1 Material and Method

The egg transfer experiment in this study is similar to the no-choice experiment of Zhenjun in China (2011), where he surveyed the larval development using outdoor cages. The purpose of the egg transfer experiment was not to examine the oviposition choice because the egg batches were put deliberately equally on every plant, but to examine the life cycle of *O. communa* and to answer the question if *O. communa* can develop on other plant species than on the host plant species, if eggs are laid (even by accident). To minimize the possibility of migration of the beetles the plant species were held in outdoor cages. *Ambrosia artemisiifolia* was put in a separate cage as a control.

### 3.1.1 Study species and study sites

The study species *A. artemisiifolia, A. annua, H. annuus* (Extrasol, Girasol, Italy and LG5687), *H. tuberosus* and *Z. elegans* were used in the egg transfer experiment (Annex 2). The experiment took place in the study sites Abbiategrasso, Magnago and Magenta during the period of July-August 2014 and during the period of August-September 2014 (Table 3; Annex 13). The egg batches used in the experiment were collected from leaves of *A. artemisiifolia* plants in the study site environments.

### 3.1.2 Experimental setup and measurement

A plot of 3x4m was mown to have the lowest possible amount of vegetation around the study species. The plants were planted in a way that they didn't touch each other to minimize larval migration. The non-target plant species *A. annua, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687), *H. tuberosus* and *Z. elegans* were planted in the fringe of 1x2m cages (Fig. 26), while the target species was planted separately in the middle of a 1x1m cage. Even though the plants were planted randomly always one pair per plant species was planted next to each other (Fig. 26). They were watered twice a day for at least 5 days. Three egg batches with 10 eggs per batch were added to each plant individual. They were put on the top of each plant between the bud and the leaves (Annex 11). The setup in Magnago was on the 15$^{th}$ of July 2014 and the egg transfer was on the 19$^{th}$ of July 2014. The setup in Magenta was on the 23$^{rd}$ of July 2014 and the egg batches were added on the same day. The setup in Abbiategrasso was on the 27$^{th}$ of July 2014 and the egg batches were as

well added on the same day. The exposure was for four weeks from the 30th of July to the 19th of August 2014 in Abbiategrasso and Magenta and the 20th of August 2014 in Magnago (Annex 13). There was a weekly assessment. After four weeks of exposure the plants were cut and the fresh biomass was measured in grams. Then, the egg transfer was repeated. Again 2 plants per species were planted randomly in pairs and exposed for 4 weeks from the 26th of August to the 15th of September 2014 in Abbiategrasso and from the 27th of August to the 17th of September in Magenta and Magnago (Annex 13).

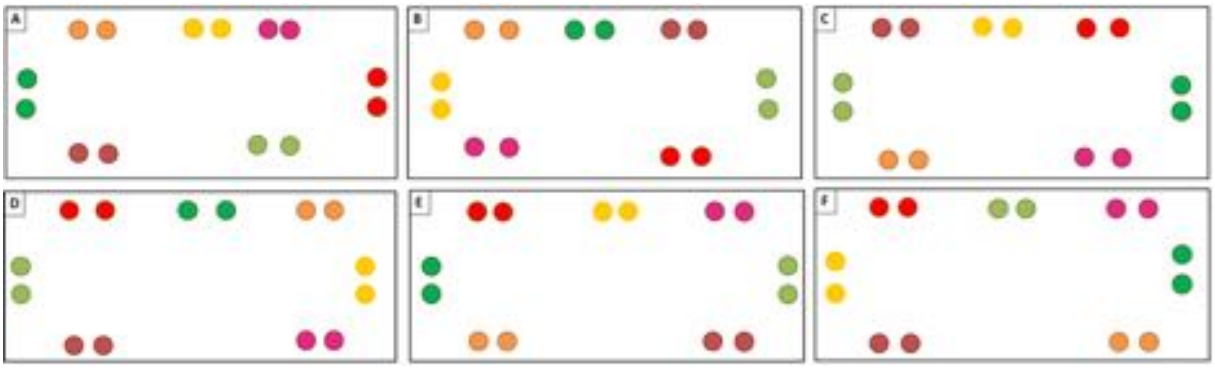

**Figure 26 :** Egg transfer experiment in Abbiategrasso, Magnago and Magenta (Table 3). Every rectangle is a 1x2m cage. Two plants per species were planted in pairs. *A. artemisiifolia* was planted in a separate 1x1m cage. The egg transfer experiments A/D were in Magnago, B/E in Magenta and C/F in Abbiategrasso. The egg transfer experiments A-C were carried out during the first cohort and the experiments D-F were during the second cohort (Annex 13). The dark red dots are *H. annuus* Girasol plants, orange *H. annuus* Extrasol, bright red *H. annuus* Italy and pink *H. annuus* LG5687. The yellow dots are *H. tuberosus* plants, dark green *A. annua* and light green *Z. elegans* (Annex 5).

### 3.1.3 Monitoring and statistical analyses

Like in the choice test the larval development and larval survival was assessed. The larval stages 1, 2 and 3 were again separated in small larvae (larval stage 1 and 2) and big larvae (larval stage 3) to minimize the probability to count the same larva twice. A distribution index was calculated for the cumulative number of larvae per plant indicating the relative attractiveness of each plant species (Palmer, 1986). The absolute number of larvae on a plant individual was divided by the total number of larvae in the experiment:

- Distribution index of larvae = $\frac{absolute\ number\ of\ larvae\ \ on\ plant}{absolute\ number\ of\ larvae\ in\ experiment}$

It was assumed that adults would hatch after one week from the cocoon and that all pupae would become adults. Assuming that, the distribution index of pupae on test plants was calculated by the absolute number of full pupae on each test plant divided by the total number of full pupae in the experiment. The distribution index of pupae was used as a value for a completed life cycle of *O. communa*.

- Distribution index of pupae = $\frac{absolute\ number\ of\ full\ \ pupae\ \ on\ plant}{absolute\ number\ of\ \ full\ pupae\ in\ experiment}$

Another value of *O. communa* was the damage of the plant tissue caused by *O. communa*. The damage level of each plant individual was determined at the final assessment. It was used as a value for the feeding preference of *O. communa* larvae. The damage level was divided into five levels (0-4).

- Level 0 = 0% damage
- Level 1 = 1-10% damaged plant tissue
- Level 2 = 11-50% damaged plant tissue
- Level 3 = 51-90% damaged plant tissue
- Level 4 = more than 90% damaged plant tissue.

The amount of larvae and pupae found on each plant was recorded weekly in an excel file. The adults found in the outdoor cages were removed to avoid additional oviposition. A general overview was taken for every study site (Annex 15). All adults found inside the cage during the assessments were removed, hence, only larval damage could be assumed. The effect of the plant species on the presence of *O. communa* was calculated using JMP statistics.

## 3.2 Results

The study site Magenta was not included in the results because almost no larvae were found on *A. artemisiifolia* and it was not possible to explain this low amount of *O. communa*. No egg predation by e.g an anystid mite, larval predation by e.g the pentatomid *Perillus splendidus* and prepupal parasitism by e.g the tachinid *Chaetonodexodes vanderwulpi* was observed during the season, which could have reduced the population (Goeden and Ricker, 1985).

### 3.2.1 Larval survival of *O. communa*

Abundance of *Ophraella* larvae

From 60 possible larvae per test plant species (30 eggs x 2 plants per species) significantly more small larvae were found on *A. artemisiifolia* than on the non-target plants ($p < 0.0001$; Fig. 27 a,b). Except in the second cohort more small larvae were found on *H. annuus* LG5687 (n=28) than on *A. artemisiifolia* (n=16). From all non-target plant species most small larvae were found on *H. annuus* LG5687 (n=28) and no small larvae were found on *A. annua*. Significantly more small larvae reached

the third larval stage on *A. artemisiifolia* than on the non-target plants ($p < 0.0001$). Between 17-81% developed from small to big larvae on *A. artemisiifolia*. Expect of 6 big larvae found on *H. annuus* Extrasol and Girasol only one big larva was found on the non-target plants *H. annuus* (varieties Italy and LG5687) and *Z. elegans*. No big larvae were found on *A. annua* and *H. tuberosus* (Fig. 27 c,d).

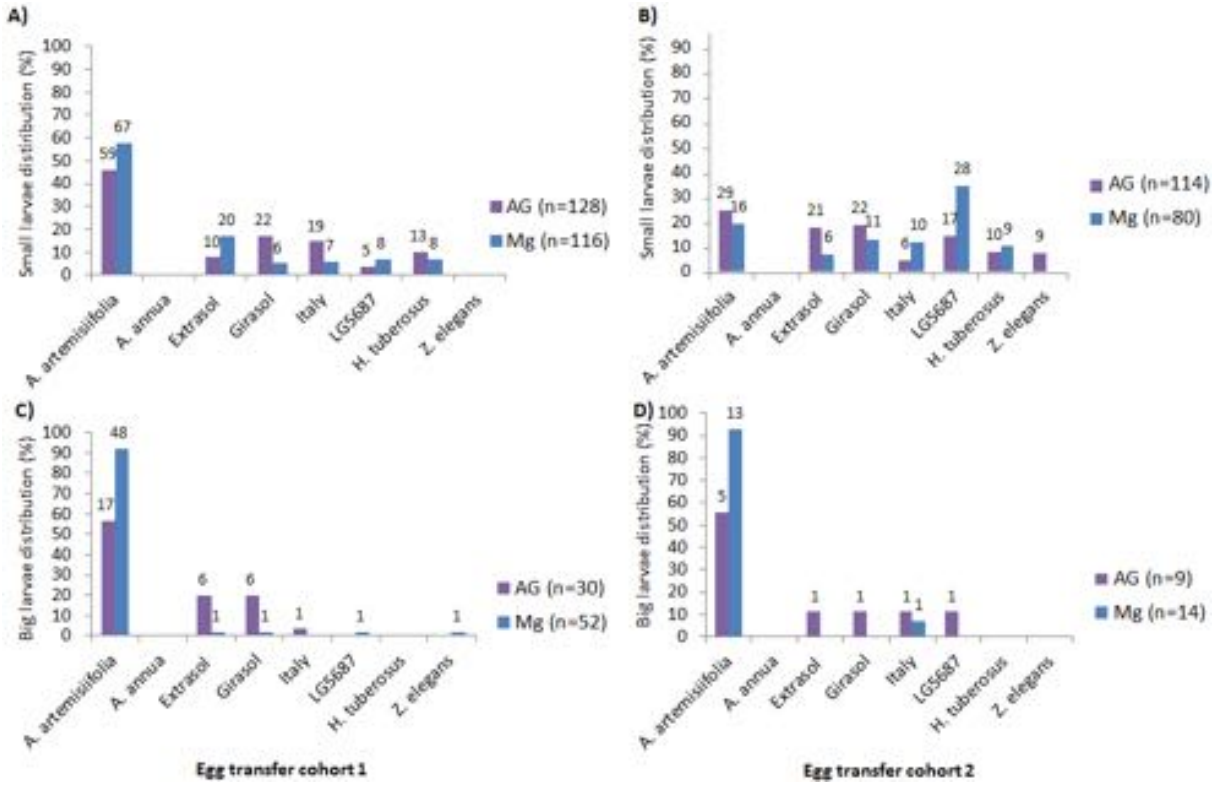


**Figure 27 :** Larval distribution among the test plant species *A. artemisiifoia, A. annua, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687), *H. tuberosus* and *Z. elegans* within the sites in the Egg transfer experiment in Abbiategrasso (AG) and Magnago (Mg). There were 2 plants per species. The exposure was for 4 weeks (Annex 13). A) Small larval distribution among the test plants during the first cohort. B) Small larval distribution among the test plants during the second cohort. C) Big larval distribution among the test plants during the first cohort. D) Big larval distribution among the test plants during the second cohort. The numbers above the columns indicate the absolute number of larvae on the test plant species out of a total of 60 eggs transferred per plant species.

Abundance of *Ophraella* pupae

Significantly more pupae were found on *A. artemisiifolia* than on the non-target plants during the first and the second cohort in Abbiategrasso and Magnago ($p < 0.0001$). During the first cohort in Abbiategrasso 91.21% of the pupae were found on *A. artemisiifolia* (n=83) and during the second cohort 100% of the pupae were found on *A. artemisiifolia* (n=3). In Magnago 83.10% of the pupae were found on *A. artemisiifolia* (n=59) during the first cohort and 59.09% were found on *A.*

*artemisiifolia* (n=13) during the second cohort (Fig. 28). Pupae were found on all *H. annuus* varieties but not on *A. annua, H. tuberosus* and *Z. elegans*.

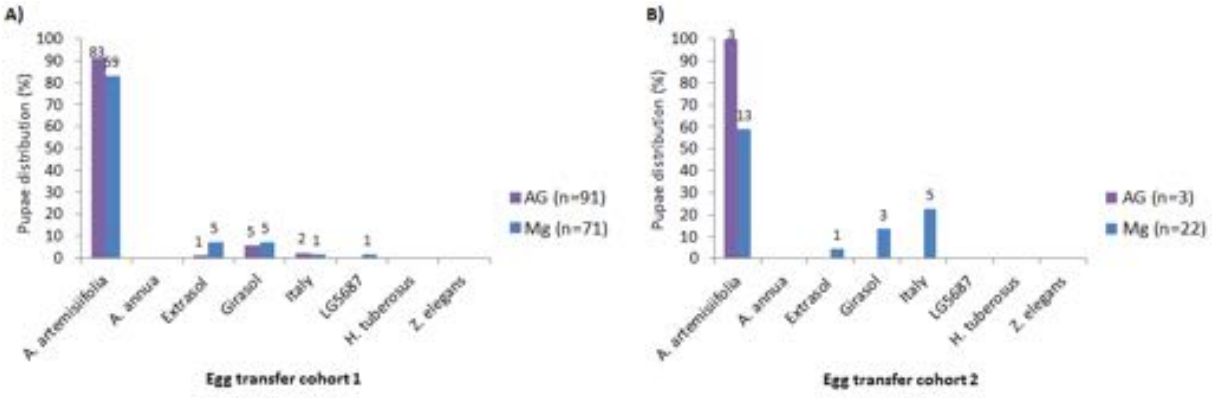


**Figure 28 :** Pupae distribution among the test plant species *A. artemisiifoia, A. annua, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687), *H. tuberosus* and *Z. elegans* within the sites in the Egg transfer experiment in Abbiategrasso (AG) and Magnago (Mg). There were 2 plants per species. The exposure was for 4 weeks (Annex 13). A) Pupae distribution among the test plants during the first cohort. B) Pupae distribution among the test plants during the second cohort.

### 3.2.2 Damage to test plants

After 4 weeks of exposure during the first cohort in the egg transfer experiment in Abbiategrasso (Annex 13), the *H. annuus* variety Italy was significantly more damaged than the other non-target plants and *A. artemisiifolia* ($p < 0.0001$). *Helianthus annus* Italy was completely damaged (damage level 4; Table 6). After 4 weeks of exposure during the second cohort in Abbiategrasso the mean damage level was significantly higher on *H. annuus* Extrasol compared to the other non-target plants and *A. artemisiifolia* ($p < 0.0001$). After 4 weeks of exposure during the first cohort in Magnago (Annex 13) *Ambrosia artemisiifolia* and the *H. annuus* varieties Extrasol, Girasol, Italy and LG5687 were significantly more damaged than *A. annua, H. tuberosus* and *Z. elegans* ($p < 0.002$; Fig. 29a; Table 6). *Artemisia annua* and *H. tuberosus* had a damage level of 0. The mean damage level at the final assessment of the second cohort in Magnago was significantly higher on *A. artemisiioflia* than on the non-target plant species ($p < 0.0003$; Table 6).

**Table 6 :** Mean damage level of the plant species in the egg transfer experiment in Abbiategrasso (AG) and Magnago (Mg) after 4 weeks of assessment in the first and the second cohort.

| **Plant species** | **Cohort 1** | | **Cohort 2** | |
|---|---|---|---|---|
| | Abbiategrasso(AG) | Magnago (Mg) | Abbiategrasso(AG) | Magnago (Mg) |
| *A. artemisiifolia* | 2.5 | 1.5 | 1 | 3 |
| *A. annua* | 0 | 0 | 0 | 0 |
| *H. annuus* Extrasol | 1 | 2 | 2 | 1 |
| *H. annuus* Girasol | 1 | 1 | 1 | 1 |
| *H. annuus* Italy | 4 | 2 | 1 | 1.5 |
| *H. annuus* LG 5687 | 1.5 | 1 | 1 | 1.5 |
| *H. tuberosus* | 1 | 0 | 1 | 0 |
| *Z. elegans* | 1 | 0.5 | 0 | 0 |

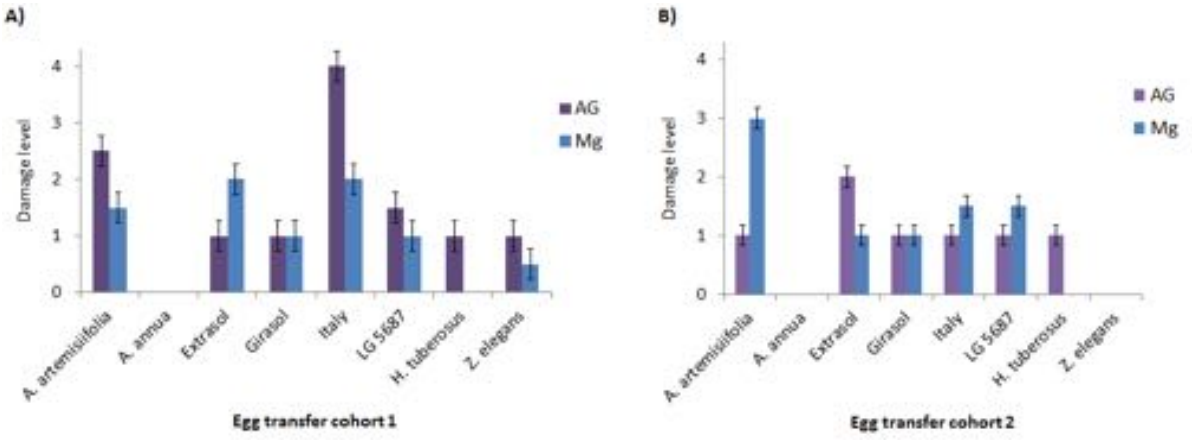


**Figure 29 :** Mean damage level of the test plant species *A. artemisiifoia, A. annua, H. annuus* (varieties Extrasol, Girasol, Italy and LG5687), *H. tuberosus* and *Z. elegans* within the sites in the Egg transfer experiment in Abbiategrasso (AG) and Magnago (Mg) during the first cohort (A) and the second cohort (B).

## 3.3 Discussion

### 3.3.1 Larval survival of *O. communa*

A small amount of larvae was able to complete the life cycle on different *H. annuus* varieties in this study. Studies in China showed similar results where the survival rate of neonate larvae in no-choice outdoor cages was significantly lower on non-target plants than on *A. artemisiifolia* (Zhenjun Cao, 2011). Under no-choice conditions the larval mortality is recorded to be higher on non-target plants than on *A. artemisiifolia*. However, no-choice tests in Japan showed that larval survival was similar between target and non-target plants species (Yamazaki et al., 2000). It may be explained by the fact that the no-choice tests in Japan were performed in small vials in the laboratory and fed with

detached leaves. Development performances under these conditions can be affected by constraints from severe spatial limits and changes in nutrients because of the detached leaves. The different results between the no-choice tests in Japan and this study could be because the outdoor cages used in this study lead to a more natural environment for the larvae and could hence lower the constraints, and therefore reduce false-positive results (Zhenjun Cao, 2011). Like in the studies of Palmer and Goeden in the United States (1991) and of Zhenjun in China (2011) the *H. annuus* varieties used in this study could sustain *O. communa* larval development although the larval mortality was very high. A possible reason that no larvae were found on *A. annua* and *Z. elegans* is larval death. No larvae were recorded on *A. annua* and *Z. elegans* in previous experiments. Another reason could be larval movement like already observed in the choice tests of this study and during the Master thesis of Stéphanie von Bergen. The larvae could possibly have migrated from *A. annua* and *Z. elegans* to the *Helianthus* plants in search of better nutrients. Larval movement would also explain why more pupae than big larvae were found on some non-target plants. For example 6 out of 10 small larvae reached the third larval stage on *H. annuus* Extrasol in Abbiategrasso, but only one big larva pupated (Fig. 27). This observation was also made in the separate *A. artemisiifolia* cage. It remains difficult to explain why for example 83 pupae, but only 17 big larvae were found on *A. artemisiifolia* in Abbiategrasso. The most reasonable explanation is an inadequate assessment.

### 3.3.2 Damage to test plants

No-choice tests in Canada showed that larvae are able to damage *H. annuus* significantly, but because the survival rate is very low the risk to *H. annuus* is considered as negligible (Dernovici et al., 2006). In this study the damage to non-target plants was lower than the damage to *A. artemisiifolia* with exception of the damage caused to the *H. annuus* variety Italy in Abbiategrasso. Overall, the results showed a potential severe damage to non-target plant species, but only in individual cases. The hypothesis that the damage is significantly higher in a no-choice environment than a choice environment could not be confirmed.

# 4. Conclusion and Outlook

The various experimental setups providing different field situations allowed getting a more differentiated overview, but overall, predicting the risk to the non-target plant species, especially to *H. annuus* remains difficult. The predictions showed in Table 2 could more or less be confirmed. The more *A. artemisiifolia* were present in the open-field experiments the lower was the damage to the non-target plants. But also the abundance of *O. communa* is crucial for the damage caused to the non-target plants. The more *O. communa* were present in the open-field experiments and in the surroundings, the higher was the damage to the non-target plants. Therefore it can be concluded that the interaction of the abundance of *A. artemisiifolia* and *O. communa* is of importance for the attack and damage level on non-target plants. The damage caused to the non-target plants in the no-choice test was not higher than in the choice-tests, therefore it can not be concluded that the damaged is highest under no-choice conditions (Table 2). Even though every *O. communa* stage was found on the non-target plant species, especially on *H. annuus*, the abundance was that low that it can be concluded that *A. artemisiifolia* is significantly more attractive for *O. communa* than the non-target plant species in this study. Overall, it cannot be said that there is no risk for sunflower. On the other hand, *A. annua* and *Z. elegans* can be considered to be unattractive and thus safe for all *O. communa* stages and can be excluded from subsequent studies in Europe.

The results of this study showed a negligible number of *E. strenuana* galls on non-target plants. Therefore it can be concluded that it is unlikely that *E. strenuana* is able to cause economic damage to *H. annuus*.

For subsequent experiments it would be better to use damage percentages instead of levels to have a more precise indication of the damage caused to test plants. It was also quite difficult to totally exclude damage caused by other herbivorous or pathogens. In addition, subsequent experiments could include more cohorts to have more generations of *A. artemisiifolia* and *O. communa* to test. Experiments in different study sites, but with the same objective, should have the same setup to simplify comparisons among sites. For example the same number of test plants and the same number of released beetles would simplify the comparison and subsequent analysis.

## 5. Acknowledgment

First I want to thank my professor and supervisor Heinz Müller-Schärer who helped me throughout my Master thesis and gave me the chance to work in an international ecological project. I also want to thank Urs Schaffner and Suzanne Lommen, who helped me a lot for the design of my practical work. For the setup and the assessment of my practical work I am grateful to have had the help from Anne Häberle, Stephanie von Bergen, Eva Van Cleef, Simon Vandenbrande, Silvia Stefanelli and Elisa Cardarelli.

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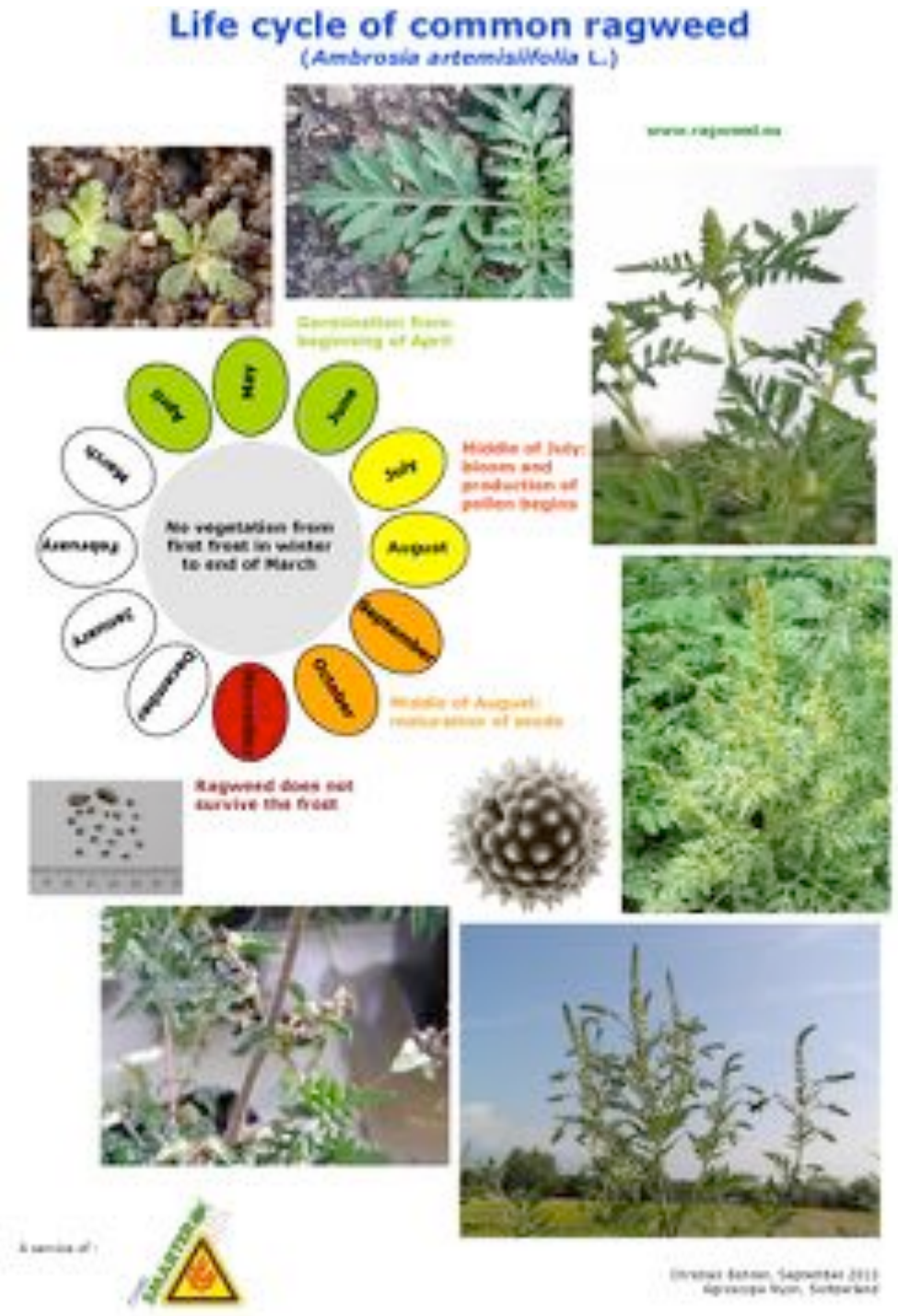
# 7. Annex

**Annex 1 :** Life cycle of *Ambrosia artemisiifolia*. Source: Agroscope.



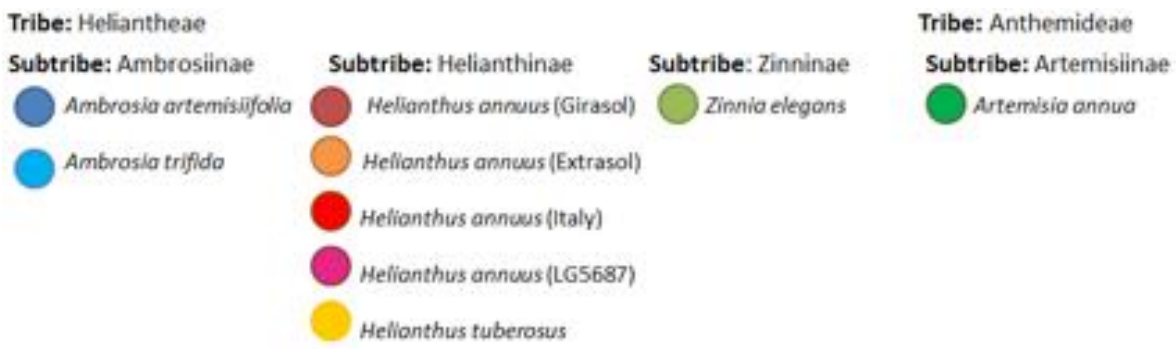
**Annex 2 :** Overview of the study species used per experiment in the different study sites.

| Study species | Study sites | Experiments |
|---|---|---|
| *A. artemisiifolia* | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT<br>5 Rovio CH<br>6 Langfang/Beijing CN | Latin square 3, 4, 5, 6<br>Interspersion 2, 4, 5, 6<br>Reverse Interspersion 6<br>Egg transfer 1, 3, 4 |
| *A. trifida* | 1 Langfang/Beijing CN | Latin square 1<br>Interspersion 1<br>Reverse Interspersion 1 |
| *A. annua* | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT | Latin square 1, 3, 4<br>Interspersion 2, 4<br>Egg transfer 1, 3, 4 |
| *H. annuus* Extrasol | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT<br>5 Rovio CH<br>6 Langfang/Beijing CN | Latin square 3, 4, 5, 6<br>Interspersion 2, 4, 5, 6<br>Reverse Interspersion 6<br>Egg transfer 1, 3, 4 |
| *H.annuus* Girasol | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT<br>5 Rovio CH<br>6 Langfang/Beijing CN | Latin square 3, 4, 5, 6<br>Interspersion 2, 4, 5, 6<br>Reverse Interspersion 6<br>Egg transfer 1, 3, 4 |
| *H.annuus* Italy | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT<br>5 Rovio CH<br>6 Langfang/Beijing CN | Latin square 3, 4, 5, 6<br>Interspersion 2, 4, 5, 6<br>Reverse Interspersion 6<br>Egg transfer 1, 3, 4 |
| *H. annuus* LG5687 | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT | Latin square 1, 3, 4<br>Interspersion 2, 4<br>Egg transfer 1, 3, 4 |
| *H. tuberosus* | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT | Latin square 1, 3, 4<br>Interspersion 2, 4<br>Egg transfer 1, 3, 4 |
| *Z. elegans* | 1 Abbiategrasso IT<br>2 Busto Arsizio IT<br>3 Magenta IT<br>4 Magnago IT | Latin square 1, 3, 4<br>Interspersion 2, 4<br>Egg transfer 1, 3, 4 |

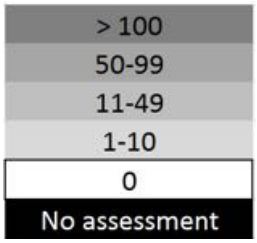
**Annex 3 :** Study sites used for the choice and no-choice tests in Italy and Switzerland. A) Abbiategrasso. B) Busto Arsizio 1. C) Busto Arsizio 2. D) Magenta. E) Magnago. F) Rovio.


**Annex 4 :** Choice tests in the open field in Langfang/Beijing, China, 2014. A) Central plot of the Latin square experiment. B) Interspersion experiment. C) Reverse Interspersion experiment.

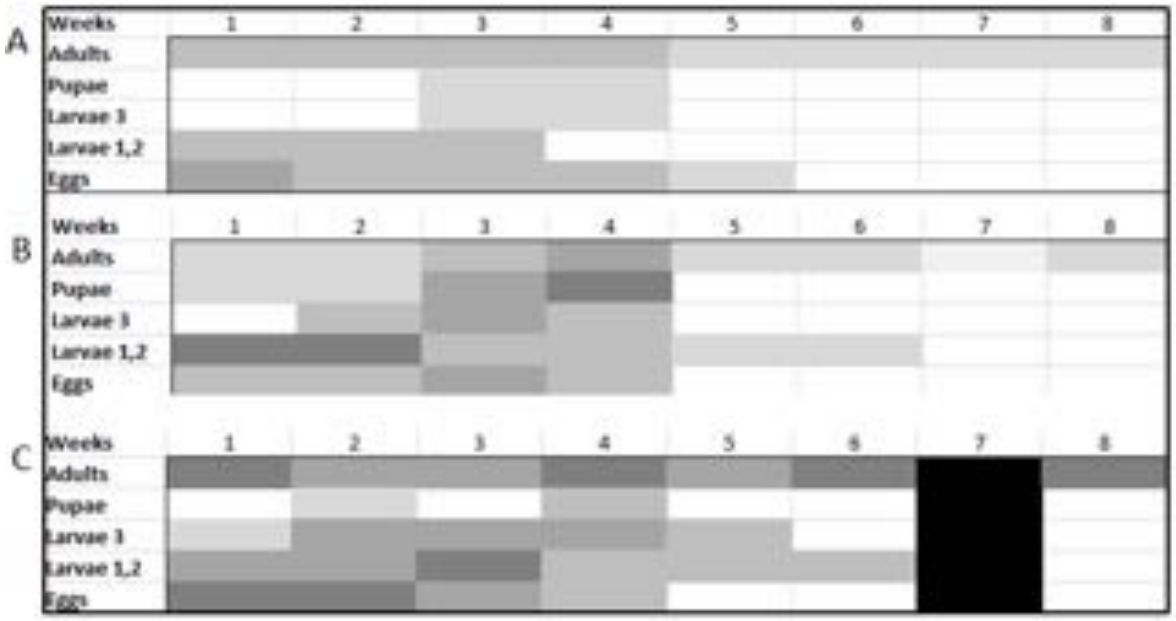

**Annex 5 :** In the study designs we used specific colour to identify the plant species or varieties. Different species in the same subtribe had similar colours. The subtribe Ambrosiinae had blueish colours and the subtribe Helianthinae reddish colours. As *Z. elegans* and *A. annua* are not in the same subtribe, we used greenish colours.



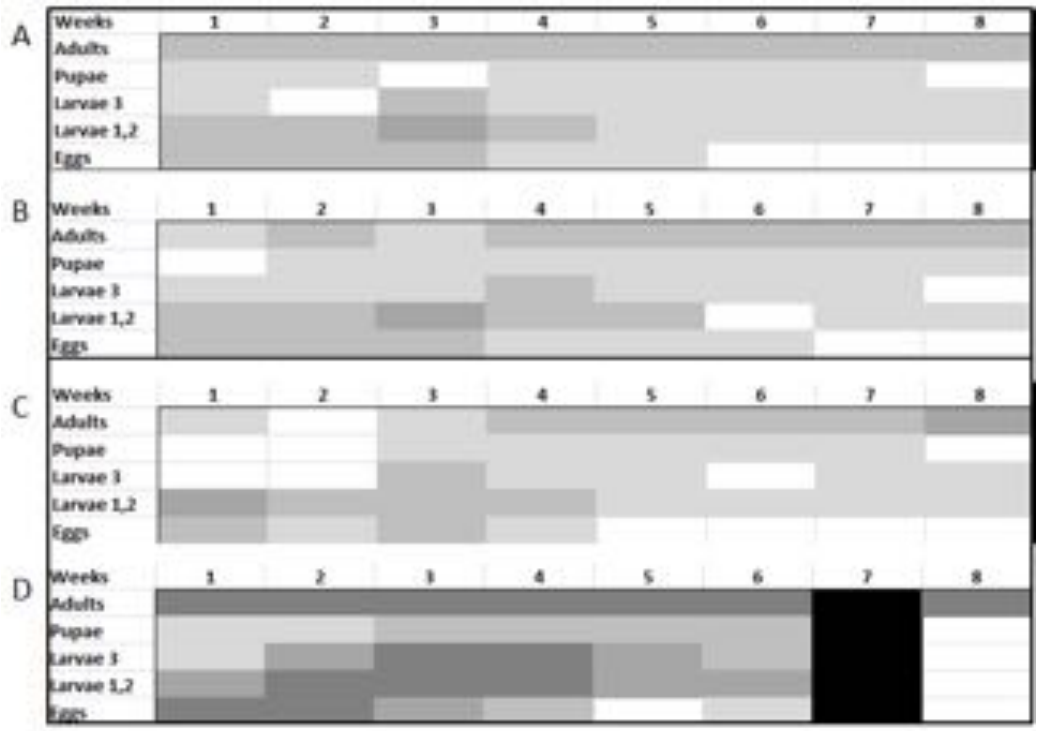
**Annex 6 :** Relative occurrences of the various developmental stages of *O. communa* over the duration of the experiments (cf. Annex 7, 8, 9).



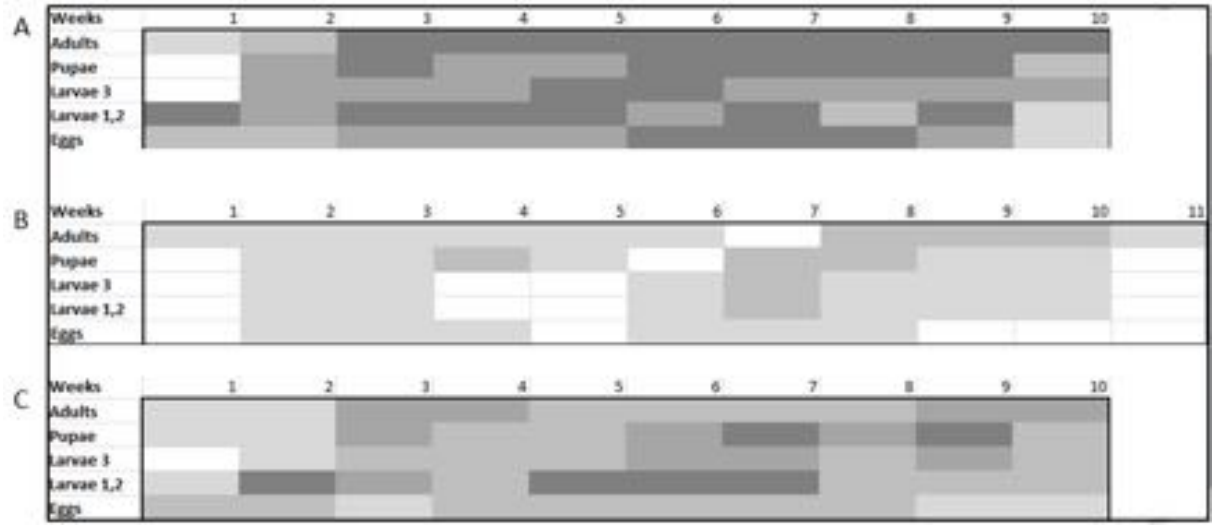

**Annex 7 :** Temporal variation of the relative occurrences of the different developmental stages of *O. communa* in the Latin square experiment in Switzerland and Italy 2014. A) Magenta. B) Magnago. C) Rovio.



**Annex 8 :** Temporal variation of the relative occurrences of the different developmental stages of *O. communa* in the Interspersion experiment in Italy and Switzerland. A) Busto Arsizio 1. B) Busto Arsizio 2. C) Magnago D) Rovio.

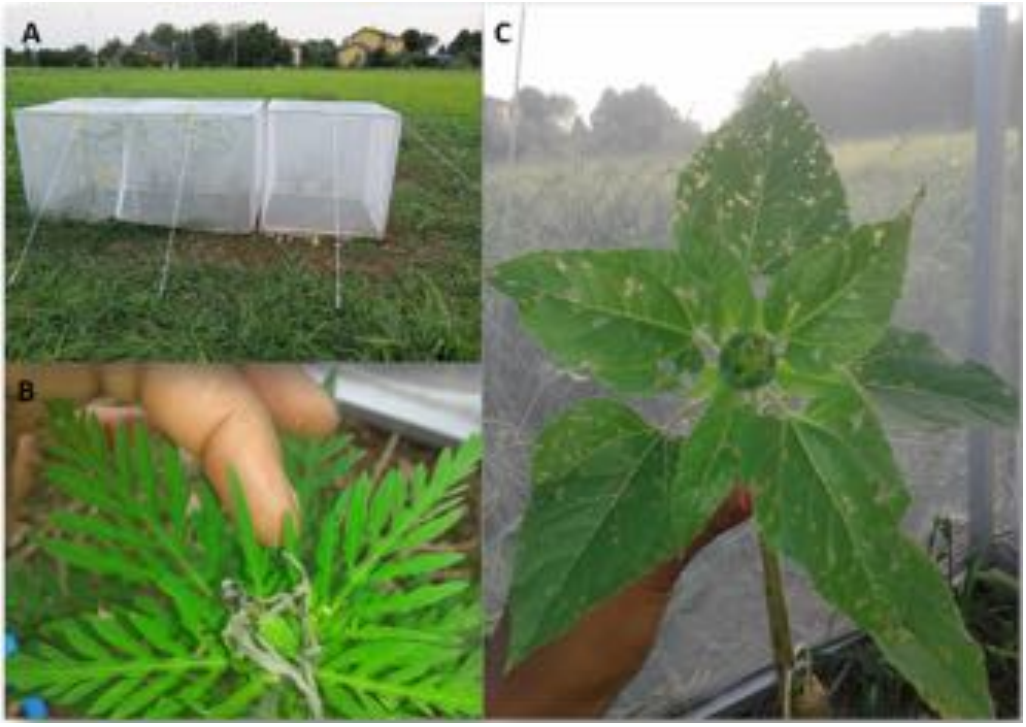



**Annex 9 :** Temporal variation of the relative occurrences of the different developmental stages of *O. communa* in the choice experiments in Langfang/Beijing 2014. A) Latin square experiment. B) Interspersion experiment. C) Reverse Interspersion experiment.



**Annex 10 :** Damage to sunflowers in the Interspersion experiment in Rovio 2014. A/B) Two different plants assessed on the 15th of September 2014. C/D) The same plant as above assessed on the 1st of October 2014.

**Annex 11 :** Egg transfer experiment in Magenta 2014. A) A 2x1m non-target plants cage and a 1x1m target plant cage. B) The egg batches were put between the leaves. C) Larval damage to *H. annuus* Italy after 4 weeks of exposure.




**Annex 12 :** Period of exposure (in weeks) in the choice and no-choice tests in the summer season 2014.

| Experiment | Study site | Number of weeks of experiment | Number of assessments |
|---|---|---|---|
| Latin square | Abbiategrasso<br>Magenta<br>Magnago | 8 | 4/4 |
| Latin square | Rovio | 8 | 4/3 |
| Latin square | Langfang/Beijing | 11 | 10 |
| Interspersion | Busto Arsizio 1<br>Busto Arsizio 2<br>Mangago | 8 | 8 |
| Interspersion | Rovio | 8 | 7 |
| Interspersion | Langfang/Beijing | 11 | 11 |
| Reverse Interspersion | Langfang/Beijing | 11 | 11 |
| Egg transfer | Abbiategrasso<br>Magenta<br>Magnago | 8 | 4/4 |

**Annex 13 :** Assessment dates and dates of insect release in the different choice and no-choice experiments in 2014. The study sites are numbered as follows: Abbietegrasso (1), Busto Arsizio1 (2), Busto Arsizio2 (3), Magenta (4), Magnago (5), Rovio (6) and Langfang/Beijing (7).

| **Experiment** | **Study site** | **Cohort** | **Setup/ planting of test plants** | **Initial assess-ment** | **First assess-ment** | **Final assess-ment** | ***O.communa* (adults) release** | **# released adults** | ***O.communa* egg transfer** | **# released egg batches** | ***E. strenuana* (galls) release** | **# released galls** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Latin square | 4 | 1 | 17.07.14 | 23.07.14 | 30.07.14 | 22.08.14 | 22.07.14 | 120 | no transfer | 0 | no release | 0 |
| | 5 | 1 | 15.07.14 | 23.07.14 | 30.07.14 | 20.08.14 | 22.07.14 | 120 | no transfer | 0 | no release | 0 |
| | 6 | 1 | 04.08. 14 | 04.08.14 | 11.08.14 | 01.09.14 | 04.08.14 | 30 | no transfer | 0 | no release | 0 |
| | 7 | 1-2 | 08.07. 14 | 11.07.14 | 20.07.14 | 28.09.14 | 12.07.14 | 100 | no transfer | 0 | 12.07.14 | 100 |
| | 4 | 2 | 22.08. 14 | 22.08.14 | 27.08.14 | 17.09.14 | 22.08.14 | 120 | no transfer | 0 | no release | 0 |
| | 5 | 2 | 20.08. 14 | 20.08.14 | 27.08.14 | 16.07.14 | 20.08.14 | 120 | no transfer | 0 | no release | 0 |
| | 6 | 2 | 01.09. 14 | 01.09.14 | 08.09.14 | 01.10.14 | 01.09.14 | 30 | no transfer | 0 | no release | 0 |
| Interspersion | 2 | 1-2 | 22.07. 14 | 22.07.14 | 31.07.14 | 16.09.14 | no release | 0 | no transfer | 0 | no release | 0 |
| | 3 | 1-2 | 22.07. 14 | 23.07.14 | 31.07.14 | 16.09.14 | no release | 0 | no transfer | 0 | no release | 0 |
| | 5 | 1-2 | 19.07. 14 | 19.07.14 | 30.07.14 | 16.09.14 | no release | 0 | no transfer | 0 | no release | 0 |
| | 6 | 1-2 | 04.08. 14 | 04.08.14 | 11.08.14 | 01.10.14 | no release | 0 | no transfer | 0 | no release | 0 |
| | 7 | 1-2 | 13.07. 14 | 13.07.14 | 20.07.14 | 28.09.14 | 08.07.14 | 200 | no transfer | 0 | 08.07.14<br>12.07.14<br>13.07.14 | 20<br>13<br>100 |
| Reverse Interspersion | 7 | 1-2 | 29.06. 14 | 11.07.14 | 20.07.14 | 28.09.14 | 12.07.14<br>13.07.14<br>14.07.14 | 100<br>20<br>30 | no transfer | 0 | 12.07.14 | 100 |
| Egg transfer | 1 | 1 | 27.07. 14 | 27.07.14 | 30.07.14 | 19.08.14 | no release | 0 | 27.07.14 | 30 | no release | 0 |
| | 4 | 1 | 23.07. 14 | 27.07.14 | 30.07.14 | 19.08.14 | no release | 0 | 23.07.14 | 30 | no release | 0 |
| | 5 | 1 | 15.07. 14 | 23.07.14 | 30.07.14 | 20.08.14 | no release | 0 | 19.07.14 | 30 | no release | 0 |
| | 1 | 2 | 22.08. 14 | 22.08.14 | 26.08.14 | 15.09.14 | no release | 0 | 22.08.14 | 30 | no release | 0 |
| | 4 | 2 | 22.08. 14 | 22.08.14 | 27.08.14 | 17.09.14 | no release | 0 | 22.08.14 | 30 | no release | 0 |
| | 5 | 2 | 22.08. 14 | 22.08.14 | 27.08.14 | 17.09.14 | no release | 0 | 22.08.14 | 30 | no release | 0 |

**Annex 14 :** Assessment sheet of the choice and no-choice tests. The column "Epiblema" was only used in Langfang/Beijing.

| Plant | | | Damage | Ophraella | | | | | Epiblema |
|---|---|---|---|---|---|---|---|---|---|
| Plant location | Plant size | Phenological stage | Damage level (0-4) | # Egg batches | # Larvae 1-2 | # Larvae 3 | # Pupae | # Adults | # Galls |
| Location of the randomly chosen plant, according to the plan. number_column.number_line | Measure the height from the bottom to the meristem in cm. | Vegetative stage Flowering stage Seed producing | 0 = 0% damage 1 = 1-10% damage 2 = 11-50% damage 3 =51-90% damage 4 = 91-100% damage | The total number of eggbatches on the whole plant (hatched and unhatched). Make sure to turn the leaves to check underneath . | Approximate number of larvae with larval stage 1 and 2 on the plant | Approximate number of larvae with larval stage 3 on the plant | Approximate number of full pupae on the plant | Approximate number of adults on the plant | Approximate number of galls on the plant |

| General site information | Surrounding A. artemisiifolia | | | Surrounding Ophraella |
|---|---|---|---|---|
| General site information | # Ambrosia present: | ø size of Ambrosia : | ø damage level of Ambrosia | Ophraella present inside Briese 1 (y/n) |
| Write in 2-3 sentences how the site looks like. | Average number of Ambrosia present inside plot (don't count seedlings < 1cm) | Average size of Ambrosia present inside plot | 0 = 0% damage 1 = 1-10% damage 2 = 11-50% damage 3 =51-90% damage 4 = 91-100% damage | Yes, Ophraella is present in any stage (eggs, larvae, pupae, adults) but not on the test plants No, no Ophraella is present inside the Briese 1 plot |