Susceptibility of sunflower to *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), a candidate for the biological control of common ragweed (*Ambrosia artemisiifolia* L.)

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Short Title

Ophraella communa – host specificity

Serghei Dernovici

Abstract

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The suitability of sunflower (*Heliantus annuus* L.) as a host of *Ophraella communa* Le Sage (Coleoptera: Chrysomelidae) was evaluated under greenhouse and field conditions. Population dynamics of *O. communa* on sunflower and on ragweed (*Ambrosia artemisiifolia* L.) were determined using a life table approach. Sixty percent of *O. communa* females died during the first 30 days on sunflower while only 14% died on ragweed plants. Only 20% of fertile females laid eggs on sunflower plants as compared with 100% on ragweed plants. Fecundity, life duration, egg viability, and other biological parameters were significantly higher on ragweed plants than on sunflower plants. Ragweed is the main host plant for *O. communa*. Nevertheless, in specific situations (no-choice) adults and larvae of *O. communa* can damage sunflower plants. However, *O. communa* cannot complete its life cycle or increase its population on sunflower plants.

Résumé

Maitrise en Science Serghei Dernovici Malherbologie

La valeur du tournesol (*Heliantus annuus* L.) en tant qu'hôte pour *Ophraella communa* Le Sage (Coleoptera: Chrysomelidae) a été évaluée en serres et en champ. La dynamique des populations de *O. communa* sur le tournesol et sur l'herbe à poux (*Ambrosia artemisiifolia* L.) a été déterminée en utilisant une approche par table de survie. Soixante pourcent des femelles de *O. communa* sont mortes au cours des 30 premiers jours sur le tournesol tandis que seulement 14% des femelles son mortes sur l'herbe à poux. Seulement 20% des femelles fertiles ont pondu des oeufs sur les plants de tournesol comparativement à 100% sur l'herbe à poux. La fécondité, la longévité, et la viabilité des œufs et plusieurs autres paramètres biologiques étaient significativement plus élevés sur l'herbe à poux que sur le tournesol. L'herbe à poux est la plante d'hôte principale pour *O. communa*. Néanmoins, dans les situations spécifiques (l'aucun choix) les adultes et les larves *d'O. communa* peut endommager les plantes de tournesol. Cependant, *O. communa* ne peut pas compléter son cycle de vie ou augmente sa population sur les plantes de tournesol.

Dedication

I dedicate this work to my family.

Acknowledgements

I wish to express my gratitude to my thesis supervisor Dr. Alan K. Watson, whose knowledge and advice have been invaluable. I appreciate his support and attention during my studies at McGill University as well as his diligence in supervising and reviewing various stages of this thesis. The author is deeply grateful to Dr. Miron Teshler, research associate, for his continuing support, guidance, and sharing his extensive knowledge of *Ophraella communa*. I am grateful to Josée Boisclair of Institut de Recherché et de Development en Agroenvironmenment (IRDA) who served on my advisory committee. Financial support from Phytodata Inc. and AAFC/NSERC Research Partnership Grant is gratefully acknowledged. The author is indebted to Richard Smith, the greenhouse manager, for his assistance in providing facilities and support during all greenhouse experiments. I appreciate the assistance of Luciano Germani and Helen Cohen-Rimmer for providing audio-visual services, and advice. Thanks to Scott Couture and Sophie St-Louis for facilitating my field experiments and to M. Ameur Manceur, undergraduate student, McGill University, who assisted in my experiments during the summer of 2001.

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1. Literature review

1.1. *Ambrosia artemisiifolia* L

1.1.1. Biology

Ambrosia artemisiifolia L. (common or short ragweed) is a native annual North American species whose pollen has been found in interglacial deposits of southern Ontario and Québec dating back more than 60,000 years ago (Harris and Piper, 1970). It belongs to the Heliantheae subtribe of the Heleniae tribe of the Compositae (Asteraceae) family (Gleason and Cronquist, 1963) and the genus *Ambrosia* consists of 41 species (Fernald, 1950; Payne, 1964). *A. artemisiifolia* can be found throughout Canada but eastern Canada, particularly southern Ontario and Québec, has the most abundant population of this weed (Bassett and Crompton, 1975). This might be due to the clearing of the land upon the arrival of the European settlers and intensified agriculture which favoured the spreading of the weed that *A. artemisiifolia* became a serious pest in eastern Canada. *A. artemisiifolia* is a "secondary noxious weed" under the Federal Seeds Act and a "noxious weed" in many provincial statues. It is the most abundant of the four Canadian *Ambrosia* species and its pollen is the primary cause of allergenic hay fever in North America (Bassett and Crompton, 1975).

A. artemisiifolia is a monoecious, wind-pollinated plant with numerous staminate flowers containing proliferous numbers of pollen grains. Persons who are susceptible to ragweed allergies from August to September suffer each year as a result of the histamine reaction to common ragweed pollen. There are at least nine allergens that have been isolated from common ragweed and the most important is "*Amb* a I-antigen E" (Bierman *et al.*, 1996).

This weed is a pioneer species that flourishes in disturbed habitats such as along rights-of-way and in vacant lots. In southwestern Québec and Ontario, it has become a serious agricultural weed. Its seeds germinate in the spring, the vegetative phase is from May to August, flowering commences in the first week of August, and copious quantities of airborne pollen are

produced until frost. Individual plants produce 3,000 to 62,000 seeds that can remain viable for 39 years or more when buried in soil (Bassett and Crompton, 1975). *A. artemisiifolia* is highly plastic and varies in height, inflorescence form, leaf shape, and life form strategy; for example plant height can range from 5 cm to 200 cm.

1.1.2. Control measures

In most soil types, *A. artemisiifolia* can easily be uprooted, but readily adapt to mowing by quickly developing new stems below the cutting height (Vincent and Ahmim, 1985). It is susceptible to several herbicides including 2,4-D [(2,4-dichlorophenoxy) acetic acid], MCPA [(2 methyl-4-chlorophenoxy) acetic acid], 2,4-DB [4-(2,4-dichlorophenoxy butanoic acid)], MCPB [4-(4-chloro-2-methylphenoxy) butanoic acid], mecoprop [2-(4-chloro-2-methylphenoxy) propionic acid] and dicamba (3,6-dichloro-2-methoxybenzoic acid). Bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] and imaze-thapyr [2-[4,5-dihydro-4-methyl-4(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl)-5-ethyl-3-pyridi-necarboxlic acid] provide good control of *A. artemisiifolia* in soybean. Various herbicides and herbicide mixtures provide control in corn. However, populations of *A. artemisiifolia* have developed resistance to atrazine (triazines) and linuron (ureas) (Heap, 1997; St-Louis *et al.*, 2000), thus restricting control options in vegetable crops.

Herbicides such as 2,4-D and dicamba have been the mainstay of *A. artemisiifolia* control strategies in urban areas; however, the wide-scale use of herbicides has dramatically declined in recent years because of increasing public concern over health and environmental effects (St-Louis *et al.*, 2000). These reductions have resulted in increased *A. artemisiifolia* infestations and associated increases in incidence of allergenic reactions. Effective non-chemical strategies to control this weed in urban and sub-urban areas, as well as in agricultural fields are, therefore, required.

Harris and Piper (1970) suggested that *A. artemisiifolia* is amenable to biological control programs because it lies outside the geographic center of origin of the genus *Ambrosia*. These researchers also indicated that mountainous regions of Mexico and South America are potential source areas for biotic agents adapted to a cold climate that could be introduced for the control of common ragweed in Canada.

Faunistic surveys in Canada, southern California, and Mexico listed 894 insect species (86 monophagous and 31 oligophagous) known to attack the 15 most common plant species representing all of the genera within the North American Ambrosiinae (Goeden and Palmer, 1995). In Canada, some native insects and fungi of *A. artemisiifolia* are being studied as inundative biological control agents. Phytocenotic plant competition is also being studied.

In North America, phytophagus insects are important natural enemies of common ragweed that have been successfully used for classical biological control in other countries (Goeden and Teerink, 1993). More than 30 insect species were shipped to USSR in 1978 by Agriculture Canada and USDA-ARS entomologists for the control of *A. psilostachya* and *A. artemisiifolia*. Of these, only two species, *Tarachidia candefacta* (Lepidoptera: Noctuidae) and *Zygogramma suturalis* (Coleoptera: Chrysomelidae) passed extensive host specificity testing and were successfully established (Gilstrap and Goeden, 1974; Goeden and Teerink, 1993; Julien and Griffiths, 1998). *Z. suturalis* rapidly spread throughout the infested areas of southern Russia attaining population densities as high as 5000 insects per m^2 and controlling *A. artemisiifolia* within localized areas. This new biological phenomenon was called a solitary population wave (SPW). Formation of the SPW in crops was accompanied by an increased yield (Kovalev and Vechernin, 1986).

In 1975, a ragweed biological control project was initiated at the Macdonald Campus of McGill University. The objectives of the program were to investigate the potential of endemic

fungal plant pathogens and native herbivorous insects for the inundative control of common ragweed.

The fungus, *Albugo tragopogi Persoon ex S.F. Gray*, occurs locally and has a restricted host range. When inoculated onto *A. artemisiifolia* seedlings at the two-leaf stage, *Albugo* reduced pollen production by 99%, seed production by 98%, and top weight by 79% for systematically infected plants. However, difficulties in mass production of *A. tragopogi* have limited its potential use (Hartmann and Watson, 1980).

A highly virulent *Phoma sp.* was isolated from several diseased ragweed plants in the fall of 1993. *Phoma sp.* can cause substantial seedling mortality and reduction in pollen production but rarely kills mature plants. Studies were conducted on the effect of the combination of *Phoma sp.* and *O. communa* on ragweed and was found that *O. communa* predisposed the host to the attack of the fungal pathogen. Moreover, the combination of *O. communa* and *Phoma sp.* showed a synergistic effect resulting in high levels of plant mortality (Teshler *et al.*, 2002).

In 1994, the potential of two native herbivorous insects, *Zygogramma suturalis* and *Ophraella communa* (Coleoptera: Chrysomelidae), as biological control agents for common ragweed, were evaluated at the Macdonald Campus of McGill University under controlled environment and field conditions.

During 1996 to 1998, *O. communa* and *Z. suturalis* host-specificity, biotic potential, life table, and methods of culturing were examined and compared. Life tables were constructed and the feeding potential of the different life stages of *Z. suturalis* and *O. communa* were determined at three temperatures (20, 24, and 28^{0}C) and three relative humidities (RH) (50, 60, and 80 %) (Teshler *et al.*, 1996). Under near optimal conditions for *Z. suturalis* and *O. communa* of 26-28^{0}C and 40 to 60 % RH, the intrinsic rates of increase were 0.098 and 0.190, respectively. Females of *Z. suturalis* and *O. communa* lay an average of 140 (max 400) and 620 (max 1500)

eggs, respectively. Within five days, eight *Z. suturalis* and *O. communa* 1st and 2nd instar larvae consumed 40-45 % and 75-90 % of the foliage of one *A. artemisiifolia* plant (8-to10-leaf stage), respectively. Mass rearing of *O. communa* on potted common ragweed plants in the greenhouse was feasible due to its intrinsic reproductive rate with no obligatory diapause and direct pupation on the ragweed plant (Teshler *et al.*, 1999). In contrast, the adaptive behavior of *Z. suturalis* (reduced or no oviposition on extensively damaged ragweed plants) and pupation occurring in the soil, presented considerable obstacles for mass rearing.

1.2. *Ophraella communa* (Coleoptera, Chrysomelidae)

Ophraella communa LeSage (Coleoptera, Chrysomelidae, subfamily Galerucine) was previously described as *Galerucella notulata* (Horn, 1893) and then as *Ophraella notulata* (F.) (Welch, 1978; Goeden and Ricker, 1985). LeSage (1986) provided the most complete morphological description, bionomics and distribution of *Ophraella* spp. and separated *O. notulata* as a new species, now known as *O. communa.*

O. communa is an oligophagous insect (*i.e.*, it feeds on several species within the same genus). LaSage (1986) reported that *O. communa* had only one host species, *A. artemisiifolia*, although other hosts were known or suspected. It was reported to also feed upon other members of the subtribe Ambrosiinae (Asteraceae, Heliantheae), including *Parthenium hysterophorus* L., *Xanthium strumarium* L. and *Ambrosia psilostachys* DC (Palmer and Goeden, 1991).

All of the developmental phases of this native, multivoltine insect occur on common ragweed. The adults (fertile females) overwinter in the soil debris and are observed, along with their eggs, on ragweed seedlings in early spring. Adults can fly on short distances (about 2 m). *O. communa* eggs are pale yellow when deposited, darkening to orange as they mature. They are generally deposited in clusters on the host plant (LeSage, 1986). About 24 hrs before hatching, the larval head capsule darkens and can be observed through the chorion. The development time

of each of the three instars is three to four days. All 1st instar and most 2nd instar larvae appear to be gregarious at night, but scatter throughout the plant during daylight. Instars can be distinguished by the size and color of the head capsule (Welch, 1978). Under favorable conditions, *O. communa* adults and larvae can completely defoliate ragweed plants (LeSage, 1986). Neonate larvae typically skeletonize leaves while adults and older instar larvae can devour entire leaves. Prior to pupation, the 3rd-instar larvae spin loosely woven cocoons on the upper or lower leaf surface. Cocoons are constructed as a clear viscous maxillary secretion which hardens and darkens soon after being extruded. Development time for the pupa stage is about seven days. Adult emergence occurs only during the day. The total development time from oviposition to adult emergence is 21.8 ± 0.9 days (Welch, 1978).

1.3. Host specificity

Biological control can be applied by conserving existing natural enemies, adding large numbers of natural enemies to the environment, protecting natural enemies from their predators, or by importing new natural enemies (Wapshere *et al.*, 1974). Criticism of biological control has been based on the fear that after the introduction of the insects that destroyed their weed host, the insects would attack economic crops. The cornerstone of biological control policy has been to introduce only those agents that have a very restricted host range and desirable plant species are not being attacked by the agents.

Host specificity tests are an important component of biological weed control, and without the knowledge of the host range, no biological control agent should be released. Organisms imported for the biological control of weeds should not, therefore, cause any significant damage to plants of economic or ecological importance (Shepherd, 1988).

Caged insects usually lay on and survive on more plant species than they attack in their natural environment. At best, this behavior is inconsistent with proving the safety of the insect

for release and it may result in the rejection of a potentially useful species. For example, the moth *Utetheisa pulchella* (L.), which is one of the principal enemies of common heliotrope (*Heliotropium arborescens* L.) in the Mediterranean, would not pass current standards for introduction to Australia. However, the moth is already present there and causes severe damage to the weed with no recorded damage to any useful plant (Wilson, 1960).

The most important constraint on the choice of biotic agents for use in weed control is that they must not attack any cultivated or socially important plant in the region into which they are to be used. Harris and Zwölfer (1968) suggest that the method for the safety testing of insects should be biologically relevant, being based on investigations of the physiological and chemical basis of host restriction, combined with a limited amount of testing of the selected plants. Host specificity testing should include the following criteria:

a) they are related to the host;

b) a host plant of related insects;

c) plants on which the agent has occasionally been recorded; and

d) plants having characteristics in common with the weed.

As safety usually implies specificity of the organism, either to its weed host, and/or a small group of unimportant related plants, a centrifugal phylogenetic sequence of testing described by Wapshere (1974) is commonly used (Table 1).

Table 1. The centrifugal phylogenetic host specificity method as applied to *Ambrosia artemisiifolia*

Testing sequence	Plants to be tested
1st stage	Other *Ambrosia* species
2nd stage	Other members of tribe *Heleniae*
3rd stage	Other members of family *Asteraceae*

O. communa was studied as a potential biological control agent for introduction into Australia for common ragweed control (Palmer and Goeden, 1991). In pre-release no-choice feeding tests conducted under laboratory conditions, sunflower (*Helianthus annuus* L.) sustained some feeding damage by *O. communa*. The insect was, therefore, rejected even though it had not been recorded on *H. annuus* under field conditions (Shultz, 1978; Hilgendorf and Goeden 1981), except for one record of *O. communa* feeding on Texas blueweed (*H. ciliaris)* (Futuyma, 1990). Similar studies by Palmer and Goeden (1991) were conducted at the Macdonald Campus of McGill University, for multiple as well as no-choice testing for *O. communa* imago on lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), carrot (*Daucus carot* sativus Hoffman), sunflower, and ragweed. Insect feeding studies showed that *O. communa* caused significantly more damage to ragweed than to sunflower. Average estimated daily defoliation caused by *O. communa* on sunflower and ragweed was 6.2 + 2.1% and 15.7 + 4.9%, respectively (M. Teshler, unpublished).

O. communa is a native species and, thus, its use as a biocontrol agent does not have the degree of risk associated with the introduction of an exotic agent. However, if *O. communa* was to be mass released, the risk to the closely related economic crop, sunflower, must be fully assessed.

2. Is sunflower of risk to attack from *Ophraella communa*?

2.1. Introduction

Common or short ragweed, *Ambrosia artemisiifolia* L, is a native, annual North American species and its pollen is considered to be a biological pollutant that is the primary cause of allergenic hay fever, asthma, and eczema. In southwestern Québec and Ontario, it has become a serious agricultural weed. *A. artemisiifolia* plants vary greatly in size and shape and are very competitive, with a high level of allelopathic activity. In most soils, it can easily be uprooted, but it can readily adapt to mowing by quickly developing new systems below the cutting height (Vincent and Ahmim, 1985). It is susceptible to herbicides, but populations of *A. artemisiifolia* have developed resistance to atrazine and linuron (St-Louis *et al.*, 2000), thus restricting control options in vegetable crops.

Classical biological control, involving insect introductions, has been successfully used to control weeds (Julien and Griffiths, 1998). At the same time, native biological control agents have potential merits over classical biological control agents since native biocontrol agents do not require foreign research and strict quarantine procedures. Moreover, an important advantage of using an endemic species is the potential decrease in risk of undesired effects on non-target species since, presumably, native species have co-evolved with local consumers and are less probable to impact existing equilibriums among herbivores, predators, and parasites.

Various insect species have been evaluated as biological control agents against common ragweed (Harris and Piper, 1970; Gagne, 1975; Goeden and Ricker, 1985). *Ophraella communa* Le Sage (Coleoptera, Chrysomelidae) is being evaluated as a potential biological control agent for common ragweed in the United States (Futuyma and Floyd, 1997; Palmer and Goeden, 1991). *O. communa,* previously referred to as *Gallrucella notulata* or *O. notulata,* is native to Québec and can be found throughout most of the United States and Canada (Horn,1893) feeding chiefly, and in eastern North America exclusively, on *Ambrosia* species (Wilcox,1965; Wood 1973;

Futuyma *et al.,* 1993). All stages of *O. communa* occur on common ragweed and total development time from egg laying to adult emergence 21.8 ± 0.86 days (Welch, 1978).

In previous laboratory and field evaluations at McGill University *Ophraella communa* has been shown to be a promising native biological agent of common ragweed. It causes significant ragweed damage, especially at the seedling stage, has a restricted host range, and a high intrinsic reproductive rate (Teshler *et al*, 1998).

Host specificity of *O. communa* was studied by Palmer and Goeden (1991) with the purpose of introducing this insect into Australia for common ragweed control. However, in no-choice experiments under laboratory conditions, the insect attacked sunflower plants and, therefore, was rejected as a potential biological agent without being tested under field conditions. However, the results obtained by Palmer and Goeden (1991) for common ragweed control in Australia do not necessarily rule out the prospects of using *O. communa* in a mass rearing-mass release program in Québec considering that the insect is native to the area. It is hypothesized that in multiple-choice situations, *O. communa* will prefer to feed and reproduce on ragweed plants, and would cause insignificant or no damage to sunflower plants. As well, if under field conditions *O. communa* cannot complete its life cycle on sunflower plants, *O. communa* becomes a potential biological control agent for ragweed control.

Therefore, the main goals of this research were:

- To determine the susceptibility of sunflower cultivars to *O. communa;* and
- To determine the risk of damage to sunflower if *O. communa* was mass released for ragweed control.

2.2. Materials and methods

2.2.1. Experimental cages and containers

For mass rearing and no-choice experiments for groups of insects, 30 x 40 x 26 cm cages covered with 32 x 32 mesh nylon screening fabric (BioQuip, USA) were used (Fig. 1A). For emerged adults, plastic pupation-containers (34 x 20 x 9 cm) with a wick sponge inside and the top covered with 32 x 32 mesh screen were used (Fig. 1B). For field experiments, open cages (70 x 90 x 90 cm) were used (Fig. 2). The cages were 20 % open on each of the four sides as well as on the top (Dunn, 1978). For collection, storage, transportation, and release of *O. communa* adults, 125-ml plastic specimen containers (Container I) were used (Teshler *et al.*, 2001) (Fig. 1C). For the no-choice experiments with individual insects, a plastic container (Container II) with a mesh-covered top (27 x 9 cm) was used (Fig. 1D).

2.2.2. Mass production of *Ophraella communa*

2.2.2.a. Facilities

All development stages of *O. communa* were reared on ragweed plants at the Macdonald Campus of McGill University research greenhouse. The experiments were conducted in an isolated greenhouse section equipped with a thermostat controlled electrical fan and six sodium 400 w lamps. Conditions were maintained at 26 ± 2°C, 40-60 % RH, and 16-8 light-dark photoperiod as these were the optimum rearing conditions based on previous life table studies (Teshler *et al.*, 1996). An entomological aspirator was used to collect and transfer the insects during the mass rearing process (Fig.1 E).

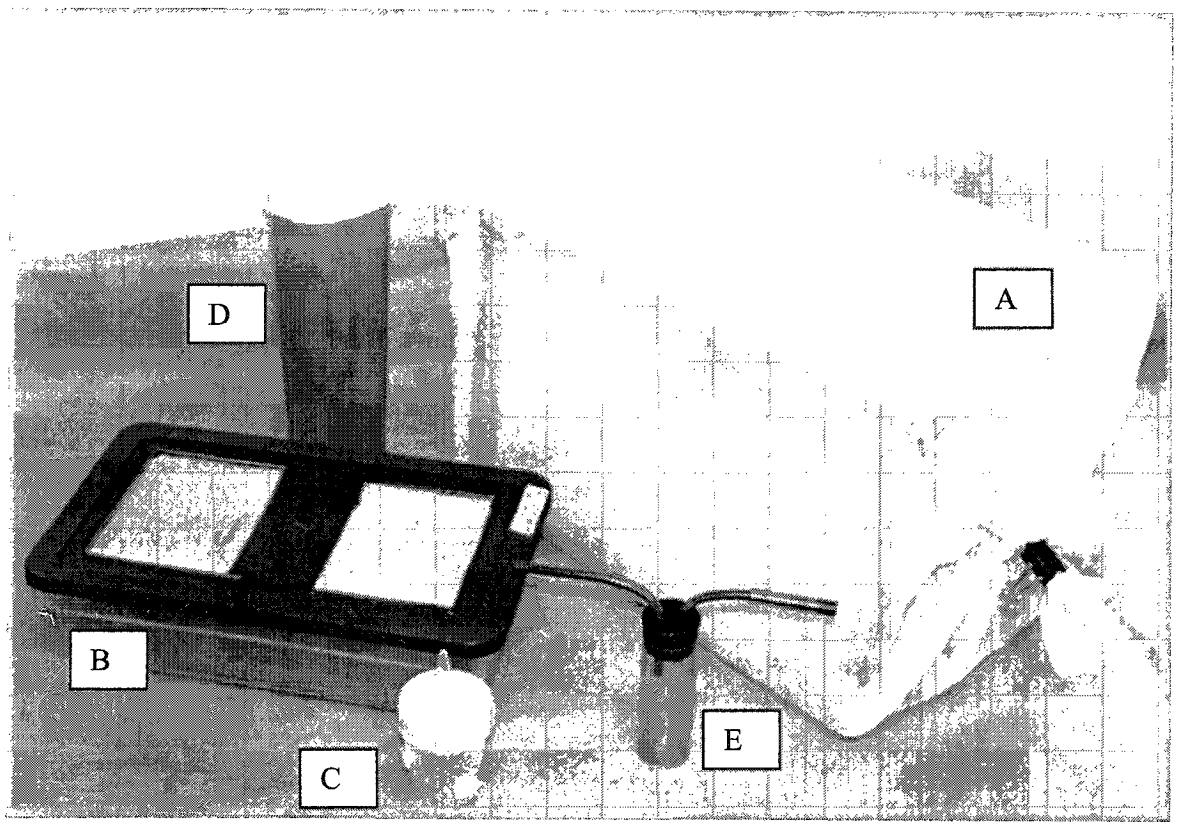

Figure 1. Various containers and the entomological aspirator used in the mass rearing of insects and in experiments. A) Cage for no-choice experiments and mass rearing of *Ophraella communa*; B) Plastic pupa container used for mass rearing; C) Container I (multi-use); D) Container II (life tables experiment); E) Entomological aspirator.

2.2.2.b. Production of ragweed plants

Ragweed seeds were collected from September to October in 1998 and 1999 from natural infestations in the Macdonald campus research fields, mixed with moist sand, and stored at 4 ± 1°C for three months. Then seeds were removed from the sand, placed into paper bags and stored at 4 ± 1°C for one year. To break the dormancy, seeds were treated with 95% sulphuric acid for 10 minutes (I. Teshler, personal communication), rinsed with cold tap water for 12 h, and sown in 30 x 90 x 7 cm^3 plastic trays filled with Pro-mix B (Premier Horticulture Ltd., Dorval, QC, Canada) potting mixture. Six ragweed seedlings (2-to 4-leaf stage) were transplanted into Styrofoam boxes (17.5 x 30 x 6 cm^3) filled with Pro-mix. Plants were fertilized once a week by watering with a 3% solution of 20-20-20 fertilizer (Plant Products, Brampton, ON, Canada).

2.2.2.c. Insect culturing

The laboratory colony of *O. communa* was established by Dr. M. P. Teshler in 1994 from insects collected from a natural population in southwestern Québec. The laboratory colony was annually supplemented with insects from natural population. Mass production of *O. communa* on ragweed plants in the greenhouse had been previously developed in the McGill laboratory (Teshler *et al.*, 1998), however, in this study the method was modified in order to facilitate the production of uniform *O. communa* populations. Insects were divided among three cages depending on their development stage as follows: Cage I – egg masses, Cage II – developing larvae, and Cage III – emerged adults. Imagoes (10 to 50) were placed into the cages with one flat of six ragweed plants for 10 days. After 10 days, ragweed plants with eggs were transferred into a separate cage for pupa development for 10 to 14 days, and *O. communa* adults were then fed with new ragweed plants.

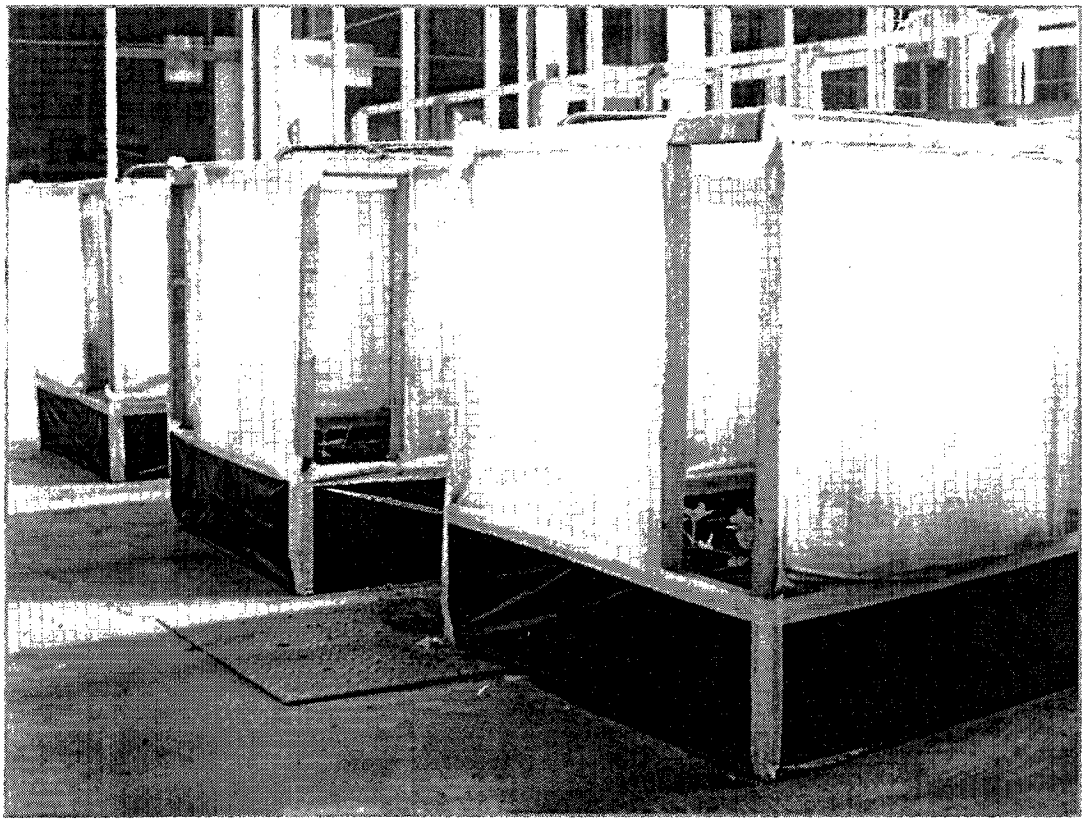

Figure 2. Twenty percent open cages (70 x 90 x 90 cm) at the Macdonald campus of McGill University research greenhouse. The cages were 20 % open on each of the four sides as well as on the top (Dunn, 1978).

Ragweed plants with pupa were cut and placed in pupation containers. Containers were examined every day (morning), and newborn adults were transferred into new cages. All cages were numbered and accurate records were maintained. For short-term storage, adults from the greenhouse were collected in a plastic specimen container (Container I), kept for four hours at room temperature (21 ± 2°C), and then placed in a refrigerator (4 ± 1°C) for 30 days. After cold storage, adults were kept at room temperature for six hours, and then placed in cages containing ragweed plants in the greenhouse.

2.2.3. Sunflower varieties and hybrids

The following sunflower (*Heliantus annuus* L.) varieties and hybrids were tested: Prado and Big Smile (garden varieties); P6230, XF4729, VS 8350 (Western Canada oil and seed hybrids), and 231, T 46-R9 (Northern Corn Belt oil and seed hybrids). All plants were grown at the Macdonald Campus of McGill University research greenhouse and transplanted at the 2-to 4-leaf stage into 9 cm pots (for the life table experiment), into 25 x 23 cm nursery containers for the greenhouse host-specificity studies, or into experimental field plots.

2.2.4. No-choice experiment for a group of insects (cohort)

A total of five cages with 14 sunflower plants (two plants from each sunflower cultivar) were used. *O. communa* adults (male and female) were released in a cage at a ratio of 1:1 male - female per plant (28 adults per 14 sunflower plants). Sunflower damage (%) and oviposition were evaluated every two days for 20 days. Visual feeding damage was estimated using a 5-point scale, where 0 = no damage, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4=51-85%, and 5=86-100% defoliated plant (Kovalev *et al.*, 1983).

2.2.5. Life table approach for individual insects

Life tables of *O. communa* on sunflower and ragweed were constructed following the method of Andrewartha and Birch (1954). Newly emerged adult beetles were kept on ragweed plants in the greenhouse for seven days for feeding and mating. Males and females were then separated using sexual dimorphism, and only females were used for the experiment. Females were held individually on ragweed plants for two days to test fertility. Only females that passed the fertility test on ragweed plant were used in the life table experiments. The fertile females were separated into two groups; the first group was placed on sunflower plants and the second group on ragweed plants.

Observations were carried out every second day until all the females died. When eggs were found on the previously used sunflower plant, the female was transferred onto a new sunflower plant. Ragweed plants with eggs were kept in a separate container (container II) to record egg hatchability, larvae, and pupa mortality, and adult emergence. Sequential generations of *O. communa* on sunflower were continued to be examined using the life table approach.

2.2.6. Multi-choice experiments

One day prior to the start of the experiment, *O. communa* were collected from the stock population in container I and stored in a refrigerator overnight at 10°C.

A. Host-specificity experiment 1 (Hs-1).

The experiment was conducted at the Macdonald Campus of McGill University research greenhouse in a separate room (7 x 3.5 m^2) from July 16 to July 30, 2001. The experiment consisted of three treatments: treatment one (20 %-open cage with 50 insects per cage);

treatment two (20 %-open cage with 100 insects per cage); and treatment three (closed control cage with 50 insects per cage). Treatments one and two consisted of three 20%-open cages, and treatment three consisted of two covered cages. Seven individually potted sunflower plants (one plant of each hybrid) were placed into each of 20 %-open and control cages (Fig. 3A). No ragweed plants were potted in this room. Insect releases were conducted at 1700 h, and the first sampling occurred at 1000 h the following day. During the first week of release, samples were taken every day, and subsequently, samples were taken every second day. The monitored responses included: percent plant damage, oviposition, sunflower seed production, and distribution index (Palmer, 1986). Distribution index is correlation of an average number of beetles on plant to beetles in cage, and reflects the relative attractiveness of the different plants tested.

When the experiment was completed, all insects were collected from the sunflower plants, and all cages were removed. Plants in pots were labelled and kept in the greenhouse until seed production. At the same time, 30 sunflower plants (six from each of the five hybrids) were planted in an isolated room in the greenhouse. Five sunflower cultivars representing the Western Canada and Northern corn Belt oil and seed hybrids were grown until seed production (undamaged control plants). For statistical analysis, a two-way analysis of variance was used.

B. Host-specificity experiment 2 (Hs-2)

The experiment was conducted at the Macdonald Campus of McGill University research greenhouse from July 16 to August 16, 2001. The experimental design and monitored responses were similar to Hs-1 except that seven pots with ragweed plants (one plant per pot) were placed into each 20% open cage with the sunflower plants (Fig. 3B).

C. Host-specificity experiment 3 (Hs-3)

The experiment was conducted at the Macdonald Campus of McGill University research greenhouse from July 31 to August 10, 2001. The experimental design and monitored responses were similar to Hs-1 except that the ragweed plants (one pot with six plants) were placed 1.0 m away from the experimental cages (Fig. 3C).

2.2.7. Field experiment

The experiment was conducted in the Plant Pathology field of the Macdonald Campus of McGill University from July 18 to July 27, 2001. The experimental plots were 0.9 x 0.9 m (Fig. 4). One sunflower plant of each of the seven hybrids and seven ragweed plants were planted into each plot. There were six replications, 50 *O. communa* adults were released in the center of the plot, and monitored for 20 days. The numbers of *O. communa* beetles and egg masses were recorded daily. The distribution index was applied to evaluate *O. communa* feeding preference on ragweed and sunflower plants.

Various insect species that fed on sunflower and ragweed plants were collected. Data were recorded every two hours from 0900 h until 1900 h on the first day, every four hours from 0900 h until 1700 h on the second day, and subsequently twice a day at 0900 h and 1700 h.

Figure 3. Host specificity multi-choice experiments at the Macdonald campus of McGill University research greenhouse. A) Host specificity experiment -1 – sunflower plants in the cage and a 125-ml specimen container I; B) Host specificity experiment -2 – sunflower and ragweed plants in the same cage and 125-ml specimen containers I; C) Host specificity experiment -3 – ragweed plants placed 1.0 m away from experimental cages with sunflower plants.

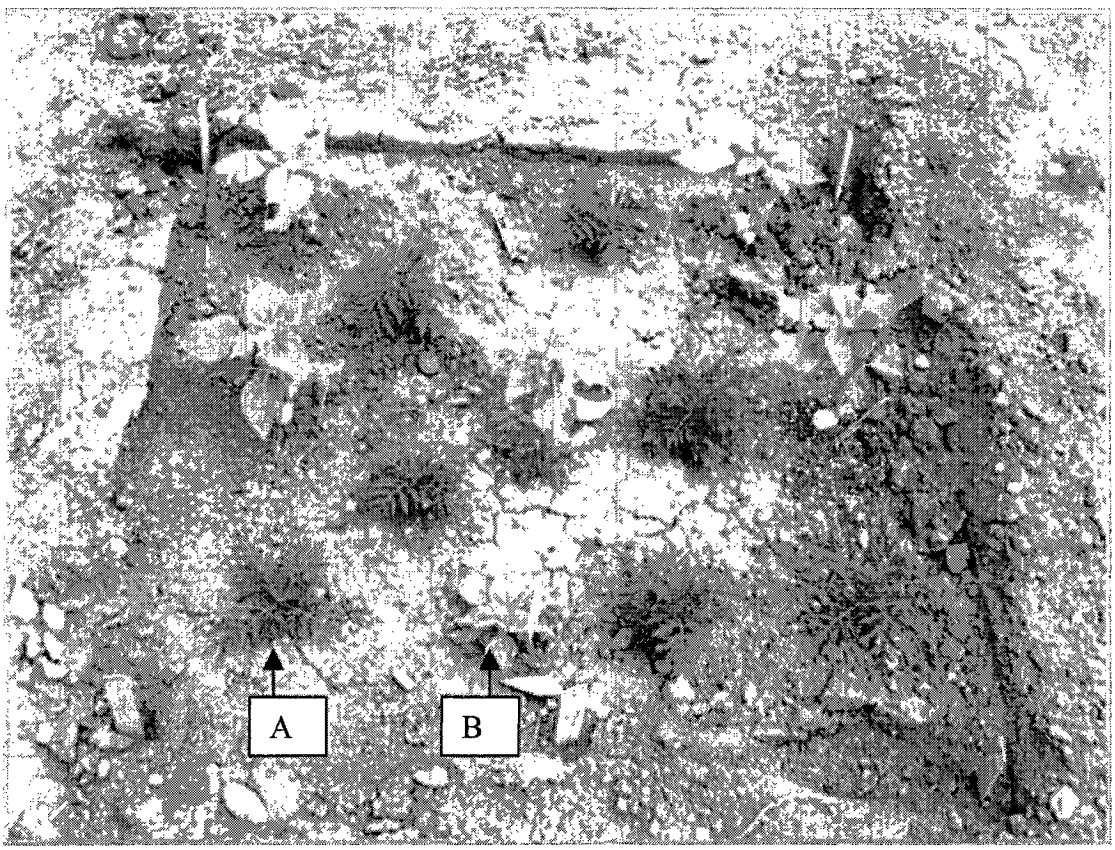

Figure 4. Experimental field plot (0.9 x 0.9 m) located in the Plant Pathology field of the Macdonald Campus of McGill University with ragweed (A) and sunflower (B) plants.

2.3. Results

Life Table.

In the no-choice experiments with a group of insects and an insect-sunflower ratio of 2:1, *O. communa* caused a small amount of damage on sunflower plants (3.3 ± 1.0 %), and no oviposition was observed. No significant difference in damage was found among sunflower cultivars ($P = 0.0106$).

More detailed information was obtained from the no-choice experiment for individual insects. No significant difference was found in the mean life duration of *O. communa* adult females when fed with either sunflower or ragweed plants (Table 2). However, within 30 days 60% of the females died on sunflower plants, while only 14% died on ragweed plants (Fig. 5). The first month is a very important period in *O. communa's* life cycle as substantial population increases occur during this period. During this month, 67.7% of the eggs were laid on ragweed plants (Fig. 6). Table 2 indicates that only 20% of the fertile females placed on sunflower laid eggs, whereas 100% of the females on ragweed plants laid eggs. *O. communa* females started laying eggs on sunflower just three days before they died. It was also observed that *O. communa* females that fed on ragweed plants produced significantly more eggs (16 eggs/day) than females that fed on sunflower plants (0.4 eggs/day) (Table 2). Moreover, egg clusters produced on ragweed plants were significantly larger (23 eggs/cluster) than those produced on sunflower plants (14 eggs/cluster) (Table 2). Egg hatchability on sunflower did not exceed 40%, as compared to 86.7% on ragweed (Table 2). More than 50% of the larvae produced on sunflower plants died during the first three to four days (Table 2).

Table 2. Comparative life table parameters for *Ophraella communa* on sunflower and ragweed plants.

Ophraella communa	Sunflower plants (mean ± 95 % CI)	Ragweed plants (mean ± 95 % CI)
Life duration (days)	30.34 ± 7.4 (a) *	43.7 ± 16.3 (a)
Fecundity (number eggs/female/day)	0.39 ± 0.36 (a)	15.79 ± 1.9 (b)
Ovipositing (percent ovipositing females)	23 ± 0.85 (a)	100 (b)
Size of egg masses (number of eggs/cluster)	13.9 ± 1.8 (a)	22.9 ± 3.1 (b)
Egg hatchability (%)	40.4 ± 27.02 (a)	86.7 ± 9.9 (b)
Larvae mortality (%)	49.74 ± 43.7 (a)	1.3 ± 3.2 (b)

* Means with same letter within a row are not significantly different at the 5% level according the t-test.

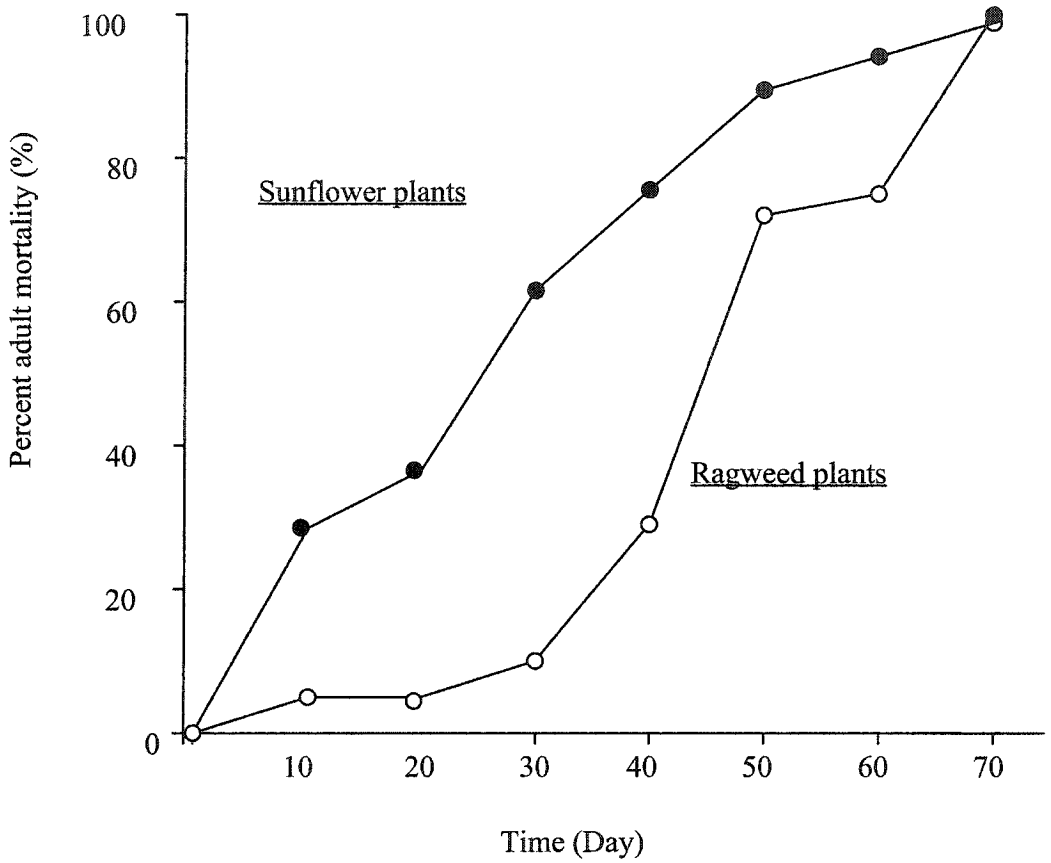

Figure 5. *Ophraella communa* female mortality on sunflower and ragweed plants (Life Table Experiments).

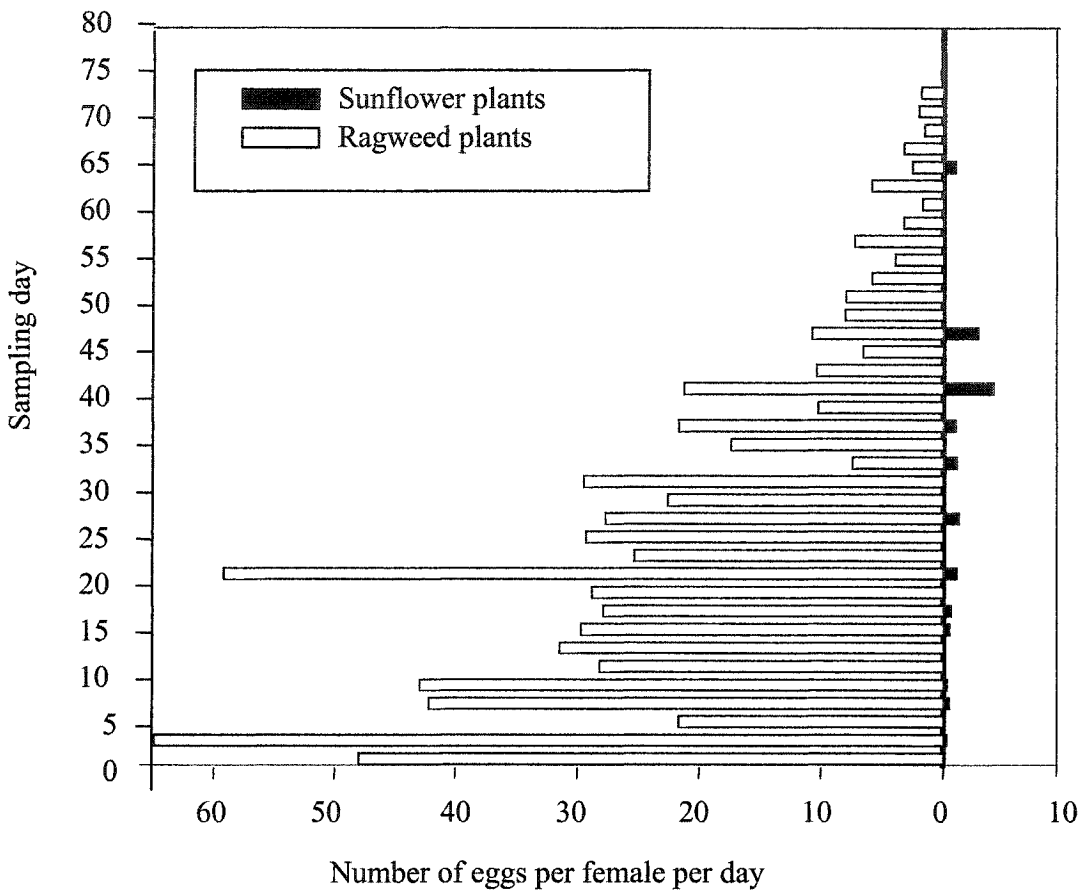

Figure 6. *Ophraella communa* daily fecundity on sunflower and ragweed plants (Life Table Experiments).

The combination of all biological parameters of *O. communa* on ragweed and sunflower plants clearly demonstrates that *O. communa* beetles can feed on sunflower plants, but the *O. communa* population rapidly declines in the no-choice situation. Comparative *O. communa* growth rates on sunflower and ragweed plants, based on mortality and reproduction parameters, are presented in Table 3. Within 30 days, the *O. communa* population increased 208 times when feeding on ragweed and decreased 4.2 times when feeding on sunflower.

Of the 70 gravid females individually placed on sunflower, only six produced a next generation cohort of 14 adults (seven females) on sunflower. Only two of these females from this first generation on sunflower were reproductive and produced a sequential generation of 15 adults (nine females) (Fig. 7). These nine females laid 27 eggs, and none survived to adulthood.

Hs-1

In the no choice situation, *O. communa* caused significantly greater ($P = < 0.001$) damage to sunflower plants in the closed cages than in the open cages. No significant difference in damage among sunflower cultivars or between different densities of *O. communa* in the open cages was found. Twenty-six eggs were found in the open cages, and only five eggs were noted in the closed control cages during the duration of the experiment. The distribution index in the open cages was 0.006, indicating that 99.3% of the *O. communa* adults left the open cages with the sunflower plants. In the no-choice situation, the distribution index was higher in the closed cage than in the open cage (Fig. 8). No significant difference in seed production was found between non-attacked (control) sunflower plants (445 ± 23 seeds/plant) and plants damaged by *O. communa* in open cages (359 ± 13 seeds/plant) (Fig. 10).

Table 3. Population growth of *Ophraella communa* on sunflower and ragweed plants based on Life Tables.

(A) Sunflower plants	
Biological characteristics	Number of insects
Initial insects	100 adults (♀)
Female fertility - 20%	20 adults (♀)
Fecundity (eggs/day) - 0.6% and Adult mortality (30 days) - 60%	126 eggs
Egg viability - 40%	50 larva
Larva 1st instar mortality - 50%	25 pupa
Pupa mortality 5%	24 adults (♀) 2nd generation
Population growth	**Decreases in 4.2 times**
(B) Ragweed plants	
Initial insects	100 adults (♀)
Female fertility - 100%	100 adults (♀)
Fecundity (eggs/day) - 17% and Adult mortality (30 days) - 14%	23715 eggs
Egg viability - 95%	22530 larva
Larva 1st instar mortality - 3%	21854 pupa
Pupa mortality 5%	20760 adults (♀) 2nd generation
Population growth	**Increases 208 times**

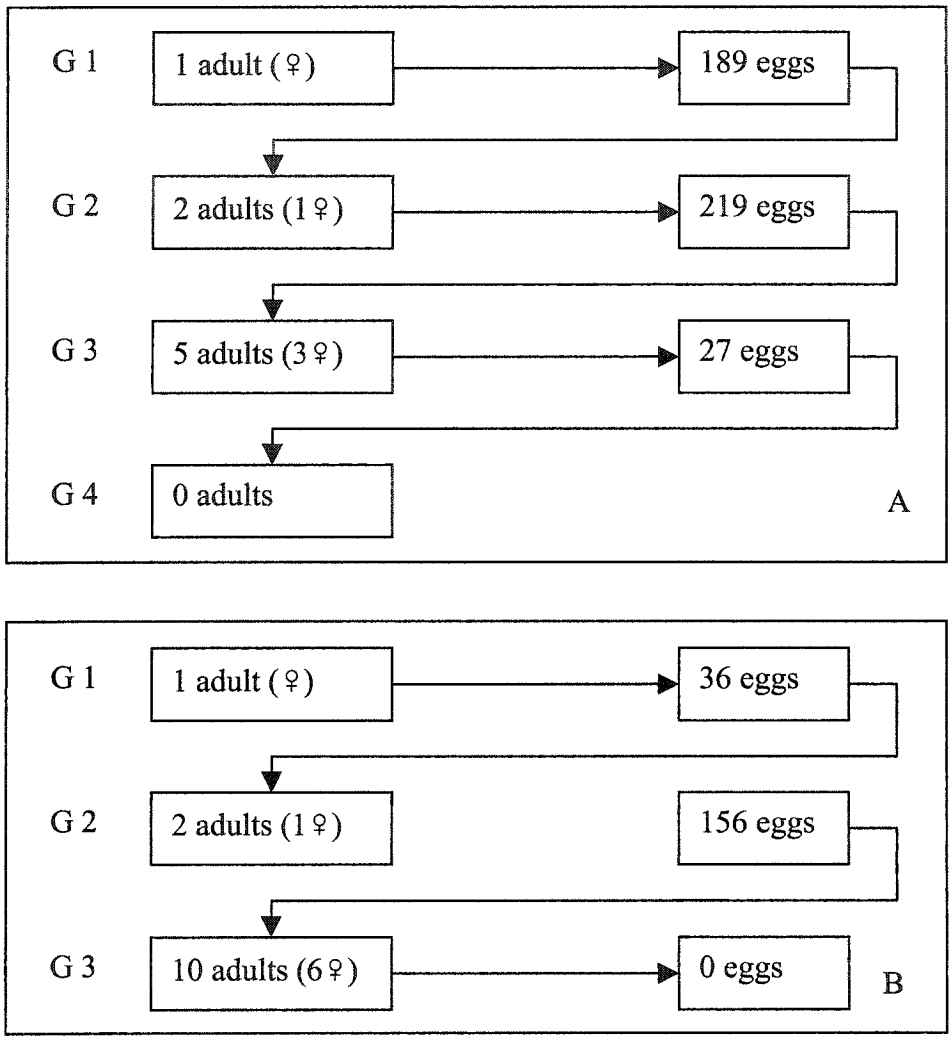

Figure 7. Reproduction of *Ophraella communa* on sunflower; G-generation

A) *O. communa* female - number - 5VS8300; B) *O. communa* female - number -3XF4729.

Hs-2

No difference in damage among sunflower cultivars was found and *O. communa* density (50 or 100 adults per cage) did not cause significant damage to the sunflower plants ($p = 0.298$). During the first 18 days, damage on ragweed plants ($n = 41$) was significantly higher ($74.2 \pm 9.2\%$) than on sunflower plants in open ($n = 41$) or closed ($n = 14$) cages (8.7 ± 3.0 and 16.9 ± 5.4 %, respectfully) (Fig. 11A). No eggs were found on sunflower plants, but 192 ± 29.7 eggs/plant were laid on ragweed (Fig. 11B).

The distribution index on sunflower plants in open cages was 0 (no adults were observed on sunflower plants), whereas on ragweed plants, the average was 0.02 (Fig. 9). After 36 days, damage on ragweed plants was significantly greater ($94 \pm 3.9\%$) ($p < 0.001$) (Fig. 11A). There was a significant difference between the damage on sunflower plants in open cages ($54.7 \pm 6.4\%$) and closed cages ($18.5 \pm 5.1\%$) ($p < 0.001$). No significant difference in oviposition between sunflower plants in closed and open cages was observed, but significantly more eggs (152 ± 22.7) were laid on ragweed plants ($n = 76$) ($p < 0.001$) (Fig. 11B). The number of sunflower seeds produced per plant in closed cages was 71 ± 11, in open cage 134 ± 24, and on sunflower plants without damage 390 ± 31 (Fig. 10). Even when *O. communa* adults completely defoliated ragweed plants in the open cages, the adults did not feed on adjacent sunflower plants, but adults migrated to new ragweed plants outside of the cages. Larvae of *O. communa* did not move from the place of enclosure (especially the first instar larvae). When newborn larvae hatched on ragweed plants in the open cage, the larvae had no choice but to attack sunflower plants causing damage and reducing sunflower yield.

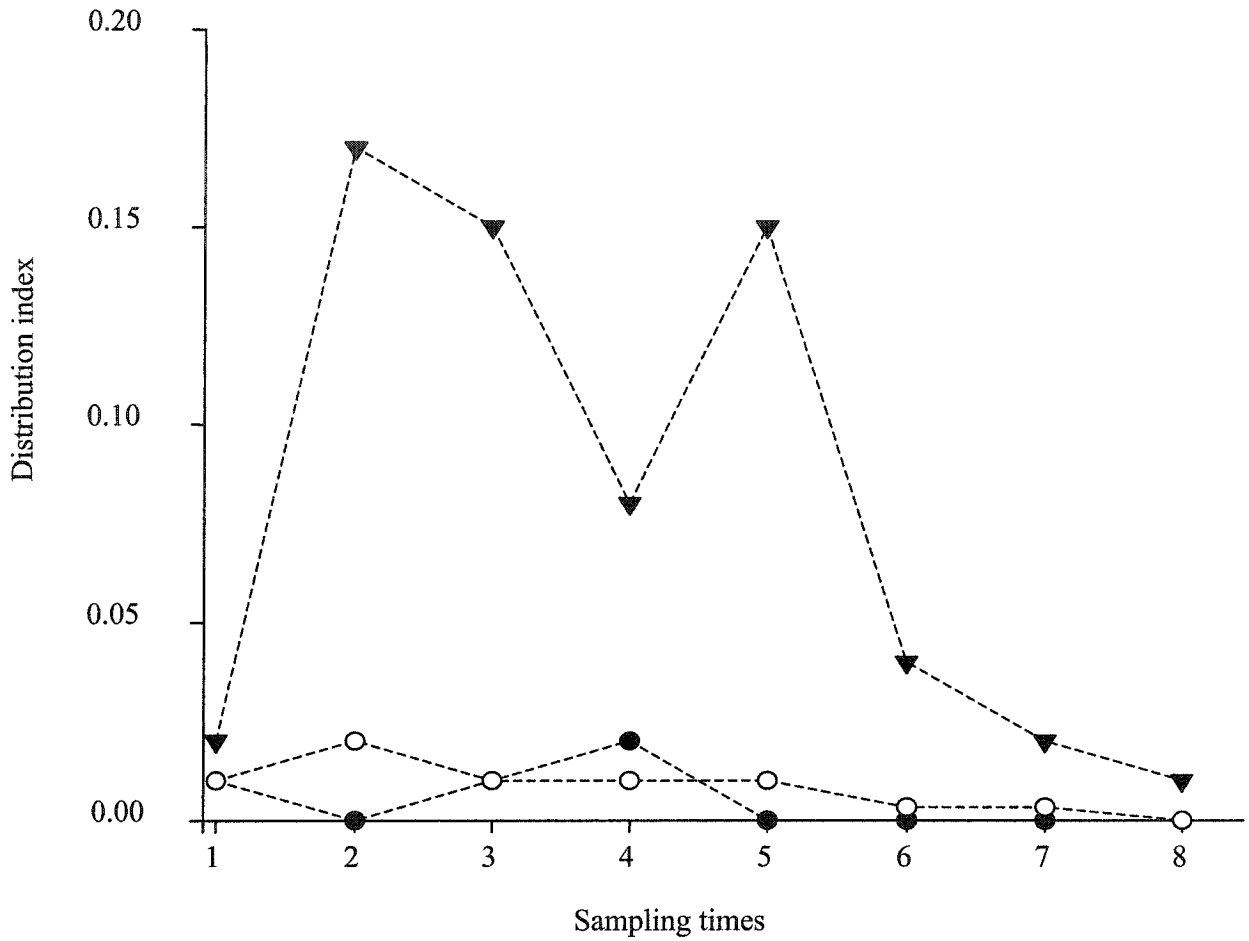

Figure 8. *Ophraella communa* distribution index (D.I.)* on sunflower plants (Host specificity-1).

--●-- In open cage with density of 50
--○-- In open cage with density of 100
--▼-- In closed cage with density of 50

$$*DI = \frac{\text{Average No. of beetles on plant}}{\text{Average No. of beetles in cage}}$$

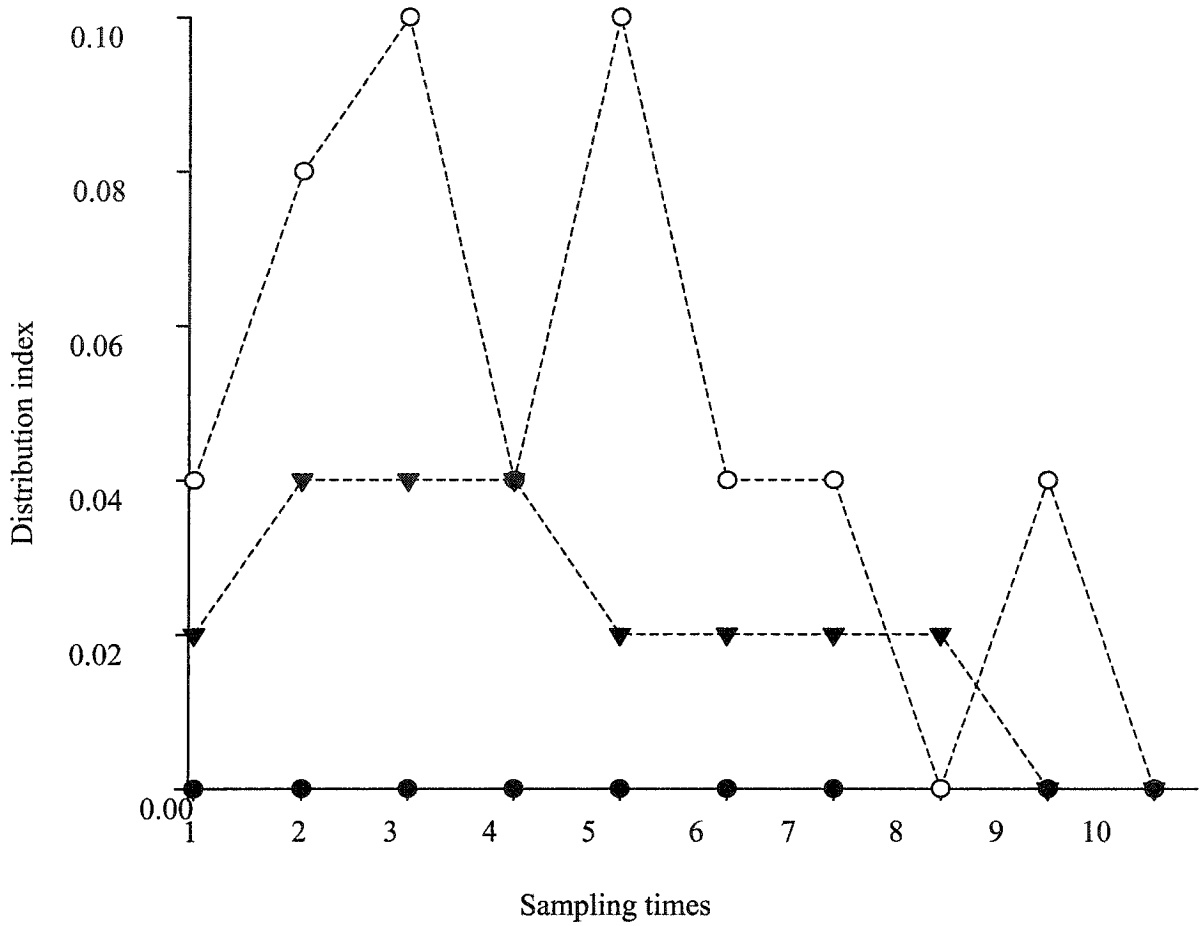

Figure 9. *Ophraella communa* distribution index (D.I.)* on sunflower plants (Host specificity-2).

In open cage with sunflower
In closed cage with sunflower
In open cage with ragweed

* DI = Average No. of beetles on plant / Average No. of beetles in cage

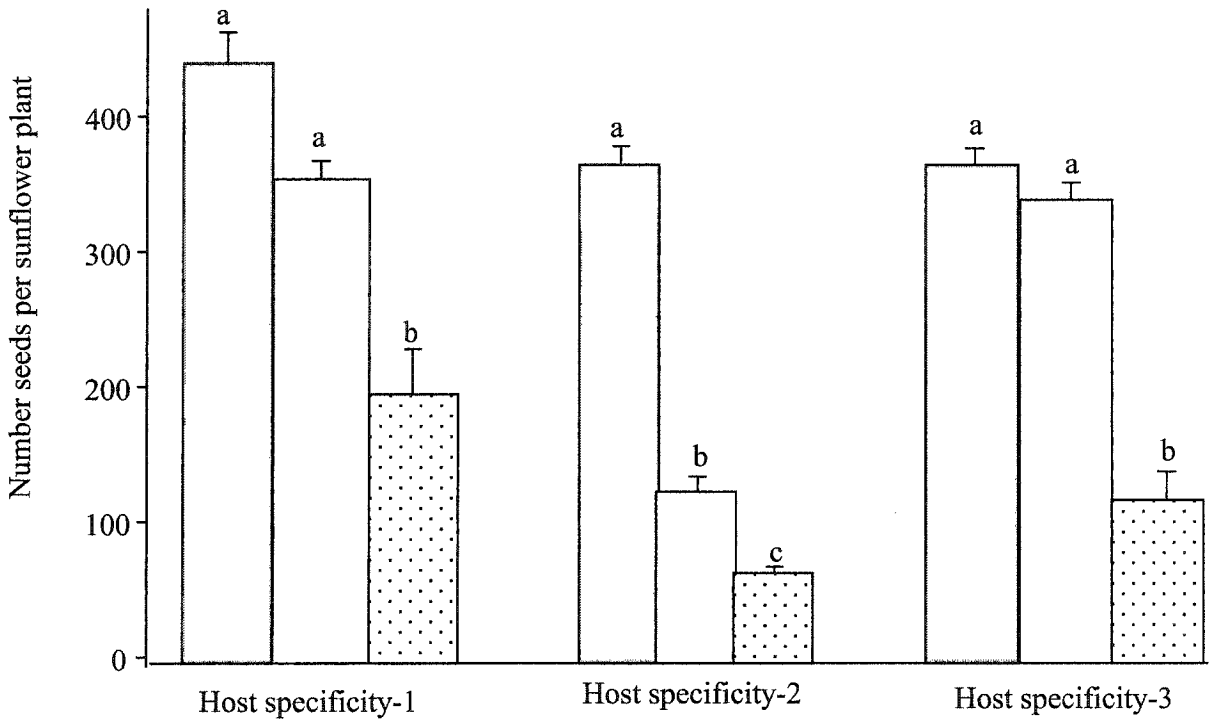

Figure 10. Effect of *Ophraella communa* damage on sunflower seed production in three multi-choice experiments (Hs-1, Hs-2, and Hs-3). Means with same letter within a row are not significantly different at the 5% level according to the Student-Newman-Keuls test.

Hs-3

Damage caused by *O. communa* on sunflower hybrids in the open cage was similar to that observed in Hs-1 and Hs-2. No significant difference was found between the damage on sunflower in closed cages (33 ± 17) and ragweed (32 ± 8) placed outside of the cages. However, significantly lower damage was found on sunflower plants in open cages (0.47 ± 0.7). During the experiment, no eggs were observed on sunflower plants in open cages, and only five eggs in the control, covered cages. During the same period, 127 egg masses (about 1800 eggs) were found on ragweed plants outside the cage. No adults of *O. communa* were observed on sunflower plants in the open cages. The distribution index on sunflower plants in covered cages was 0.02, and on ragweed plants outside the cage was 0.20. Thus, 20.4% of the insects remained on ragweed plants and only 2.2% remained on sunflower plants in the open cages. Seed production of sunflower plants without damage (control) (391 ± 27) and sunflower plants from the open cage (358 ± 27) were not significant different ($p = 1.000$) (Fig. 10).

Field experiment

O. communa preferred ragweed plants to sunflower plants for oviposition. During the first two days, 24 egg masses were found on ragweed plants while no egg masses were observed on sunflower plants. On the final sampling day (day 16), there were 93 egg masses on ragweed and only three egg masses masses were found on the interior side of the lower leaves sunflower (Fig. 12).

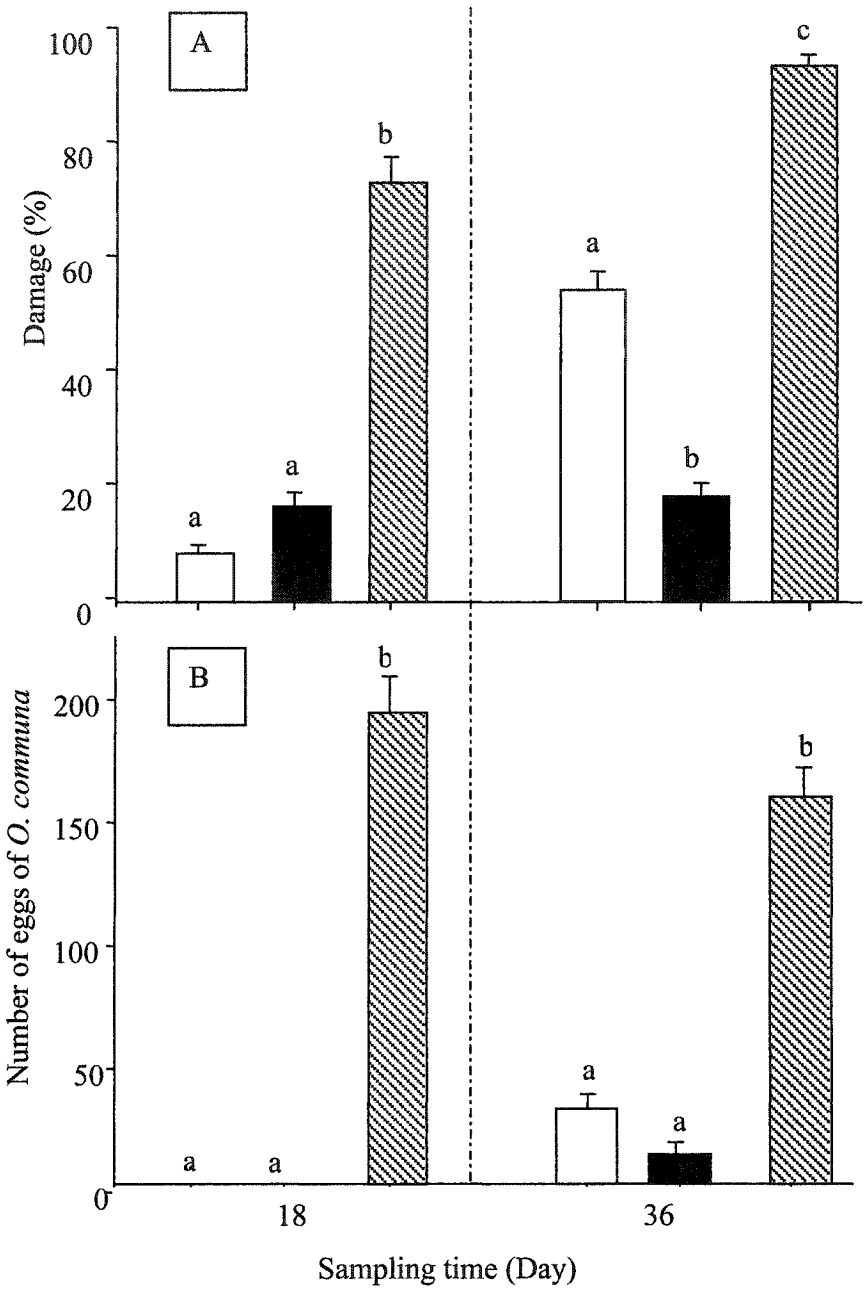

Figure 11. *Ophraella communa* damage (A) and oviposition (B) on sunflower and ragweed plants 18 and 36 days after release (Host specificity-2). Means with same letter within a row are not significantly different at the 5% level according to the Student-Newman-Keuls test (SWK).

The distribution index was significantly (P = 0.004) lower on sunflower than on ragweed (Fig. 13). *O. communa* beetles stayed briefly on sunflower plants. The density of *O. communa* on sunflower and ragweed plants stabilized five days after release with one insect per ragweed plant and 0.1 insect per sunflower plant. These ratios of *O. communa*: sunflower and *O. communa*: ragweed were maintained during the rest of the experiment.

Different polyphagous insects from the Chrysomilidae family were noted on the plants. Polyphagous beetles from Carabidae family attacked ragweed seeds. *Evaresta bella* Loew (Diptera: Tephritidae), a monophagous *A. artemisiifolia* seed feeding fly, was recorded. *E. bella* can destroy 8% of ragweed seeds (Foote, 1984). During the field studies, there were sharp decreases in egg masses (Fig. 12) due to egg predators by *Damsel bugand* (Nabidae), *Toralius* bugs, and lady bugs (Coleoptera: Coccinellidae). Spiders also attacked the first instar larvae and eggs of *O. communa.* During the monitoring experiment, predators destroyed approximately 27% of the eggs.

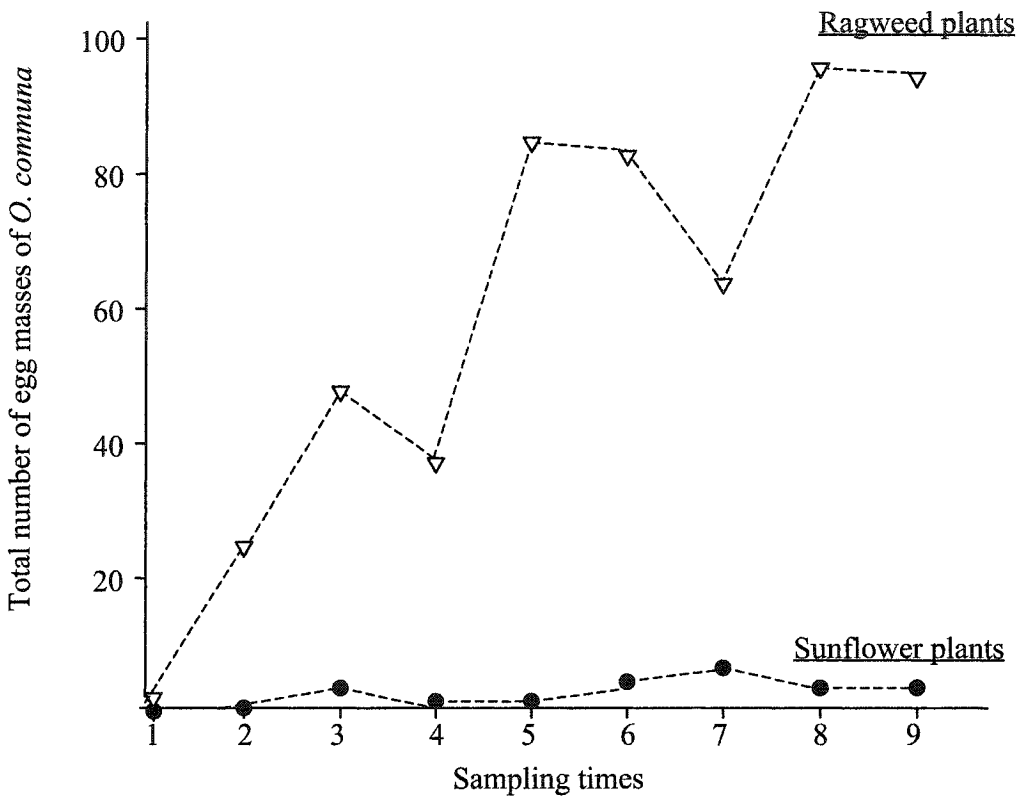

Figure 12. Oviposition of *Ophraella communa* on sunflower and ragweed plants in the field.

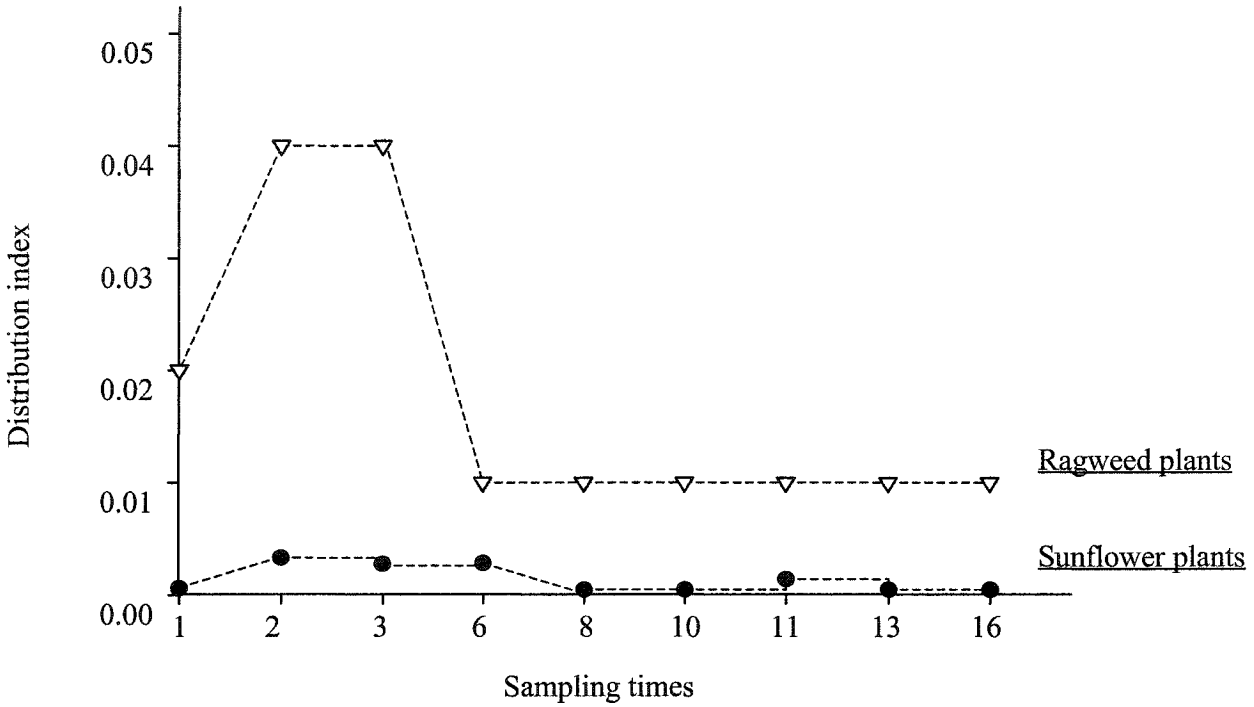

Figure 13. Distribution index*(DI) on sunflower and ragweed plants in the field.

$$*DI = \frac{\text{Average No. of beetles on plant}}{\text{Average No. of beetles in cage}}$$

3. Discussion

Classical biological control is based on two ecological principles: that one organism (biological agent) can be used to control another, and that this biological agent has a limited host range. Until now, most of the classical biological weed control organisms have been insects. The chrysomelid beetles are conspicuously more successful than any other insect group (Crawley, 1989). One chrysomelid beetle, *Zygograma suturalis,* a natural enemy of common ragweed (*A. artemisiifolia)* in North America, successfully acclimated and suppressed common ragweed in one region of the former United Soviet Socialist Republic. Common ragweed is a serious urban and agricultural weed. Ragweed pollen is a major biological pollutant being the primary cause of allergenic hay fever, asthma and eczema. More that 30 insects have been examined as potential biological agents to control common ragweed (Kovalev *et al.*, 1983).

Another ragweed insect, *O. communa* is an oligophagous insect (LaSage, 1986) reported to be a host of various members of the subtribe Ambrosiinae (Asteraceae, Heliantheae). The current tendency in biological control is not to automatically exclude oligophagous insects from the contemporary protocols (Palmer and Goeden, 1991).

In the laboratory no-choice testing, *O. communa* developed equally well on *Helianthus annuus*, and *A. artemisiifolia* (Palmer and Goeden, 1991). The insect was, therefore, rejected to be introduced in Australia even though it has never been recorded on *H. annuus* in the field (Goeden and Palmer, 1995). Analogous results were obtained in 1998 at the Macdonald Campus of McGill University (Teshler, unpublished). The selection of sunflower as a "critical test plant" is supported by faunistic surveys since approximately 21% of the *Ambrosia* insects also utilized sunflower plants as hosts.

Host specificity tests typically measure the potential of the biological agent to complete its life cycle on the target organism, as well as on non-target organisms. Scientists have used different methods for host specificity testing. Early safety evaluation was assessed by testing the

potential agent against all the crop plants that grow in the region into which the control organism was being considered for introduction. Harris and Zwolfer (1968) proposed biologically relevant testing methods based on investigations of the physiological, morphological, phenological, entomological, and chemical bases of host restriction, combined with testing plants that are related to the target host, host plants of related insects, the agent has occasionally been recorded on them, and have characteristics in common with the target weed. The centrifugal-phylogenetic testing procedure (Wapshere, 1974), a commonly used biologically relevant method, is an additive procedure that involves testing plants of increasingly distant relationship to the host until the host range is circumscribed. A margin of safety is added by testing all related plants of economic (e.g., crops and horticultural plants) and ecological (e.g., threatened and endangered plants in the native flora) value that could be considered "at risk" from the biological control agent. Wapshere's system has been successfully applied in many host specificity studies including that of *Uromysces heliotropii*, a fungal agent for the biological control of common heliotrope (*Heliotropium europaeum*) in Australia (Hasan *et al.*, 1992). Tests included inoculating 96 plants important to the Australasian region using both microscopic and macroscopic observations that looked for reactions in host and non-host plants.

Resistance of plants to herbivores insects and pathogens is mediated via constitutive or induced defense mechanisms (Mauricio *et al.*, 1997). However, because of the differences in metabolism of plant toxins some induced defenses do not protect against specialist herbivores insects, but only against generalist herbivores insects (Agraweal, 1999). Terpenes of *Helianthus* play an important role as mechanism against larvae of the sunflower moth (Lepidoptera: Pyralidae) (Roegers *et al.*, 1987). Further studies of host specificity of *O. communa* should compare defense mechanisms of sunflower and ragweed plants against this herbivores insect.

In the present research, closed cages and 20% open cages were used to determine the suitability of *H. annuus* as a host of *O. communa*. Similar results to the ones reported by Palmer and Goeden (1991) were obtained for the no-choice situation in closed cages containing only sunflower plants. In the 20% open cages, *O. communa* can remain in contact with the sunflower plants, but also have the alternative to leave the cage. Over 99% of the *O. communa* beetles left the sunflower plants in the 20% open cages with no ragweed nearby , and 98% left the 20% open cages when ragweed plants were close by.

A different approach was taken in this research. Life tables (Andrewartha and Birch, 1954, Morales-Ramos and Cate, 1992,. Cocuzza *et al.*, 1997, Southwood, 2000.) were used as an indicator of host specificity. The life table approach provided the opportunity to observe the biological potential of *O. communa* on host (ragweed) and non-host (sunflower) plants. By comparing data from host and non-host plants, survival and reproductive growth for following generations can be modeled. Life tables present an especially useful approach where developmental stages are discrete and mortality rates may vary widely from one life stage to another (Andrewartha and Birch, 1954). *O. communa* demonstrated different population growth on non-host (sunflower) and host (ragweed) plants. Population of *O. communa* increased in 208 times by feeding on ragweed, and decreased in 4.2 times on sunflower plants. From the first generation of 100 *O. communa* females on sunflower, only 24 female were born in the next generation. Clearly, *O. communa* cannot show equal development on both plants, contary to conclusions of Palmer and Goeden (1995).

Host specificity and host range tests are no guarantee of environmental safety, however. The danger of biological control organisms goes beyond their ability to consume non-target organisms and include their potential to harm non-target organisms in other ways (e.g., by direct interference or interaction via intermediate species), plus their capacity to survive, reproduce, disperse, and evolve. For example, *Galerucella calmariensis* and *G. pusilla* leaf-feeding

chrysomelids were released in the United States for control of purple loosestrife (*Lythrum salicaria*) (Schooler *et al*, 2003). This plant originates from Europe where 120 species of insects feed on it. Host specificity of the abovementioned leaf-feeding chrysomelids was studied in order to determine whether the proposed biological control agents could complete their life cycles on crepe myrtle (*Lagerstroemia indica L.*) (non-target plant) under the laboratory and field conditions (Schooler *et al*, 2003). The host specificity test results obtained by Schooler *et al.* demonstrated that larvae of these beetles could not develop on crepe myrtle and, therefore, these beetles could not complete their life cycle on the non-target plant. The results also suggested that the damage caused by these chrysomelids to the non-target plant had only temporary and minimal effects on the crepe myrtle populations. Under field conditions, the damage caused by adult beetles decreased as the distance between purple loosestrife (target plant) and crepe myrtle (non-target plant) increased. The results further confirmed that the beetles had great effect on the target plant. As biological control agents may provide the long-term success in controlling purple loosestrife, the California Department of Food and Agriculture granted permission for release of *Galerucella calmariensis* and *G. pusilla* leaf-feeding chrysomelids for control of purple loosestrife within the state (Schooler *et al.*, 2003).

Goeden and Palmer (1995) indicated that *O. communa* has never been recorded on sunflower in the field. In the present study, *O. communa* beetles stayed briefly on sunflower plants in the field experiments, with only one *O. communa* individual per ten sunflower plants staying five days after release, meanwhile on ragweed one individual per ragweed plant remained during this period.

The current situation with *O. communa* in Japan can serve as a natural confirmation of some conclusions made in this discussion. *O. communa* was discovered in 1996 after it was accidentally introduced into Japan. During the following years, *O. communa* rapidly spread in 25 prefectures and was observed on *Ambrosia artemisiifolia*, *A. trifida* and *Xanthium strumarium*.

Density of adults of *O. communa* of the second generation on *A. artemisiifolia* was five adults per plant; two adults per plant on *A. trifida;* and 1 adult per plant on *X. strumarium* (Yamazaki *et al.*, 2000). *O. communa* was not observed on sunflower plants in the field. *O. communa* was very mobile but demonstrated its friendliness to the new environment and preference to *Ambrosia* species (Yamazaki *et al.*, 2000).

O. communa has demonstrated great potential for biological control. Parameters that characterize good biological control agents: high feeding potential, high reproductive potential; and ease for mass rearing and handling (Crawley, 1989). All of the developmental stages occur on common ragweed and both *O. communa* adults and larvae can completely defoliate ragweed plants (LeSage, 1986). The total development time from oviposition to adult emergence is 21 to 22 days (Welch, 1978). Since 1997, *O. communa* has been successfully mass reared in research greenhouse at Macdonald Campus of McGill University (Teshler *et al.*, 2000). Having shown a lot of potential, further research should focus on improving mass rearing of *O. communa* by developing an artificial diet and on optimizing release schedules to minimize the influence of predators and parasites on *O. communa*.

4. General conclusions and summary

Two methods have been used to demonstrate the safety of *O. communa* for the biological control of ragweed; a life table approach for individual insects and host specificity studies with cohorts of insects. Data from life tables demonstrated the ability of *O. communa* to survive and develop on sunflower plants. Susceptibility, comparative life tables of *O. communa* were constructed on sunflower and ragweed plants to evaluate differences in oviposition, life duration, egg hatchability, larvae and pupa mortality, and generation increase. In addition, damage and distribution indices were evaluated. The life table no-choice method revealed biological differences between *O. communa* females feeding on sunflower and ragweed plants.

Host-specificity studies, a series of experiments with the 20% open cages, simulated *O. communa* behavior and feeding choices they would make in open, natural situations. The rationale behind the use of 20% open cages was to ensure that insects have ample opportunity to be in contact with the test plant, but also have the alternative to leave the cage, and search for an acceptable host plant. All experiments with 20% open cage were planned to be conducted under field conditions. However, prior to *O. communa* release, sunflower plants were found damaged by other phytophagous insects. Therefore, all experiments were moved into the greenhouse. In the different simulation situations (experiments Hs.1, Hs.2, Hs.3) with 20% open cages, no significant difference in damage among sunflower cultivars was found. Damage in open cages was significantly lower on sunflower than on ragweed. In the no-choice situation (Hs-1) without ragweed plants nearby, over 99% of the *O. communa* adult left the sunflower plants, and only three egg masses were observed during the experiment. Similar data was obtained in Hs-3 where ragweed plants were outside the cage that contained sunflower plants, wherein 98% of adult *O. communa* left sunflower plants, and no eggs were observed on sunflower plants compared to 127 egg masses on ragweed plants. Thus, it can be concluded that there is minimal risk of *O. communa* adult-stage beetles attacking sunflower plants. Usually a female *O. communa* female

prefers to die on sunflower than to lay eggs (only two out of 10 fertile female laid a few eggs on sunflower plants in the no-choice situation). However, *O. communa* larvae can cause significant damage to sunflower plants. That situation was demonstrated in Hs.2 when damage on sunflower plants affected seed production. This occurred because of the high density of the 1st instar larvae, which hatched on completely defoliated ragweed plants and had no choice to move to new ragweed plants. As a result, they attacked the closest sunflower up to (20-30 cm) away in the area. Nevertheless, by using data from Life Table experiment, 50 % of the 1st instar larvae will die feeding on sunflower plants and there will be no increase in the *O. communa* population. Newly emerged adults prefer to leave the sunflower plants.

Thus, it is highly improbable that *O. communa* would cause significant damage to sunflower plants in the field. For *O. communa* to survive on sunflower plants in the field, several simultaneous factors have to be met. The 1st generation of *O. communa* adults must completely defoliate ragweed plants and the 2nd generation of larvae, having no ragweed plant to feed and develop on, would move onto adjacent sunflower plants up to (20-30 cm away). Therefore, there is an extremely low probability of *O. communa* surviving on sunflower plants in the field. From additional life table experiments on sunflower plants, we know that *O. communa* can reproduce, however, the following generations attenuate, and cannot survive by feeding on sunflower.

An interesting phenomenon was discovered during the life table experiments. Most of the *O. communa* females died during the first 30 days, but in some cases, longevity was more than 100 days, which is two times longer than the usual survival on ragweed. The abnormal behavior was also observed with *O. communa* feeding on a non-optimized artificial diet (Teshler, unpublished). An explanation of this phenomenon is that *O. communa* female preserves biological potential by laying less number of eggs, which effects its survival longevity and, thus, extends the chance to lay eggs on the preferred host plant, *A. artemisiifolia*.

It can be concluded that *O. communa* prefers to feed and reproduce on its host plant - *Ambrosia*. However, in situations with no host plants, and taking into consideration the poor flying ability of the beetle, *O. communa* could migrate to nearby sunflower plant. Nevertheless, that transitional period on sunflower plants would occur only in a no-choice situation, and no increase in the population would be observed. The following generation of *O. communa* would return to the host plant (*A. artemisiifolia*) or die.

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