

## A Novel Device for the Collection, Storage, Transport, and Delivery of Beneficial Insects, and its Application to *Ophraella communa* (Coleoptera: Chrysomelidae)

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*A multi-use device was developed for the collection, short-term storage, transport, and delivery of Ophraella communa (Coleoptera: Chrysomelidae), a biological control agent of common ragweed, Ambrosia artemisiifolia. The device is made from a 125-ml plastic specimen container that can hold O. communa adults or pupae. When used as an aspirator, insect collection and counting times are reduced. O. communa adults and pupae can be stored inside the container at 3°C with median survival of 41 and 21 days, respectively. A cotton wick saturated with water or a 5% sugar solution nourishes insects during transport and the container design minimizes insect mortality by providing an optimum microclimate during insect storage and transport. Designed to protect insects from rainfall and to limit encounters with predators and parasites, the containers can be used for field releases of O. communa adults and pupae. Although the container has been designed and tested for O. communa, it is highly versatile and could possibly be used with a variety of insect species.*

**Keywords:** multi-use device, biological control, beneficial insects, *Ophraella communa*, collection, storage, transport, field delivery

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## INTRODUCTION

*Ambrosia artemisiifolia* L. (common ragweed) (Asteraceae) is a native annual North American herbaceous species whose pollen is the primary cause of allergenic hay fever, asthma, and other serious illnesses (Bagarozzi & Travis, 1998). Furthermore, *A. artemisiifolia* is an important agricultural weed of both field and vegetable crops in eastern North America including south-western Québec (Uva *et al.*, 1997). Populations of *A. artemisiifolia* have developed resistance to some commonly used herbicides (Heap, 1997), thus restricting control options in both crop and non-crop systems. Wide scale herbicide use has declined in recent years in urban areas because of increasing public concern about health and environmental effects. These reductions have resulted in increased *A. artemisiifolia* infestations, and associated increases in allergenic reactions. Thus, there is a need to find alternate strategies of controlling this troublesome weed that are both environmentally and economically acceptable.

The native chrysomelid-defoliator *Ophraella communa* LeSage [*O. notulata* (F.)], and several plant pathogenic fungi have been evaluated as potential inundative biocontrol agents of *A. artemisiifolia* (Teshler *et al.*, 2002). Under favorable conditions, *O. communa* adults and larvae can completely defoliate and kill *A. artemisiifolia* plants (Lesage, 1986). All developmental phases of this multivoltine oligophagous insect occur on this plant. The mated adults overwinter in the soil and are observed in early spring along with eggs on *A. artemisiifolia* seedlings (Goeden & Ricker, 1985). Recent observations in Japan have shown that *O. communa* has great vagility and host-discriminating ability – neither eggs nor larvae were found on plants not suitable for its progeny (Yamazaki *et al.*, 2000).

In Québec, cage releases of *O. communa* against common ragweed were made in various vegetable crops in 1999 and 2000 (Teshler *et al.*, 2000), and open field releases were conducted in an organic soybean (*Glycine max* (L.) Merrill) field in 2001. These studies demonstrated the potential for using *O. communa* for the inundative biological control of ragweed. However, several predators and parasites have been reported to reduce *O. communa* density (Goeden & Ricker, 1985; Futuyma, 1990; Teshler *et al.*, 2002).

Mass rearing of *O. communa* on transplanted ragweed plants under glasshouse conditions is feasible due to the high intrinsic reproductive rate of this insect (Teshler *et al.*, 1996) and ease of control of the adult diapause stage (Watanabe, 2000). Although *O. communa* is relatively simple to rear on transplanted ragweed plants under glasshouse conditions (Moriya, 1999; Teshler *et al.*, 2000), it is difficult to maintain a significantly large colony to ensure a continuous and uniform supply of insects. Thus, it would be desirable to store mass-reared insects for a time, without deterioration in quality, until sufficient numbers are available for field release.

The success of the inundative biological control tactic using insects is highly dependent upon the effective mass rearing of target agents such that manipulation of agents and losses during collection, storage, transport, and field releases are minimized.

Current methodologies for the collection or sampling of living insects involve the use of mouth aspirators and mechanical suction devices (Southwood & Henderson, 2000). Plastic or carton containers and fabric bags (Knudsen, 1966; Boldt & Drea, 1980; O'Neil *et al.*, 1998) have been used for the transport/shipment of insects. Field distribution of beneficial insects to control agricultural pests has been carried out using carton cards, capsules, and plastic cylinders (Van Schelt & Ravensberg, 1990; Kiku & Teshler, 1993; Mahr & Ridgway, 1993). Cold storage at low temperatures has been widely employed in biological control programs to hold beneficial insects temporarily in the laboratory, or to synchronize releases with the development of pest insects or weeds in the field (Gilkeson, 1990; Tauber *et al.*, 1997; Leopold *et al.*, 1998; Li & Otvos, 1998; Gagne & Coderre, 2001).

The main goal of this work was to develop an efficient and simple method to facilitate year-round research using *O. communa* insects reared on transplanted ragweed plants under glasshouse conditions. Here, we present a simple device for (1) collection, (2) low-

temperature storage, (3) transport, and (4) small-scale field delivery of *O. communa* insects cultured under controlled environments.

## MATERIALS AND METHODS

The *O. communa* laboratory colony was established with insects collected in 1998 from a natural population in Mount-Rigaud, southwestern Québec, Canada (longitude 74°18'W and latitude 45°27'N). All developmental stages of *O. communa* were reared on ragweed plants inside 32-mesh nylon screen cages (25.5 × 40 × 30 cm) housed in a research glasshouse at 24 ± 4°C, 60 ± 20% RH, and a 16-h photoperiod.

### Device description

A multi-use device was constructed from a 125-ml polypropylene specimen container (a) with a screw cap (b) equipped with a 1.5 ml transparent microcentrifuge tube (c) (Canadawide Scientific Ltd., Ottawa, ON, Canada) (Figure 1). The device has three 10-mm openings (d) to facilitate insect collection and field release and to allow air circulation during storage and transport. Two of the openings have a removable plastic cap, while the third has a plastic cap with an 8-mm diameter circular area of 32-mesh nylon screen. The protruding 1.5-ml tube is used to secure the container in the soil and prevent the container from being dislodged by strong winds. A water-saturated 4 by 10 mm cotton wick (e) projecting into the container permits insect feeding during transport and maintains high humidity during storage and transport. Two removable flexible silicone tubes (f) (9.5-mm outside and 6.5-mm inside diameter) (Canadawide Scientific Ltd., Ottawa, ON, Canada) can be fitted into the release-collection openings, thereby converting the container into an aspirator. The tube size was chosen based on *O. communa* adult length and width dimensions of 4.1 by 2.1 mm for females and 3.7 by 1.9 mm for males (LeSage, 1986). To protect the user from inhaling debris, one end of the tube is covered with a fine nylon screen (g). The 125-ml specimen

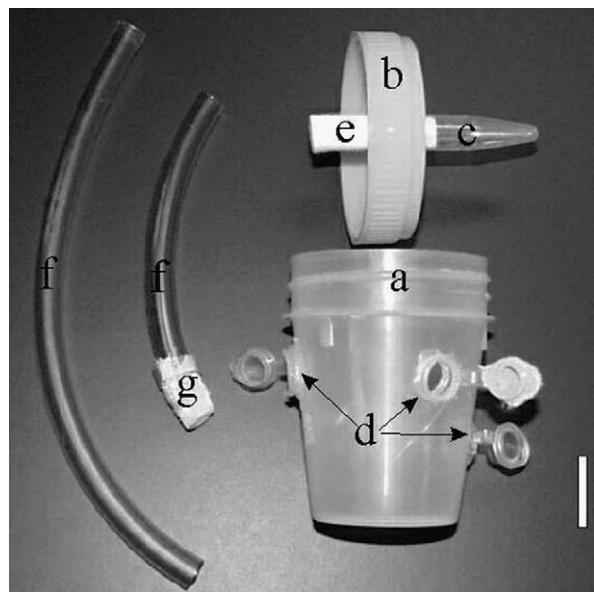


FIGURE 1. Disassembled view of the collection, storage, transport, and delivery device (description is in the text). Scale bar = 20 mm.

container can hold up to 200 *O. communa* adults. The aspirator reduced collection and recording time up to four-fold compared with insect collection using a fine paintbrush. *O. communa* adults reared under greenhouse conditions on transplanted ragweed plants were collected in specimen containers for low-temperature storage, transport and small-scale field releases (Figure 2).

### Storage

Adults and pupae of *O. communa* were stored inside the described containers, with air circulation provided through the mesh-covered opening while the other two openings were kept closed. The containers were placed in a low temperature incubator (Sheldon Manufacturing, Cornelius, OR, USA) at  $3 \pm 0.5^\circ\text{C}$  under constant darkness. A hygrometer (Testo 605-H1- LabCor Inc., Anjou, QC, Canada) with a 125-mm probe stem was used to monitor RH inside the containers.

*Adult stage.* Twenty-five non-diapausing *O. communa* adults (sex-ratio ca. 1:1) were collected in each of 20 specimen containers.

*Acclimation prior to storage.* Containers with insects were placed for three hours in a refrigerator at  $10^\circ\text{C}$ , and then for 72 hours at  $5^\circ\text{C}$ . Containers were subsequently transferred to the low temperature incubator ( $3^\circ\text{C}$ , constant darkness). Ten containers were equipped with a water-saturated cotton wick (hereafter referred to as w-containers); the mean RH inside these containers was  $95.7 \pm 2.9\%$ . The 10 remaining containers did not receive cotton wicks (hereafter referred to as nw-containers); the RH inside these containers was similar to



FIGURE 2. Different uses of the container: aspiration of *Ophraella communa* adults into the container (1); storage at low temperatures (2); transport to the field (3); and release in a soybean field (4).

the incubator ambient humidity of  $64.6 \pm 2.4\%$ . Weekly, w-containers were refilled with water as needed.

*Recovery from storage.* Containers with insects were transferred from the incubator into a refrigerator set at  $5^{\circ}\text{C}$  for 24 hours,  $10^{\circ}\text{C}$  for three hours, and subsequently placed on a laboratory bench at ambient conditions ( $22 \pm 1^{\circ}\text{C}$ ).

Once weekly for ten weeks, one w-container and one nw-container were removed from storage, and insects within the containers released in glasshouse cages containing ragweed plants as described above. Insect mortality was determined within 24 hours. Five female insects were randomly selected from each cage and placed individually into  $12 \times 14$  cm transparent plastic cylinders containing a single 4- to 6-leaf stage ragweed plant. Twice weekly, fecundity and egg viability were evaluated for each female. *O. communa* adults and eggs reared on ragweed plants under glasshouse conditions served as controls.

*Pupal stage.* Twenty-one containers with 15 pupae each comprised the low-temperature storage experiment. Procedures for pupal acclimation, prior storage, and recovery after storage were similar to those described for adult insects. Once a week, seven w-containers were removed from storage and placed on a laboratory bench under ambient conditions ( $22 \pm 1^{\circ}\text{C}$ ). *O. communa* pupae taken from the same laboratory population and not subjected to cold storage served as controls. Adult emergence and mortality were determined daily.

### Transport

An experiment simulating the transport of *O. communa* adults was carried out in controlled environment chambers set at a high temperature ( $28 \pm 0.5^{\circ}\text{C}$ ), constant darkness and a RH of  $50 \pm 3\%$  (Controlled Environments Ltd., Winnipeg, MB, Canada). To simulate vibration during the transport, containers were fixed on a slowly-agitating (50 rev/min) orbital shaker (Lab-Line Instruments Inc, Melrose Park, IL, USA).

*Adult stage.* *O. communa* adults emerging from pupae within one to two days were collected and placed for seven days on ragweed plants in a glasshouse. Ten adult insects (1:1 sex ratio) were placed inside (1) w-containers, (2) nw-containers, and (3) containers equipped with a cotton wick saturated in a 5% sugar solution (w5-containers). Each treatment was replicated 10 times. Adult mortality inside containers was recorded daily for 10 days.

*Egg stage.* Newly deposited *O. communa* eggs were placed inside w-containers and on water-moistened filter paper in Petri dishes (control treatment). Experimental and control treatments were replicated 20 times and consisted of one egg cluster (ca. 25 eggs) attached to ragweed leaves. Egg viability (number of emerged larvae) was measured daily for six days.

*Water evaporation from the cotton wick.* Experiments were conducted in controlled environment chambers ( $28 \pm 0.5^{\circ}\text{C}$ ) in the dark. Ten containers with one nylon mesh-covered opening were used to evaluate the rate of water evaporation from the cotton wick placed inside the container in the 1.5 ml vial. Each  $10 \times 40$  mm cotton wick received 2.0 ml of water with an additional 0.5 ml of water pipetted into the 1.5 ml vial above the cotton wick. Cotton wicks saturated with a similar amount of water, wrapped inside the 1.5-ml plastic vials and placed into the controlled environment chambers, served as controls. Weights of each container (and cotton wick) for the experimental treatment and weights of the cotton for the control treatment were taken daily for 14 days.

### Output from the specimen container

Experiments were conducted both in a glasshouse and in the field (Ste-Anne-de-Bellevue, QC, Canada). Specimen containers with one, two, or three openings were used. Thirty *O. communa* adults were placed in each container. Individual containers were then placed into a wooden cage ( $25.5 \times 40 \times 30$  cm) covered with a 32-mesh nylon screen and placed either in

the glasshouse, or a nearby soybean field-plot. All trials were initiated at 8 a.m. and the number of insects in each container was recorded every four hours for 24 hours.

The effects of various plant species: common ragweed (main host), sunflower *Helianthus annuus* L. (surrogate host plant), or radish *Raphanus sativa* L. (non-preferred plant) on the output rate of *O. communa* adults from the specimen containers were compared. Containers with 100 *O. communa* adults were placed inside the wooden cages described above and transferred to controlled environmental chambers with ragweed, sunflower, or radish plants arranged outside the cage. An environmental chamber without plants placed around insect cages served as the control treatment. Each treatment was replicated four times. The number of insects remaining in each container was recorded every four hours for 48 hours.

### Field delivery

In 2001, inundative releases of *O. communa* were conducted in a ragweed-infested organic soybean field in St. Isidore, QC, Canada. *O. communa* adults were released using specimen containers, as described above, with 30 adults per container. Insects were released within  $2.5 \times 2.5$  m experimental plots (one treatment) consisting of twenty-five  $0.25 \text{ m}^2$  sub-plots. The following factors were evaluated: (1) insect density per experimental plot (10, 25, and 40 adults per  $\text{m}^2$ ) and (2) the number of release sites per plot (1, 3, and 5). Plots with no released insects served as controls. Damage caused by *O. communa* to ragweed plants was recorded twice weekly for four weeks for experimental and control plots based on 25 randomly selected plants (one plant taken from each  $0.25 \text{ m}^2$  subplot). A damage scale from 0 to 5 was used (Karel & Rweyemamu, 1984). Experimental and control treatments were replicated twice.

### Statistical analysis

One-factor ANOVA followed by a Tukey's test was used for separation of means using SigmaStat (v. 2.03, SPSS Inc., Chicago, IL, USA). Prior to statistical analysis, all percentage data were subjected to arcsine transformation (Fowler *et al.*, 1998) and checked for normality. For all tests conducted, statistical significance was set at 5% and data are presented as untransformed means  $\pm$  SE. Regression analysis was carried out for the storage and output experiments using the curve fitting procedure of SigmaPlot (v. 6.10, SPSS Inc., Chicago, IL, USA). For comparative purposes, *O. communa* survival in storage and transport was expressed as median survival (Motulsky, 1995), which is the amount of time spent in storage or transport resulting in a 50% reduction in survival (50% mortality).

## RESULTS

### Storage

*Adult stage.* *O. communa* adults could tolerate short term storage at  $3^\circ\text{C}$  inside the specimen containers (Figure 3). The high relative air humidity (ca. 95%) produced by the water-saturated cotton wick probably facilitated *O. communa* survival. Within the first 21 days of storage, no significant difference ( $P < 0.05$ ) was found between the mean survival of adults within nw-containers and w-containers. After 21 days of storage, however, significantly higher mortality ( $P < 0.05$ ) was observed for insects stored inside nw-containers (Figure 3). The median survival time of *O. communa* adults was 41 and 28 days for w-containers and nw-containers, respectively (see dotted lines, Figure 3). Median survival time of insects maintained inside w-containers under glasshouse conditions (i.e. control treatment) was  $6.2 \pm 2.5$  days.

On average, storage at  $3^\circ\text{C}$  caused a two- to three-day delay in oviposition by *O. communa* females after they were removed from the incubator; however, the cold treatment did not affect *O. communa* egg viability. For instance, eggs laid by females stored for six weeks in

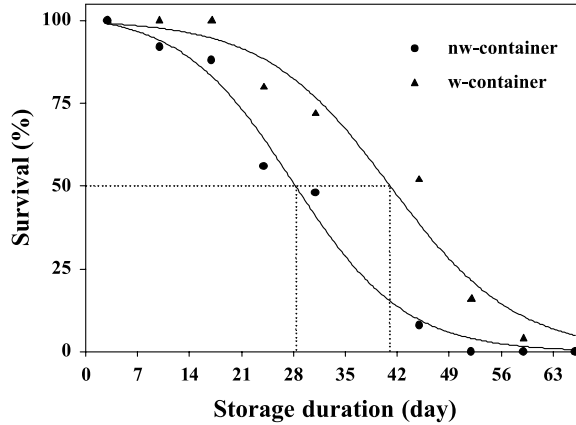


FIGURE 3. Effect of storage duration (days) on *Ophraella communa* adult survival inside a container equipped with a water-saturated cotton wick (w-container) or without a cotton wick (nw-container). Nonlinear regression equations are:  $Y_{nw} = 102.8/1 + e^{(-3.70+0.14x)}$ , ( $R_{nw}^2 = 0.99$ ) and  $Y_w = 100.2/1 + e^{(-4.85+0.12x)}$ , ( $R_w^2 = 0.97$ ), where,  $Y_{nw}$  and  $Y_w$  = survival (%) inside w- and nw-containers, respectively;  $x$  = storage period (day);  $e$  = base of natural logarithm (2.718281). Dotted line = mean survival time.

w-containers and nw-containers had mean viabilities of  $93.0 \pm 2.5\%$  and  $88.2 \pm 6.5\%$ , respectively. This was not significantly different ( $P = 0.301$ ) from egg viability of adult insects not subjected to the cold treatment ( $96.3 \pm 1.3\%$ ).

After four weeks of storage in w-containers, mean daily egg oviposition per female ( $13.7 \pm 4.8$ ) was also not significantly different ( $P = 0.448$ ) from that of comparably aged *O. communa* females fed on ragweed plants under glasshouse conditions ( $8.4 \pm 1.9$ ).

**Pupal stage.** *O. communa* pupal survival based on adult emergence was  $76.2 \pm 4.6\%$  when stored for up to 14 days inside w-containers at  $3^\circ\text{C}$ , and was not significantly different ( $P < 0.05$ ) from pupal survival for the control treatment ( $68.3 \pm 7.5\%$ ). Median survival time of pupae in cold storage was 21 days.

**Transport**

**Adult stage.** The specimen container is a convenient and safe method for transporting and/or shipping live insects. The cotton roll soaked in water or 5% sugar solution significantly reduced ( $P < 0.001$ ) adult mortality compared with the control treatment, where insects were kept inside the specimen containers without a supply of water or sugar solution. For example, after seven days at  $28^\circ\text{C}$ ,  $95 \pm 5\%$  of females and  $66.7 \pm 11.8\%$  of males survived when nourished with the 5% sugar solution (w5-container). When insects were supplied only with water (w-container),  $85 \pm 10.7\%$  of females and  $55.5 \pm 15.5\%$  of males survived. No significant difference ( $P = 0.397$ ) was found between the percentage of adults surviving inside w-containers and w5-containers. However, only  $15.0 \pm 10.7\%$  of females and no males survived after seven days in the control treatment, where insects were held in nw-containers.

**Egg stage.** Under ambient conditions of  $23\text{--}24^\circ\text{C}$ , *O. communa* eggs attached to ragweed leaf surfaces could be properly maintained inside w-containers for up to six days. Within this interval, most eggs hatched (egg viability  $73.8 \pm 5.7\%$ ). No significant difference was found ( $P = 0.897$ ) between the viability of eggs placed on moistened filter paper in Petri dishes (control treatment) or those inside w-containers.

**Moisture evaporation.** The wrapping of the cotton wick inside of the plastic vial provides a slow-evaporation system for maintaining humidity when storing or transporting insects. For example, based on experiments conducted under controlled chamber conditions,  $21.8 \pm 1.5\%$  of water evaporated from cotton wick within four days,  $35.9 \pm 0.9\%$  within seven days, and  $72.9 \pm 1.5\%$  within 14 days. Alternatively, the cotton wicks wrapped in plastic vials and maintained under the same temperature conditions outside the specimen container (i.e. control treatment) were dry within 20 hours of the start of the trial. Median water evaporation from cotton wick inside w-containers and from cotton wicks placed outside the containers (control) was 10 days and 10 hours, respectively.

### **Insect output from the container**

**Number of openings and temperature conditions.** The 125-ml specimen containers were used for *O. communa* adult field and glasshouse releases. Within 24 hours at mean ambient temperature of  $18 \pm 2^\circ\text{C}$  (field trial), no significant difference ( $P = 0.155$ ) was found in the number of insects leaving the w-containers having one or two openings, but a significantly greater ( $P < 0.05$ ) number of insects abandoned the containers when three openings were provided. The departure rate of *O. communa* was significantly ( $P < 0.005$ ) greater at  $27^\circ\text{C}$  in a glasshouse than at  $18^\circ\text{C}$  in the field. For instance, within the first 14 hours, more than 80% of insects vacated the containers at  $27^\circ\text{C}$  while only 50% left the containers at  $18^\circ\text{C}$ .

**Attractiveness of plant species.** In the environmental chamber experiment, no significant differences in the departure of *O. communa* adults from containers was found after 2, 4, 8 and 24 hours from the start of the trial when ragweed, radish, sunflower, or no plants were placed outside the containers. For example, 24 hours after the initiation of the trial, the percentage of adult insects leaving the containers was:  $68.5 \pm 8.2\%$  (ragweed),  $72.4 \pm 6.0\%$  (radish),  $77.0 \pm 4.8\%$  (sunflower),  $73.3 \pm 3.1\%$  (no plants).

### **Field release**

In the open non-caged field experiment, initial insect-plant ratios ranged from one insect per two ragweed plants to one insect per 10 ragweed plants. Despite high initial ragweed field infestation levels and low insect densities, *O. communa* caused notable ragweed damage largely because of significant increases in larval density. In some treatments, as many as 13 larvae per plant were recorded. The following time sequence of ragweed damage was observed in the field for treatments with initial densities of one adult insect per two ragweed plants: (1) first week after release, moderate ( $12.6 \pm 3.8\%$ ) ragweed damage caused by adult feeding; (2) second week, no increase in ragweed damage ( $9.9 \pm 4.5\%$ )-at this time, most female beetles began to deposit eggs and disperse; (3) third week, significant increases ( $32.4 \pm 7.7\%$ ) in plant damage from larval feeding were observed.

## **DISCUSSION**

The container described can be used for collection and transport of different developmental stages of live insects (e.g., adults, fragile egg masses, larvae, or pupae) or the convenient and safe shipment of living insects to the field. Its construction diminishes damage, dehydration, or death of the target organism. In addition to aspiration, a hand-operated pump or modified vacuum sweepers (Fisher & Finney, 1964) could be used to facilitate insect collection. The cotton wick wrapped inside the plastic tube and saturated either with water or a carbohydrate-rich nutrient source (e.g. sugar solution) functions as a slow-evaporation system. It can support insect feeding and maintain optimum moisture level inside the container for up to seven days during *O. communa* transport at up to  $28^\circ\text{C}$ , or storage at low temperatures.

Non-diapausing *O. communa* adults and pupae tolerate short-term storage at low temperatures (3°C) inside the containers. Adults may be stored for up to six weeks and pupae for up to two weeks without significant mortality or reductions in fecundity and egg viability. *O. communa* adults could be stored for longer periods if they were to undergo diapause.

Field observations demonstrate that the containers could protect insects against rainfall and minimize *O. communa* mortality by unspecialized predators or parasites. Based on data collected from five different sites, *O. communa* adult mortality inside containers did not exceed  $1.3 \pm 0.7\%$ ,  $n = 2000$ . Many parasitic and predatory insects rely on olfactory cues, such as volatiles from frass, and visual signals when searching for host or prey (Whitman, 1988; Klowden, 2002); *O. communa* may be shielded by the container, giving off fewer cues and are, thus, less susceptible to parasites and predators. The use of these containers is especially important for quiescent insect stages like pupae or eggs that are more vulnerable to attack. For example, unprotected pupae of *O. communa* are readily parasitized by the gregarious hymenopteran parasite *Asecodes mento* (Walker) (Teshler *et al.*, 2002).

Based on our field experiments, within a week of release, *O. communa* adults feed on ragweed plants in the vicinity of the release site, lay eggs and migrate extensively throughout the field. Approximately two weeks after release, when *O. communa* density increase due to larval eclosion, it was difficult for insects to substantially impact growth and development in this fast growing weed species. Therefore, if common ragweed is to be controlled successfully by *O. communa*, releases should occur early in the growing season, with combined releases of adult and pupal stages. The container devices described can be used for *O. communa* adults or pupal releases and are equally suited for combined releases of these two developmental stages.

An important advantage of the container is that it is inexpensive and easy to construct. The cost of making one container using readily available laboratory materials is about 0.45\$(US), which includes specimen container, glue, cotton wick, 1.5 ml vial, and labor. One person can produce approximately 20 containers per hour. However, one drawback of releasing insects in these containers is the additional cost for their removal from test fields prior to crop harvest. To reduce such expenses, containers could be made from biodegradable and yet heat-resistant material, such as a heavy papier-mâché.

Several specialized techniques and devices have been developed for collecting, packing, and storing beneficial insects pending shipment and field releases (Fisher & Finney, 1964; Krysan, 1981; Hendrickson *et al.*, 1987). A similar approach using a device that combines all the functions described in this paper including collection/aspiration, storage, transport, and delivery in one container or vessel was described by Stary (1970). Stary used the exchangeable plastic containers for mass-collection, storage, transport and release of parasitic hymenopteran species (*Aphidius smithi* Sharma & Subba Rao) for the control of pea aphids in perennial and annual crops. Despite some similarities, however, Stary's method was designed for relatively small (less than 1 mm in size) parasitic insect species, and lacks several important features of our method and device. Firstly, Stary's (1970) method includes no moisture regulation system inside the containers. The moisture level during insect storage and transport is instead regulated by placing a damp cotton wool on the mesh outside the plastic container which needs to be frequently moistened. Secondly, in his approach, insects are released by opening the container's cap, thus the plastic container does not protect insects against rainfall or predators and is not appropriate for combined releases of different developmental stages.

The method and device described herein can be modified for use with various beneficial insects against glasshouse pests including aphidophagous (*Coleomegilla maculata* (DeGeer), *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guerin), cocidophagous (*C. montrouzieri* Timberlake) and aleurodophagous (*Cryptolaemus montrouzieri* Mulsant) coccinellids. Containers could be helpful for general use in experimental research with insects and for field

collection of various stages of living insects, and plant parts for safe transport and laboratory testing.

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